STANDARDISATION AND QUALITY EVALUATION OF 'TEMPEH' AND TEMPEH BASED INSTANT SOUP MIXES

By

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes"** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

Vellanikkara
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CERTIFICATE

Certified that this thesis entitled **"Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes"** is a bonafide record of research work done independently by **Mrs. Lakshmy, P. S.** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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'Feeling gratitude and not expressing it
is like wrapping a present and not giving it.'
(William Arthur Ward)

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

ABBREVIATIONS

A.O.A.C.	- Association of Official Analytical Chemists
cfu	- Colony forming units
DAS	- Days after storage
DMRT	- Duncan's multiple range test
g	- Gram
h	- Hour/Hours
IMTECH	- Institute of Microbial Technology
IVPD	- *In vitro* protein digestibility
IVSD	- *In vitro* starch digestibility
kg	- Kilogram
LAB	- Lactic acid bacteria
mg	- Milligram
ml	- Millilitre
mm	- Millimetre
MTCC	- Microbial Type Culture Collection
ND	- Not detected
NS	- Not significant
ppm	- Parts per million
µg	- Microgram
µl	- Micro litre
º C	- Degree centigrade
%	- Per cent

Introduction

1. INTRODUCTION

The wise men consider that health is the greatest of human blessings and as the age old saying *"Let food be thy medicine and medicine be thy food* - (Hippocrates)*"* is certainly a truth that one must accept.

Fermented foods, whether from plant or animal origin, are an intricate part of the diet of people in all parts of the world. Indigenous fermented foods were known before recorded history, but only recently the world has taken a closer look at it as these are not only low cost and nutritious, but survived for centuries and time tested to be safe and wholesome. The preparation of many indigenous or 'traditional' fermented foods remains as a house hold art (Farooque and Nautiyal, 1999). Fermentation is considered as one of the oldest and most economical methods for food production and preservation (Buckenhuskes, 2001). It is the diversity of raw materials used as substrates, methods of preparation and sensory qualities of finished products that are astounding as one begins to learn more about the eating habits of various cultures.

More than anything else, man has known the use of microbes for preparation of food products for thousands of years and all over the world, a wide range of fermented foods and beverages contributed significantly to the diets of many people. In traditional fermented food preparation, microbes are used to prepare and preserve food products, adding to their nutritive value, flavour and other qualities associated with edibility (Achi, 2005).

Fermented foods play an important socioeconomic role in developing countries as well as making a major contribution to the protein requirements of the population. These processes are characterized by their limited need for energy input, allowing microbial fermentations to proceed without external heat sources. Fermented foods play an unusually extensive role in East Asia or Oriental food systems generally, the traditional methods of preparing indigenous fermented foods are simple and inexpensive. During last few years, much interest has been generated in the fermented foods of the Orients, where such foods are still being manufactured at cottage industry scale by means of natural micro flora from the staples and surrounds (Devi, 2004).

Tempeh is a solid fermented soybean product that is consumed widely in Indonesia. Tempeh is the name ordinarily used for soybean fermented product. In recent years, there has been considerable interest in the West in popularising tempeh as an alternative protein source. In general, fresh tempeh of good quality is defined as a compact and sliceable mass of cooked particles of raw materials covered, penetrated and held together by dense non-sporulated mycelium of *Rhizopus* spp. (Nout and Kiers, 2005). Tempeh is considered as the collective name for various plant materials fermented with the fungi belonging to the genus *Rhizopus*. The most important characteristics of tempeh fermentation are that the key microorganism belongs to the genus *Rhizopus* and that the final products are mycelial-knitted compact cakes (Annor *et al.,* 2010).

The major desirable aspects of tempeh are its attractive flavour and texture, certain nutritional properties, and the reduced cooking time compared with the raw materials (Shurtleff and Aoyagi, 2001). Any available plant material can be used as a substrate, provided it can support the growth of *Rhizopus* spp. and it is suitable for human consumption after fermentation. Tempeh flour has also been produced by drying and milling tempeh (Cuevas-Rodriguez *et al.*, 2004; Reyes-Moreno *et al.*, 2004). The nutritional improvement by fermentation is important for tempeh flour production, but the tempeh structure itself is not.

Legumes are one of the richest and least expensive sources of proteins in the human diet and contribute substantially to protein content of the diets of a large part of the Indian population. The supplementation of cereals with high protein legumes is considered to be one of the best solutions to the protein calorie malnutrition, particularly in the developing countries. However, the presence of anti-nutrients such as protease inhibitors and phytates, reduces the digestibility or bioavailability of proteins and minerals in legumes (Gibson *et al.*, 2006), and the presence of flatulence producing compounds, particularly in beans, may cause adverse effects in the gastrointestinal tract. In Indian situation where a variety of pulses, cereals and millets are used, tempeh could be prepared with pulses other than soybeans thereby increasing the digestibility of the common pulses and cereals. Tempeh preparation does not require any special skills. Because of its high nutritional value and acceptability tempeh and its second generation products will have an impact on the consumers. This low cost simple technology can be easily adopted as an income generating activity. Tempeh and its second generation products can solve the problem of under nutrition and malnutrition.

Hence, the present study entitled "Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes" was undertaken with the following objectives

1. To standardise the fermented food tempeh with green gram, cowpea, soybean, rice and wheat, and to evaluate its quality attributes
2. To evaluate the nutritional and shelf life qualities of tempeh flour
3. To develop instant soup mixes with tempeh flour and to evaluate its quality attributes

2. REVIEW OF LITERATURE

"*Leave your drugs in the chemist's pot if you can cure the patient with food*" the age old quote by Hippocrates is certainly the tenet of today. The use of food as medicine is nothing new, however with the current trend of treating illness and disease with nothing more than prescribing drugs, seems to suggest that mainstream medicine has turned its back on food as medicine. According to Osawa (1998), the primary function of food is to provide essential nutrients, the secondary function is to satisfy sensory attributes and the tertiary function is to prevent diseases at the molecular level. Fermented foods can boast of all these properties and our indigenous fermented foods are one of the best health foods man has ever known.

Tempeh is a solid fermented soybean product. This traditional food was originated about hundred years ago in Java, but in recent years, tempeh has become one of the fastest growing categories in the western food industry even as dairy to meat alternatives. Of all the fermented foods, tempeh with its high rating in sensory qualities, nutritional benefits and simple processing techniques, appears to be a valuable food for common people especially for children, women and undernourished.

The literature connected to the study entitled "Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes" is presented under the following heads.

2.1 Definition and history of fermentation
2.2 Benefits of fermentation
  2.2.1. Nutritional benefits
  2.2.2. Health benefits
2.3 Indigenous fermented foods
2.4 Trends in tempeh processing
2.5 Microorganisms in tempeh production
2.6 Nutritional and health benefits of tempeh
  2.6.1. Nutritional benefits
  2.6.2. Health benefits
2.7 Tempeh products

2.8 Safety of fermented foods

2.1. DEFINITION AND HISTORY OF FERMENTATION

Pasteur originally defined fermentation as "respiration without air". He performed careful research and concluded; "I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development and multiplication of cells.... If asked, what causes the chemical act whereby the sugar is decomposed .... I am completely ignorant of it."

According to Rose (1983) fermentation is a metabolic process in which carbohydrates and related compounds are oxidised with the release of energy by the action of microorganisms.

Campbell-Platt (1987) defined fermented foods as those foods, which have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modification to the food. However, to the microbiologist, the term”fermentation” describes a form of energy-yielding microbial metabolism in which an organic substrate, usually a carbohydrate, is incompletely oxidised and an organic carbohydrate acts as the electron acceptor (Adams, 1990).

Badi (1991) defined fermentation as the chemical transformation of organic substances into simpler compounds by the action of enzymes - complex organic catalysts - which are produced by microorganisms such as molds, yeasts, or bacteria. Fermentation can also be defined as the incomplete oxidation of complex organic compounds particularly carbohydrates with the help of enzymes produced by microorganisms (Khader, 2001). In another definition, Pszezola (2002) defined it as the gradual change brought to any substrate by enzymes of some bacteria, moulds and yeasts. Klein *et al.* (2005) defined fermentation as the process of deriving energy from the oxidation of organic compounds, such as carbohydrates, using an endogenous electron acceptor, which is usually an organic compound.

Microbiologically, fermentation is any process for the production of useful products through mass culture of microorganisms and conventionally it can be defined as the breakdown of larger molecules into simple ones under the influence of microorganisms or their enzymes. (Bohra and Parihar, 2006).

The fermentation process developed with the discoveries of Louis Pasteur in 19^{th} century was of the anaerobic fermentation by yeast (Racker, 1974). Louis Pasteur described the scientific basis for fermentation in wine-making, and in the brewing of beer (Dubos, 1998).

Fermentation is one of the oldest technologies discovered by man which developed during the primitive pottery age *i.e*. between 8000-3000 BC (Cavalier *et al*., 2003).The earliest evidence of wine making dates from eight thousand years ago, in Georgia, in the Caucasus area (Anon., 2003).

Anthropologists have suggested that it was the production of alcohol that motivated primitive people to settle down and become agriculturists (Pedersen, 1979). The first fermented food consumed probably was fermented fruits. Hunter-gatherers would have consumed fresh fruits but at times of scarcity, would have eaten rotten and fermented fruits and repeated consumption would have led to the development of the taste for fermented fruits (Stanton, 1985). There is reliable information that fermented drinks were being produced over 7,000 years ago in Babylon (now Iraq), 5,000 years ago in Egypt, 4,000 years ago in Mexico and 3,500 years ago in Sudan (Dirar, 1993).

Bread-making probably originated in Egypt over 3,500 years ago (Sugihara, 1985). According to Steinkraus (2004), fermentation of milk started in many places with evidence of fermented products in use in Babylon over 5,000 years ago. Consumption of live lactic acid bacteria that contribute to lactic acid fermentation has been a regular part of food intake of humans since prehistoric times (Farnworth, 2005).

China is thought to be the birth-place of fermented vegetables and the use of *Aspergillus* and *Rhizopus* moulds to make food. The book called "Shu-Ching" written in the Chou dynasty in China (1121-256 BC) refers to the use of "chu" a fermented grain product (Yokotsuka, 1985). There is also evidence of fermented meat products being produced for King Nebuchadnezer of Babylon (Kearney *et al.,* 1990). According to Ramakrishnan (1993), *idli* and *dosa* have been used as basic foods in South India since at least AD 1100.

The foundation stone for industrial fermentation was laid when Robert Koch obtained pure cultures of microbes from separated colonies in 1881 (Karlson, 1963).

Following the discoveries by Pasteur and Koch during the end of 19^{th} century and beginning of 20^{th} century, microbiology and fermentation technology grew hand in hand (Dellatt, 1979).

2.2. BENEFITS OF FERMENTATION

Fermentation makes the food easier to digest and the nutrients easier to assimilate and also it retains enzymes, vitamins, and other nutrients that are usually destroyed by food processing (Tabera *et al.*, 1995). Fermentation has been used for several thousand years as an effective and low cost means to preserve the quality and safety of foods (Devi, 2004). Animal and plant tissues subjected to the action of microorganisms and or enzymes, caused desirable biochemical changes and significant modification of food quality (Elkhalifa *et al*., 2004).

2.2.1. NUTRITIONAL BENEFITS

According to Adams (1990), fermentation results in a lower proportion of dry matter in the food and the concentrations of vitamins, minerals and protein appear to increase when measured on a dry weight basis. Tabera *et al.* (1995) reported that fermentation is associated with many chemical changes that enhance organoleptic response, contents of free sugars and vitamins, as well as bioavailability of minerals and results in the breakdown of some of the antinutritional endogenous compounds. Generally, a significant increase in the soluble fraction of a food is observed during fermentation (Liu *et al.,* 2007).

Traditional methods such as germination and fermentation tend to improve the nutrient quality of foods (Sotomayor, 1999). Fermentation is one of the household food technologies reviewed extensively as means by which the nutritive value of plant foods could be improved (Obadina *et al*., 2008).

Single as well as mixed culture fermentation of pearl millet flour with yeast and *Lactobacilli* significantly increased the total amount of soluble sugars and reducing and non-reducing sugar content, with a simultaneous decrease in its starch content (Khetarpaul and Chauhan, 1990). Valverde *et al*. (1997) have shown that natural fermentation caused an improvement in the available starch to total starch ratio.

A significant decrease in starch content was first observed after four hour fermentation of pearl millet and further significant reductions at 6, 8, 10, 12 and 14 hours. The starch content decreased from 67 to 59 per cent for standard variety and from 69 to 63 per cent for *Ugandi* variety (Hag *et al*., 2002).

The digestibility of starch in bengal gram, cowpea and green gram was increased by fermentation. Cooking of these fermented legumes further increased the starch digestibility (Urooj and Puttaraj, 1994). According to Lilieberg *et al*. (1995) sour dough fermentation has been reported to improve nutritional properties of starch such as starch digestibility. This effect could be attributed to organic acids produced during sour dough fermentation which could ameliorate glucose disposal, delaying gastric emptying or suppressing enzymatic activity. Elkhalifa *et al*. (2004) reported that *in vitro* starch digestibility was markedly increased in sorghum as a result of fermentation, while resistant starch and total starch decreased and fermented sorghum had more soluble starch and swelling power at 100°C than at 85°C. According to Bhandal (2008), fermentation caused an appreciable enhancement (96–133%) in starch digestibility of moth bean with increase in period and temperature of fermentation. In a study conducted by Arora *et al*. (2009), the *in vitro* starch digestibility of barley based fermented mixture was significantly higher than unfermented mixture.

Bhatia *et al.* (2009) reported that increase in starch digestibility of indigenous fermented foods could be attributed to the significant reduction in phytic acid during fermentation.

Fermented foods are widely exploited as a source of valuable protein (Wanink *et al*., 1994). According to Lin *et al.* (2007), the protein content of foods increased significantly after fermentation. Chen *et al.* (2009) reported a slight increase in total protein content (2%) in fermented milk.

Fermentation may not increase the content of protein and amino acids unless ammonia or urea is added as a nitrogen source to the fermentation media (Reed, 1981). The protein content of cassava decreased from 2.36 to 1.61 g/100g during fermentation (Padmaja *et al.*, 1994).

Ragaee *et al*. (1986) observed that natural fermentation of lentils for four days at 32 °C increased the availability of total amino acids and improved *in vitro* protein digestibility. The improved *in vitro* protein digestibility caused by fermentation could be attributed to the partial degradation of complex storage proteins to more simple and soluble products and it could also be attributed to the degradation of tannins, polyphenols and phytic acid by microbial enzymes (Chavan and Kadam, 1989).

According to Kozlowska (1996), natural fermentation removed a high amount of trypsin inhibitor activity, tannins, galactosides and phytic acid and thus improved the protein digestibility. Antony and Chandra (1998) revealed that fermentation of finger millet flour using endogenous grain micro flora resulted in a significant increase in *in vitro* protein digestibility (23%). According to Hag *et al*. (2002) a significant increase was first observed at two hour fermentation and further significant increase was observed at 4, 6, 8, 10, 12 and 14 hour in the *in vitro* protein digestibility of two pearl millet cultivars. The increase was from 72.7 to 83.6 per cent for Standard cultivar and from 70.4 to 81.6 per cent for Ugandi cultivar. Bhandal (2008) conducted a study on the effect of fermentation with varying temperatures and time periods on the nutritive value of moth bean and reported that at 30 °C, protein digestibility increased from 60 per cent in the non-processed moth bean to 77 per cent, 78 to 80 per cent at 35 °C, and 81 to 83 per cent following 12, 18 and 24 hour of fermentation (controlled) period, respectively.

Bhatia *et al*., (2009) reported significant reduction in the phytic acid content during fermentation culminated in a marked improvement in protein (28-50 per cent) digestibility. A significant ($P < 0.05$) reduction in the contents of phytic acid and polyphenols was noticed due to cumulative effect of germination, autoclaving and fermentation, which ultimately caused significant improvement in protein digestibility by about 44 per cent in non-germinated and 55 per cent in germinated food mixtures, (Ljarotimi and Esho, 2009).

Fermentation of legumes for making *dhokla* and fermentation of millet for making *ambali* did not show any improvement in the values reported for protein efficiency ratio, biological value and net protein utilisation in relation to the unfermented products (Aliya and Geervani, 1981).

Antai and Obong (1992) reported a significant increase in total fiber content in fermented foods. But Cabrejas *et al*. (2004) revealed that fermentation significantly decreased the soluble dietary fiber content, and no significant changes were noticed in the insoluble dietary fiber content of beans. Cellulose content of all the samples was also reduced by fermentation.

Fermented products have high contents of minerals, vitamins and pigments, in accordance with the contents of these components in the initial raw material (Rakin *et al*., 2007).

Aliya and Geervani (1981) found that the content of thiamine and riboflavin in *dhokla* and *ambali* was about 50 per cent higher after fermentation. Chavan and Kadam (1989) observed that changes in the vitamin content of cereals with fermentation vary according to the fermentation process, and the raw materials used in the fermentation. According to the author, the B group vitamins generally show an increase during fermentation. According to Steinkraus (1996), the vitamin content of *pulque* a fermented plant sap, increased from 5 to 29 mg for thiamine, 54 to 515 mg for niacin and 18 to 33 mg for riboflavin per 100g during fermentation.

During fermentation, certain micro-organisms produce vitamins at a higher rate than others do (Campbell-Platt, 1987). In the fermentation of maize or kaffir corn, the thiamine levels were virtually unchanged, but riboflavin and niacin contents were almost doubled (Nnam, 2000). Lactic acid bacteria synthesize vitamins and antimicrobials and increase their contents in fermented products (Crittenden *et al*., 2003).

According to Sharada (2004), during fermentation of *idli* a fermented food of India, sulphur amino acids and B vitamins increased, chymotrypsin activity was reduced, and flatulence causing sugars were also decreased. Liu *et al*. (2007) reported a significant increase in the riboflavin content of wheat after fermentation with *Aspergillus niger*.

Alm (1982) reported that fermented milk products showed an increase in folic acid content (ropy milk exhibited a two fold increase) and a slight decrease in the concentration of vitamin B_{12} and according to the author, other vitamins were affected only slightly. Fermented dairy products consistently revealed an increased level of folic acid as well as pyridoxine, B vitamins, riboflavin and biotin depending on the strains of bacteria present. (Kneifel and Mayer, 2007).

Among dairy products, fermented milks are considered as a potential matrix for folate fortification because folate binding proteins of milk improve folate stability and the bioavailability of both 5-methyl tetrahydrofolate and folic acid (Aryana and McGrew, 2007). According to LeBlanc *et al*. (2007) many dairy products are processed using microbial fermentations in which folate can be synthesized, significantly increasing folate concentrations in the final product.

Fermented plant foods (like *tempeh*, *miso,* or *tofu*) are the most commonly consumed food sources of vitamin B_{12}, although none of these plant foods (before fermentation) can be counted on to be a consistently excellent or very good source of this vitamin (Areekul, 1990).

According to Keuth and Bisping (1993) the most important vitamin produced during fermentation is vitamin B_{12}, which is normally not found in vegetarian foodstuffs and is formed by bacteria that accompany the fermentation process.

The nutritive value of soymilk, especially vitamin B_{12}, could be improved by using non-sequential mixed fermentation of soybean with *Rhizopus oligosporus* and *Propionibacterium shermanii* before hot water extraction process for soymilk preparation, as reported by Krusong and Yongsmith (1996).

Martens (2007) reported that cultured and fermented bean products like *tofu, tempeh, miso, tamari* and *shoyu* may or may not contain significant amounts of B_{12}, depending upon the bacteria, molds, and fungi used to produce them and the B_{12} content of sea vegetables also varies according to the distribution of microorganisms in the surrounding sea environment.

According to Watanabe (2007), the microbial B_{12} production is a convenient strategy to achieve natural enrichment of fermented foods, notably from vegetable sources.

Vegetables that have undergone lactic acid fermentation as in the case of *sauerkraut* and *kimchi*, showed an increase in the activity of vitamin C and vitamin A (Wee *et al.*, 2006).

Steinkraus (1996) observed that although it would not be expected that fermentation would alter the mineral content of the product, but the hydrolysis of chelating agents such as phytic acid during fermentation, improves the bioavailability of minerals.

Fermentation does not usually increase the level of minerals present in foods unless unusual circumstances are present (as in fermenting food in a metal or earthen container), but it decreases the activity of phytic acid naturally present in grains (Kozlowska, 1996).

Vaishali *et al.* (1997) studied the effect of natural fermentation on *in vitro* zinc bioavailability from cereal-legume mixtures and found that fermentation increased the zinc solubility (2-28%) and the zinc uptake by intestinal segment (1-16%) to a significant level.

Antony and Chandra (1998) observed that fermentation of finger millet flour using endogenous grain micro flora resulted in a significant reduction of antinutrients (phytate by 20%, phenols by 20%, tannins by 52%, and trypsin inhibitor activity by 32%) at the end of 24 h. There was a simultaneous increase in HCl mineral extractability (Ca, 20%; P, 26%; Fe, 27%; Zn, 26%; Cu, 78%; Mn, 10%).

According to Sindhu *et al*. (2005), fermentation process significantly ($P<0.05$) improved the HCl-extractability of minerals viz. iron (54-67%), calcium (22-32%), sodium (25-30%) and potassium (17-24%) in a probiotic food blend.

Natural fermentation of precooked pearl millet flour brought about a significant increase in non-phytate, inorganic and HCl-extractable phosphorus with a corresponding decrease in phytate phosphorus. HCl-extractability of calcium, copper, iron, zinc and manganese were also improved significantly (Mahajan and Chauhan, 2006). Reddy and Salunkhe (2006) reported that fermentation of rice alone for 8 hr resulted in complete hydrolysis of phytate phosphorus with simultaneous increase in the quantities of nonphytate phosphorus. According to them no apparent changes were observed in the concentrations of calcium, magnesium, zinc, and iron.

Lactic acid fermentation enhanced iron absorption not only by increasing iron solubility after digestion, but also by increasing the efficiency of cellular iron uptake. (Bergqvist *et al*., 2006).

According to Bhandal (2008), fermentation of moth bean resulted in 24 - 34 per cent reduction in phytic acid content at 30 °C and 33 - 42.5 per cent at 35 °C. Polyphenol content was reduced by 42 per cent, 48 per cent and 51 per cent at 30 °C and by 44 per cent, 49 per cent and 54 per cent at 35 °C after 12, 18 and 24 hour of fermentation period, respectively.

Ljarotimi and Esho (2009) reported that the antinutritional content of fermented bambara groundnut seed flour was low compared with germinated and roasted bambara groundnut seed flour.

Fermentation drastically reduced the contents of phytic acid, polyphenols and trypsin inhibitor activity while significantly improving the *in vitro* digestibilities of starch and protein (Reddy and Salunkhe, 2006).

2.2.2. HEALTH BENEFITS

The claimed health benefits of fermented foods are expressed either directly through the interaction of ingested live microorganisms (bacteria or yeast) with the host (probiotic effect) or indirectly as a result of ingestion of microbial metabolites produced during the fermentation process (biogenic effect) (Nakamura *et al*., 1996). Although still far from fully understood, several probiotic mechanisms of action have been proposed, including competitive exclusion, competition for nutrients and/or stimulation of an immune response (Stanton *et al*., 2005). The biogenic properties of fermented functional foods result from the microbial production of bioactive metabolites such as certain vitamins, bioactive peptides, and organic acids or fatty acids during fermentation (Parvez *et al*., 2006).

Fermented foods contain beneficial bacteria (probiotic bacteria) which are needed in our intestines. It is said that such 'living' foods can help to repopulate the intestine with proper bacterial strains. This helps with digestion and also provides a protective mechanism against other invasive bacteria and fungi which can cause serious problems when they get out of control (Patel *et al*., 2008).

According to Heller (2001), fermented foods and dairy products play a predominant role as carriers of probiotics. These foods are well suited for promoting the positive health image of probiotics for several reasons: 1) fermented foods and dairy products in particular, already have a positive health image; 2) consumers are familiar with the fact that fermented foods contain live microorganisms (bacteria); and 3) probiotics used as starter organisms combine the positive images of fermentation and probiotic cultures.

Clinical symptoms that have been reportedly treated or have the potential to be treated with probiotic foods or fermented foods include cancer, diarrhoea, gastroenteritis, irritable bowel syndrome, inflammatory bowel disease, depressed immune function, inadequate lactose digestion, infant allergies, hyperlipidaemia, hepatic diseases and *Helicobacter pylori* infections. (Bengmark, 2000; Benchimol, and Mack, 2004; Brown and Valier, 2004).

Food products with a short shelf-life (2–3 weeks) such as yoghurt and fermented milks are the most common probiotic foods available, although products with a longer shelf-life, such as probiotic cheddar cheeses, have also been developed (Ross *et al*., 2002).

Anticarcinogenic activity

According to Aso and Akazan (1992), fermented foods containing beneficial bacteria might suppress the growth of harmful bacteria that convert procarcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine.

Fermented milk and yoghurt when consumed have shown to lower the incidence of colon cancer or lower propensity to develop large adenocarcinomas (Bourtan, 1996).

Another hypothesis for the prevention or delay of tumour development by probiotic bacteria in foods is that they might bind to mutagenic compounds in the intestine thereby decreasing the absorption of these mutagens (Murch, 2001). Rowland (2004) reported a potential protective role of fermented milks containing probiotic cultures against colo rectal cancer in humans and in animals.

According to Parvez *et al*. (2006) probiotc cultures in fermented foods decrease the exposure to chemical carcinogens by producing compounds that inhibit the growth of tumor cells by stimulating the immune system.

Yan and Spitznagel (2009) suggested that consumption of fermented soy foods is associated with a reduction in prostate cancer risk in men.

Anti hypertensive activity

Animal and human clinical studies documented the antihypertensive effects of probiotic ingestion indicating that probiotic bacteria or their fermented products play a role in blood pressure control. A fermented milk product with the biologically active peptides valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) was shown to lower blood pressure in spontaneously hypertensive rats (Nakamura *et al*., 1996). Elderly hypertensive patients who consumed fermented milk with a starter containing *Lactobacillus helveticus* and *Sacchromyces cerevisiae* experienced reduction in systolic and diastolic blood pressure (Hata *et al*., 1996).

According to Yamamoto and Takano (1999), fermentation of milk proteins produced peptides that have an angiotensin converting enzyme inhibiting action and thus a blood pressure lowering effect can be achieved. Seppo *et al*. (2003) also reported that *L. helveticus* which is seen in fermented milk, in normal daily use, has a blood pressure lowering effect in hypertensive subjects and is thus potentially useful in the dietary treatment of hypertension.

Sachie *et al*. (2009) reported that the angiotensin converting enzyme inhibitor from fermented soybean food *natto* appears to moderately reduce blood pressure in hypertensive patients.

Control of blood cholesterol and hyperlipidaemia

Mann and Spoerry (1974) observed a decrease in serum cholesterol levels in men fed large quantities of milk fermented with *Lactobacillius* and this may be due to the production of hydroxymethyl glutarate by lactic acid bacteria which inhibit hydroxymethyl glutaryl CoA reductases, required for the synthesis of cholesterol. In another study, Mann (1977) concluded that consumption of large quantities of cultured yoghurt lowered serum cholesterol levels in human volunteers.

Rao *et al*. (1981) reported that metabolites from orotic acid formed during fermentation of dairy products may help lower cholesterol levels. They conducted experiments and reported that liver cholesterol levels were lower in the group receiving thermophilus milk than the group receiving skim milk.

Homma (1988) reported that feeding of fermented milk containing very large number of probiotic bacteria (10^9 cfu/g) to hypercholesterolemic human subjects lowered cholesterol levels from 300 to 150 mg/100 ml.

According to Schaafsma *et al*. (1998), adult male volunteers fed with 125ml *L. acidophilus* fermented milk, three times daily for 3 weeks showed significantly lower values for serum total cholesterol, LDL cholesterol and LDL/HDL ratio by 4.4, with no change in the levels of serum HDL cholesterol, triglycerides and blood glucose.

Anderson and Gilliland (1999) found that intake of about one cup of yoghurt with live cultures daily for one year prevented an increase in blood total and LDL cholesterol levels in adults. According to James *et al*. (1999), fermented milk containing *L. acidophilus* was accompanied by a 2.4 per cent reduction of serum cholesterol concentration (200 ml of fermented milk daily for 3 weeks).

In a well-controlled 8-week clinical trial in overweight subjects, daily consumption of 450 ml of yoghurt fermented with *Streptococcus thermophilus* and *E. faecium* resulted in an 84 per cent reduction in LDL and an increase in fibrinogen levels (Agerholm-Larsen *et al*., 2000). Parvez *et al*. (2006) reported that blood cholesterol levels can be reduced by the consumption of probiotic containing dairy foods by people with elevated blood cholesterol.

Ataie-Jafari *et al*. (2009) concluded that yoghurt containing two probiotic bacteria strains, *L. acidophilus* and *B. lactis*, had a cholesterol-lowering effect in hypercholesterolemic subjects.

Enhancing lactose tolerance

Kim and Gilliland (1983) found that feeding fermented milk to lactose intolerant subjects resulted in a significantly lower level of hydrogen in the breath when compared to the level for subjects fed unfermented milk (lower hydrogen level indicates that lactose has been metabolised prior to entering the large intestine).

According to Marteau *et al.* (1990), lactic acid of the yoghurt alleviates the symptoms of lactose intolerance in lactase-deficient individuals. The beneficial effect appears to be a consequence of the lactic acid bacteria in fermented milk increasing lactase activity in the small intestine.

Probiotic yoghurt is tolerated well by lactose malabsorbers since some lactose is hydrolysed by yoghurt bacteria during fermentation and also the coagulated milk because of its viscous nature, may pass slowly through the gut than unfermented milk (Shah *et al*., 1992).

Fresh yoghurt is more sufficient in facilitating lactose digestion than heated yoghurt. The β galactosidase activity in yoghurt drops by 80 per cent in the duodenum, one fifth of the yoghurt lactase activity is still found in the terminal ileum, suggesting a relative persistence of protein along the digestive tract. But as the bacterial β galactosidase present in yoghurt is partly resistant to luminal hydrolysis, it can hydrolyse lactose at least in the mid and distal part of the small intestine where the pH is compatible with the enzymatic activity (Shermak *et al*., 1995).

S.thermophilus, *L.bulgaricus* and other *Lactobacilli* in fermented milk products can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine and stomach (Dairy Council of California, 2000).

The beneficial effect of acidophilus milk in alleviating lactose malabsorption appears to be a consequence of the lactic acid bacteria in fermented milk increasing lactase activity in the small intestine (Pelletier *et al*., 2001).

According to Khetarpaul (2005), yoghurt tolerance is mainly due to supply of lactase activity from lactic acid bacteria present in yoghurt and the bacteria must be live and present in sufficient quantity to exert the beneficial effect (Yoghurt containing 10^{8}cfu/ml are required).

Anti diarrhoeal effect

During the fermentation process, lactic and other acids are released, lowering the pH, and possibly generating anti-microbial substances (Mensah *et al.*, 1990).

Fermented foods containing probiotic microorganism is found to be useful in the treatment of many types of diarrhoea, including antibiotic associated diarrhoea in adults, traveler’s diarrhoea and diarrhoea in young children caused by rotaviruses (Isolauri *et al.*, 1991).

Odugbemi *et al.* (1991) demonstrated that when enteropathogenic *E. coli*, the causative organism of diarrhoea were inoculated into fermented *ogi* (pH 3.6), their proliferation and survival were inhibited. Salmonella pathogens were also inhibited, but to lesser degree than *E. coli*. In Tanzania, Lorri and Svanberg (1994) monitored diarrhoea1 rates over nine months in 201 children under five years. According to them those in a village consuming fermented gruels had a 40 per cent lower frequency of diarrhoea than children in a nearby village consuming non-fermented gruels (2.1 *v.* 3.5 episodes; $P < 0.001$).

Sharada, (2004) reported that *Idli* provides protection against food poisoning pathogen induced diarrhoea and transmission of pathogens because of its acidity produced during fermentation.

According to Lei *et al*. (2006), indigenous lactic acid fermented foods may have the potential as probiotic treatment for diarrhoea, due to high levels of lactic acid bacteria. Lactic acid bacteria are known to release various enzymes into the intestinal lumen that exert synergistic effects on digestion, alleviating symptoms of intestinal malabsorption and hence reduce diarrhea (Parvez *et al*., 2006).

Vandenplas *et al*. (2007) found that, fermented foods containing selected strains of probiotics, resulted in a statistically significant but clinically moderate benefit in shortening the duration of diarrhoea caused by acute infectious gastroenteritis.

In the paediatric population, probiotics found in cultured dairy foods appear to benefit viral diarrhoea, possibly by increasing secretary IgA and decreasing viral shedding, suggesting an immunological mechanism (Shah, 2007).

Enhancement of immunity

Studies have shown that consumption of fermented dairy foods was able to confer a range of health benefits including enhancement of immunity and improved resistance to infectious illnesses and cancers (Goldin, 1998).

Clinical reports have revealed that dietary consumption of fermented foods such as yoghurt can alleviate some of the symptoms of atopy and might also reduce the development of allergies, possibly via a mechanism of immune regulation (Mack, 1999).

The majority of evidences from *in vitro* systems, animal models and humans, suggest that, probiotic organism in the fermented foods can enhance both specific and nonspecific immune responses. These effects are believed to be mediated through activating macrophages, increasing levels of cytokines, increasing natural killer cell activity and/or increasing levels of immunoglobulins (Perdigon and Alvarez 1992; Ouwehand *et al.*, 2002).

Ingestion of probiotic yoghurt has been reported to stimulate cytokines production in blood cells (Solis and Lemonnier, 1996) and enhance the activity of macrophages (Morteau *et al.*, 1997).

Controlled studies have indicated that consumption of fermented milk cultures containing lactic acid bacteria (LAB) can enhance production of Type I and Type II interferons at the systemic level (Gill *et al.*, 2000). According to Cross *et al*. (2001), lactic acid bacteria found in fermented foods have been shown to promote interferon expression, and to reduce allergen-stimulated production of IL-4 and IL-5 in some cases. They also reported that lactic acid bacteria are potent inducers of pro-interferon monokines (IL-12 and IL-18), and that cytokine secretion is stimulated by the interaction of Gram-positive cell wall components with surface receptors of mononuclear phagocytes.

2.3. INDIGENOUS FERMENTED FOODS

According to Fellows (1997), indigenous fermented foods constitute a group of foods that are produced in homes, villages, and small cottage industries at prices within the means of a majority of the consumers in the developing world. Fermentation is characterized by their limited need for energy input, allowing microbial fermentations to proceed without external heat sources (Odunfa, 1987).

Globally, there are varieties in fermented foods and beverages and so do the ingredients and recipes for making them (Abiose and Adedeji, 1992). In the Asian region, indigenous fermented foods are important in daily life. In many of these foods, yeasts are predominant and functional during fermentation. The diversity of foods in which yeasts predominate ranges from leavened bread-like products such as *nan* and *idli,* to alcoholic beverages such as rice and palm wines, and items such as *papads* and soy sauce (Aidoo *et al* ., 2005).

The *Appam* is a fermented bread usually prepared with finely powdered rice flour. In Kerala in South India, there are *Kallappam, Vattayappam* and *Palappam* (*Vellayappam*). The *Kallappam* is made on flat iron griddles. The *Vattayappam* is steamed bread, and *Palappam* is made in small shallow bottomed pans, which are kept covered while the bread cooks. *Palappam* has a thin crisp lace like strip around it (Devi, 2004).

Idli and *Dosa* are fermented foods widely consumed in South India, but are also popular in other parts of India. They are prepared by steaming fermented black gram (*Phaseolus mungo L.*) and rice (*Oryza sativa L.*) batter (Reddy *et al.,* 1982 ., Nagaraju and Manohar, 2000). *Idli* batter consists of three parts of rice and one part of black gram dhal with salt to taste. *Dosa* is a thin crisp, fried pancake made from the same ingredients used in the making of *idli* batter except that the rice and black gram are finely ground and that the fermented suspension instead of being steamed is heated with a little oil, on a flat plate (Shortt, 1998).

Dhokla is a steamed fermented food of North India made from bengal gram and rice or wheat (Chavan and Kadam, 1989). Purushothaman *et al*. (1993) reported that *Dhokla* is similar to *idli* except that bengal gram dhal is used instead of black gram dhal in its preparation and in some areas, a mixture of rice and chickpea flour is also used as the substrate for the fermentation. As in *idli* preparation, the fermented batter is poured into a greased shallow tin and steamed in an open steamer.

Masayura is an important traditional food of Nepal prepared from ground black gram and minced colocacia roots in the ratio 1:4 and is mostly used in the preparation of vegetable curry (Steinkraus, 1996). It is also prepared from split black gram or green gram, colocasia tuber and ash gourd or radish depending on their availability (Dahal *et al*., 2003).

According to Yonzan and Tamang (1998), *Gundruk* is a fermented product obtained from leafy vegetables which occupies eminent place in Nepalese diets.

Doli ki roti is an indigenous nutritional fermented bread popular among Indian Punjabis migrated from Pakistan and is a wheat-based product. Natural fermentation is carried out in an earthen pot called *doli* in vernacular language (Bhatia *et al*., 2009).

Baked foods are produced and consumed in most of the countries of the world although it originated in Egypt (Steinkraus, 1996). Bread is one of the most commonly used fermented baked foods and has traditionally been an important factor in human nutrition and the raw ingredients for making bread include wheat flour, egg, fat and yeast as the fermenting organism (Devi, 2004).

Fermented milk–wheat mixtures, known as *Kishk* (*Fugush)* in the Middle East and *tarhana* in Greece and Turkey, are important foods in the diet of many populations (Morcos, 1993). It is typically prepared by adding strained yoghurt to bulgur wheat (cracked and bran-free parboiled wheat) and allowed the mix to ferment at ambient temperature for different periods of time (Blandino *et al*., 2003).

Kenkey is fermented maize dough eaten in Ghana (McKay and Baldwin, 1990). The fermentation is dominated by a variety of lactic acid bacteria, particularly *Lactobacillus fermentum* and *Lactobacillus reuteri* (Halm *et al*., 1993).

Kisra is a traditional fermented food with sorghum widely produced in households in Sudan. Fermentation is spontaneous and uncontrolled thus resulting in a product of variable quality (Ali and Mustafa, 2009).

Ogi is a fermented cereal gruel processed from maize, although sorghum or millet is also employed as the substrate for fermentation. It is considered the most important weaning food for infants in West Africa although it is also consumed by adults (Anukam and Reid, 2009).

Injera (*Enjera*) is the undisputed national food of Ethiopians and it can be made from different cereals, including sorghum, corn, finger millet and barley, although tef (*Eragrostis tef*) is the major cereal ingredient in Ethiopian *injera*. (Mohammed *et al*., 2010).

According to Blandino *et al*. (2003), *Pozol* is fermented maize dough with the form of balls of various shapes and sizes. It is consumed in South-eastern Mexico by Indians and Mestizo groups, for whom it can be a main component of the daily diet.

Puto is a leavened steamed rice cake of Philippines mainly used as a dessert or break fast item (Collado and Lilia, 1992).

Dawa Dawa is a traditional Nigerian food made of fermented locust beans. It is usually sold as a black small molded mound and is used in flavouring rich soups and stews (Fellows, 1997). With a very peculiar aroma, it has an amazing way of enriching traditional Nigerian soups and is also known as "*ogili okpe".* (Wokoma and Aziagba, 2001).

Natto is a traditional Japanese food made from soybeans fermented with *Bacillus subtilis* and is popular especially as a breakfast food (Fujita, 1993). Red fermented rice, red *koji* rice or *ang-kak*, is bright reddish purple fermented rice used in

Chinese cuisines and medicines, acquires its colour from the fermenting mold *Monascus purpureus* (Wei *et al*., 2005). *Miso* is a traditional Japanese seasoning produced by fermenting rice, barley and/or soybeans, with salt and the fungus. The most typical *miso* is being made with soy (Ken, 2007). *Tofu* or “Bean curd” originated in China and is a fermented coagulated soymilk (Melissa, 2007).

Tempeh, or *tempe* in Indonesia, is made by a natural culturing and controlled fermentation process that binds soybeans into a cake form (Hachmeister and Fung, 1993). According to Astuti *et al*. (2000) tempeh is unique among major traditional soy foods in that, it is the only one that did not originate in China or Japan. It originated in Indonesia, and is especially popular in the island of Java, where it is a staple source of protein.

Among the fermented cassava products are *gari*, *fufu*, and *lafun*, (Lancaster *et al*., 1982). *Sinki* is an acid non salted fermented radish taproot consumed as the base of a soup or as a pickle by indigenous people of Sikkim, Nepal, and Northestern India (Aidoo, 1986). *Gari* is a dry granular, fermented product of cassava which is widely consumed across West Africa, Central Africa and in some parts of North and Southern Africa (Hahn, 1989). *Lafun* is a fermented cassava flour, popularly consumed in south west Nigeria and is usually prepared as a stiff porridge using boiling water, prior to being consumed with soup and *fufu* is a pasty cassava mash which is cooked in boiling water and consumed with soup (Oyewole and Sanni 1995).

Dry salted lime pickle undergoing lactic acid fermentation is a popular homemade product in India which is very spicy and hot (Steinkraus, 1996). Indigenous people of eastern Himalayan regions use the fermented bamboo shoot product called *mesu* as pickle and as a base of curries (Sekar and Mariappan, 2007).

Sauerkraut is finely shredded cabbage that has been fermented by various lactic acid bacteria, including *Leuconostoc*, *Lactobacillus*, and *Pediococcus* (Edward, 2003). According to Byun *et al*. (2002), *Kimchi* is Korean fermented vegetables similar to sauerkraut with salt, spices and other condiments. *Kimchi* is one of the numerous traditional Korean pickled dishes made of *Lactobacillus* fermented vegetables with varied seasonings. Almost all vegetables cultivated in Korea have been linked to *kimchi* preparation; however, Chinese cabbage (*baechu* in Korean) is the most popular (Kim and Chun, 2005).

Dahi is a lactic acid fermented product of cow or buffalo milk popular in India and it is consumed directly either as sweetened (*misty dahi*) or as salted and spiced form (Steinkraus, 1996). Tamang (1998) reported that in addition to *Dahi*, there are other traditional fermented milk products in India like buttermilk, *Paneer, Shrikhand and Churpi* (yak cheese).

Cheese is another ancient fermented milk product from the western world whose origin predate to recorded history and the type of cheese varies in different countries (Roest and Menghi, 2002). According to Platt (2004), fermented dairy products have been one of the oldest fermented foods, man has ever known.

In southern East India, the fishes are traditionally preserved by fermentation (Thapa *et al*., 2004). *Nga-pi* is actually fermented shrimp or fish paste of Burma and is prepared by pounding the fish with salt and partially drying it in the sun and fermented for 3-6 months (Hutkins, 2006).

2.4. TRENDS IN TEMPEH PROCESSING

Although extruded meat like nuggets have been popular in India in modern times, centuries ago low cost fermented vegetable protein as meat substitute or analogue was known to the Indonesians, which is called ‘tempeh kedele’ (Mukherjee *et al*., 1992).

Tempeh was originated in today's Indonesia, almost certainly in Central or East Java, prior to 1800, and perhaps as long ago as a thousand years or more (Babu *et al*., 2009).

According to Mittal and Garg (1990) in traditional method, the soybeans are washed and soaked in water overnight, during which time they undergo bacterial acid fermentation reducing the pH to 5.0 or lower. The traditional tempeh production process involved dehulling of soybean, soaking in water for 24 hours, boiling in water for 30 minutes, inoculation and fermentation (Abdel-Gawad, 1993). An alternate process is to place the soybeans in water, bring it to a boil, and beans are then allowed to soak overnight and after soaking, beans are dehulled and then given a short boil, cooled, surface-dried by winnowing, and inoculated with *tempe* mould either from a previous batch of sound *tempe* or from mould grown and dried on leaves. According to Hesseltine (1995), the dehulled soybean cotyledons which may be acidified traditionally by the addition of lactic acid prefermentation is nowadays replaced with lactic acid, citric acid or vinegar. Traditionally, the inoculated cotyledons are then wrapped in small packets using wilted banana or other large leaves and are incubated in a warm place for two or three days, during which time they are completely overgrown by the mould mycelium (Steinkraus, 1996).

Steinkraus *et al*. (1965) used freeze-dried, 4-day fermented soybeans as inoculum for the preparation of tempeh. Wang *et al*. (1975) developed *Rhizopus oligosporus* starter culture by fermenting either rice, rice: wheat bran (4: 1) or wheat: wheat bran (4:1) at a substrate to water ratio of 10:6 for 4 days at 32°C. The fermentation mass was then immediately freeze dried and ground into fine powder. Today regular tempeh without mentioning any pulse is prepared with soybeans and the fermentations are carried out with pure cultures of *Rhizopus oligosporus* (Jurus and Sundberg, 1976). A new type of tempeh in which wheat and soybeans are combined was developed by Hesseltine and Wang (1979).

According to Nout and Rombouts (1990), tempeh is one of such traditional fermented food that have received attention from all over the world, and different countries make tempeh products from locally available substrates. Heat-pasteurized cassava root, cowpeas, partially defatted peanuts, rice and soybeans were evaluated by Shambuyi *et al*.(1992) for their suitability to support growth and sporulation of the tempeh mold, and they found that molds grew best and sporulated most luxuriantly on cassava and rice incubated at 37°C. According to them, the viability of molds remained high for up to 30 weeks when dried (a_w0.48) powdered substrates on which the molds had been cultured, were stored at 5, 25 and 37°C. Survival was best when powders were stored at 5°C. Vaidehi *et al*. (1996) prepared tempeh using different pulses and vegetables.

The wrapping of inoculated soybean cotyledons are at present done in perforated polyethylene bags instead of banana leaves (Steinkraus, 1996). Steinkraus (2004) developed a new method for incubating the tempeh in plastic bags with perforations at 0.25 to 1.3 cm intervals to allow access to oxygen. In this method, the soybean cotyledons are inoculated with the mould and placed in plastic bags or in plastic tubes similar to sausage casings and can be incubated immediately or stored in a refrigerator until fermentation is desired. Then, the mould overgrows the soybeans in a day or less. The plastic-bag process has been widely adopted in Indonesia and is also being used commercially in new tempeh factories in the United States.

Current technology and new scientific advancements have enabled researchers to examine specific strains of *Rhizopus* and new substrates such as cereal grains and pulses (Steinkraus, 2004).

A patented barley tempeh procedure has been developed by fermenting whole pearled barley kernels (Gourmet korn) with selected strains of *Rhizopus oligosporus* (Berg *et al.*, 2001). The fermentation process has recently been modified and applied on a new barley genotype (Karmose) with a high amylose and β-glucan content. The modified process has been found to strongly reduce the phytate content while preserving minerals (Eklund-Jonsson *et al.*, 2006) and also lower the glycemic index of barley tempeh.

According to Cuevas-Rodriguez *et al.* (2004), any available plant material can be used as a substrate, provided it can support the growth of *Rhizopus* spp. and it is suitable for human consumption after fermentation.

2.5. MICROORGANISMS IN TEMPEH PRODUCTION

Tempeh has been produced in Indonesia for many centuries. However, it was the Dutch scientist Prinsen Geerligs, who in 1895 identified the tempeh mould for the first time (Shurtleff and Aoyagi, 2001).

The type of microorganisms involved in tempeh production has been reported by many workers (Swan and Hesseltine, 1979). Tempeh is the result of mixed culture fermentation by a diverse group of microorganisms including moulds, yeasts, lactic acid bacteria and different gram-negative bacteria (Steinkraus *et al*., 1983).

Many different moulds were found in tempeh, but species within the zygomycete genus *Rhizopus* were found to dominate (Steinkraus *et al.*, 1983). According to them *Rhizopus oligosporus* is the most preferred species in tempeh fermentation due to its properties such as rapid growth at high temperature (30-42°C), inability to ferment sucrose, high proteolytic and lipolytic activities and production of strong antioxidants. *Rhizopus* includes three species groups: *R. oryzae* group, *R. stolonifer* group, and *R. microsporus* group with species from the latter group dominating in tempeh (Schipper and Stalpers, 1984).

Rhizopus oligosporus is considered as a domesticated form of *Rhizopus microsporus* which can produce toxic secondary metabolites such as rhizoxin, and rhizonins A and B (Samson, 1985). However, *Rhizopus oligosporus* does not produce any of these metabolites in different laboratory and natural substrates, not even under prolonged incubation conditions conducive to the formation of these metabolites (Jennessen *et al.*, 2005).

Hesseltine *et al*. (1963) isolated many fungi from different lots of tempeh made in Indonesia and found that only *Rhizophus* could make tempeh in pure culture fermentation. They also found that the 40 strains of *Rhizophus* studied, 25 of them were *Rhizopus oligosporus* and others were *Rhizopus stolonifier, Rhizopus arrhizus, Rhizopus oryzae and Rhizopus formosaensis.* This finding was also confirmed by Saono *et al.* (1976) who isolated 118 cultures from 81 tempeh samples collected from markets in various parts of Indonesia.

Liem *et al*. (1977) were the first to report the growth of bacteria *Klebsiella pneumonia* which was responsible for the production of vitamin B_{12} in commercial tempeh. Mulyowidarso *et al.* (1990) also reported variable growth of bacteria during fermentation of soybeans into tempeh with *Rhizopus oligosporus*. According to Wiesel *et al*. (1997), some other moulds, such as *Rhizopus oryzae* and *Mucor* spp, may also contribute to the flavour, texture or nutritive value of tempeh.

2.6. NUTRITIONAL AND HEALTH BENEFITS OF TEMPEH

2.6.1. NUTRITIONAL BENEFITS

Tempeh has the necessary characteristics of a dietary staple which is high in protein, fiber and other nutrients (Feng *et al*., 2005) and thus it is becoming one of the most preferred and popular indigenous health foods.

Mittal and Garg (1990) reported tempeh as a highly digestible food which is free of cholesterol and a good source of vitamins and minerals. Tempeh fermentation may increase the bioavailability of proteins, lipids, carbohydrates and minerals such as iron and zinc (Astuti *et al.*, 2000).

Tempeh is a highly nutritious fermented food traditionally made from soybeans and its high protein content makes it a wonderful substitute for meat (Nout and Kiers, 2005). Babu *et al*. (2009) also reported tempeh as a source of protein which contains all the essential amino acids and same quality protein as meat or poultry.

Molins (2000) observed that, freshly made tempeh contains about 48.1 per cent protein on a dry weight basis. Posati (2000) reported that tempeh's unique amino acid composition makes it not only a basic protein source, but also a remarkable protein booster. Greshe (2006) claimed that tempeh due to its quality protein, can be used for the fortification of widely consumed cereal based food products.

Murata *et al*. (2006) reported that no large differences in protein content existed between tempeh and unfermented soybeans. Marshall *et al.* (2007) also reported that the traditional tempeh has significantly higher protein content.

Tempeh is very nutritious and serves as one of the best plant protein source containing over 40 per cent protein. The average protein efficiency ratio is 2.4 compared with 2.5 for casein. Net protein utilization (NPU) is 56 compared with 65 for chicken meat and digestibility is 86.1 per cent and also contained vitaminB_{12} (Tripathi and Misra, 2005).

According to Bejarano *et al*. (2006), *in vitro* protein digestibility (IVPD) is improved by the solid state fermentation process and the proteins of unfermented pulse flour and tempeh flour had IVPD of 72.20 per cent and 83.20 per cent, respectively. Bozena *et al*. (2008) reported that tempeh fermentation increased protein bioavailability by about 25 per cent.

Newman *et al.* (1985) observed that broiler chickens fed with barley tempeh gained more body weight than those fed with unfermented barley. However, the total or essential amino acid content and composition were not changed during barley tempeh fermentation with *Rhizopus oligosporus* alone or together with yeasts.

Lopez and Harry (1990) reported an increase in the IVPD in common beans as a consequence of tempeh fermentation and reported that this increase could be explained by the elimination of antinutritional factors (e.g. hydrolysis of phytic acid during fermentation) and protein denaturation during the cooking step, which results in proteins that are more vulnerable to enzyme action. Vaidehi (1993) reported tempeh as a complete protein food containing all essential amino acids. According to the author, the increase in the protein efficiency ratio in tempeh is due to the destruction of phytic acid present in soybeans by the fungus *Rhizopus oligosporus* during fermentation. According to Sutardi and Buckle (2008), soybeans have the highest levels of phytates, but when they are fermented to make tempeh, the amount of phytic acid decreased by one half.

Accordig to Hachmeister and Fung (1993), an important function of *Rhizopus oligosporus* in the fermentation process during the production of tempeh is the synthesis of enzymes, which hydrolyze soybean constituents and contribute to the development of a desirable texture, flavour, and aroma of the product. Enzymatic hydrolysis also may decrease or eliminate antinutritional constituents; consequently, the nutritional quality of the fermented product may be improved.

Yousif (2001) attributed the increase in protein availability in tempeh to enzymatic breakdown during fermentation to partial degradation of complex storage proteins into simpler soluble products. Trypsin inhibitors in legumes are inactivated to a great extent by germination and fermentation (Tripathi and Nath, 2002). According to Babu *et al*. (2009), tempeh is easy to digest because the fermentation process breaks down the complex proteins found in soybeans, making it more easily digested than non-fermented soy foods or whole soybeans.

Bejarano *et al*. (2006) observed that total starch values were slightly higher in untreated chickpea flour than in tempeh flour (49.2 *vs.* 48.4 g/100 g of dry flour). This may be due to the partial removal of non-starch constituents during the solid state fermentation process. They also reported that resistant starch of tempeh flour showed higher values than unfermented chickpea flour (7.6 *vs.* 1.9 g/100 g dry flour).

In a study conducted by Karyadi and Lukito (1996), it was found that the fat and carbohydrate content in raw tempeh was 4.0 per cent and 1.7 per cent respectively. According to Tee *et al*. (1997) the fat content and carbohydrate content of fresh tempeh was 7.5 per cent and 6.8 per cent respectively. According to Molins (2000), the carbohydrate content in tempeh was about 23.9 per cent. Babu *et al*. (2009) reported a fat and carbohydrate content of 9.2 per cent and 19.9 per cent respectively in tempeh.

The growth of the fungus reduced the concentration of low molecular carbohydrates and increased the dietary fiber content in tempeh (Nout and Rombouts, 1990). According to Babu *et al*. (2009) tempeh is high in soluble fiber, which has various health benefits.

Bioavailability of elements such as zinc, iron, manganese, calcium and phosphorus is high in tempeh (Sudermadji and Suparmo, 1997). Fermentation process of tempeh decreased the phytic acid and enhanced the bioavailability of minerals such as calcium, zinc and iron (Astuti *et al*., 2000). Fermentation neutralizes the phytate acid present in the soybeans; therefore tempeh does not restrict the body's absorption of minerals (Babu *et al*., 2009).

Karyadi and Lukito (1996) reported a calcium content of 69 mg/100g in tempeh. According to Tee *et al*. (1997) tempeh contains about 129 mg calcium / 100 g and in Malaysian diet, tempeh is also one of the calcium rich foods besides milk and dairy products. A study conducted by Haron *et al*. (2008) showed a calcium content of

56.8 ± 1.6 mg/100g in tempeh. Babu *et al.* (2009) also stated tempeh as an excellent source of calcium.

Nakamichi *et al*. (2008) reported that tempeh had a high calcium absorption ratio which is caused by both low phytate content and peptides that are produced during the fermentation of soybean by *Rhizopus*.

According to Karyadi and Lukito (1996) magnesium content in tempeh is about 70mg/100g. Silverstein (2002) reported that tempeh provides 21.9 per cent of the daily requirement of magnesium, in just four ounces. Tempeh contains magnesium, which plays a vital role in cardiovascular system and in more than 300 enzymatic reactions (Anon., 2009).

Many vitamins, such as thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5) and pyridoxine (B_6) can be produced by the tempeh fungus *Rhizopus oligosporus* (Nout and Rombouts, 1990; Mugula, 1992; Shurtleff and Aoyagi, 2001; Nout and Kiers, 2005).

Rhizopus oryzae, another important fungus frequently found in tempeh products (Samson *et al.*, 1987), has also been reported to produce niacin, vitamin K, ergosterol, tocopherol, pyridoxine, riboflavin and biotin (Wiesel *et al.*, 1997).

The mycelium of *Rhizopus oryzae* tends to be less dense, so it can not be used alone to produce tempeh (Sharma and Sarbhoy, 1984). However, the vitamin content of tempeh increased when it was co-inoculated with *Rhizopus oligosporus* (Mugula, 1992; Wiesel *et al.*, 1997).

Vitamin B_{12} is one of the most frequently studied vitamins produced by bacteria in fermented foods. It is normally present in insufficient amounts in vegetarian foods, while it is found in high amounts in animal food sources (Murphy and Allen, 2003).

Bacteria such as *Klebsiella pneumoniae*, *Klebsiella pneumiae spp. ozenae*, *Klebsiella terrigena*, *Klebsiella planticola* and *Enterobacter cloacae* can produce vitamin B_{12} during tempeh fermentation (Okada *et al.*, 1985). According to Suparmo (1989), *Klebsiella pneumoniae* (formerly *Aerobacter aerogenes*), is considered as being the main species producing vitamin B_{12} in soybean tempeh.

Other microbes that can produce vitamin B_{12} during tempeh fermentation belong to the genera *Propionibacterium, Pseudomonas, Clostridium* and *Streptomyces* (Hachmeister and Fung, 1993). Wiesel *et al.* (1997) reported that *Citrobacter freundii* and *Brevibacterium epidermidis* can also produce vitamin B_{12} in tempeh. Recently, the probiotic *Lactobacillus reuteri* has also been shown to produce vitamin B_{12} (Taranto *et al.*, 2003). Lactic acid bacteria can also produce other B-group vitamins and therefore, introducing lactic acid bacteria to tempeh may enhance the vitamin content of tempeh (Leroy and Vuyst, 2004; Sanna *et al.*, 2005).

2.6.2. HEALTH BENEFITS

According to Nowak (1992), tempeh is an interesting food grade ingredient for formulated functional foods. Functional foods differ from conventional foods in that they provide demonstrated physiological benefits or reduce the risk of chronic diseases, above and beyond their basic nutritional functions. Recent reports also suggest that tempeh contains a wealth of nutrients that are tied to an impressive array of health benefits, including decreased risk of heart disease and strokes, osteoporosis, cancer, digestive disorders and losing excess weight in addition to easing some of the symptoms of menopause (Babu *et al*., 2009). According to Hsu *et al*. (2009), the bio-functions of tempeh include anti-oxidant, anti-cancer, and anti-microbial activities, as well as cholesterol lowering ability.

Akesowan (2007) reported that the soy products are excellent vegetable sources of protein. Many current health recommendations suggest limiting animal protein and substituting tempeh for chicken, beef, or pork. The proteins in tempeh have the additional benefit of lowering cholesterol level, unlike the protein from animal sources, which raise the cholesterol level of a person. Thus, tempeh is an excellent alternative to meat (Nanri *et al*., 2010).

Krumhar and Carleton (2005) reported the role of soy protein in reducing the risk of cardiovascular disease, the number one killer of adults. It reduces the cholesterol level and hence, lowers the risk of heart attack or stroke. Tempeh can even lower LDL cholesterol levels, apart from raising HDL (Watanabe *et al*., 2006).

Babu *et al*. (2009) revealed that tempeh like other soy food, is rich in soluble dietary fiber, which binds fats and cholesterol and prevents their rapid absorption. Also, the dietary fiber binds the bile salts and helps throw them out of the body. As it disposes the bile, liver is stimulated to convert more cholesterol into bile salts, thereby lowering the cholesterol level in the body considerably.

Alekel *et al.* (2000) observed that soy products like tempeh inhibited the symptoms of menopause, especially hot flushes, due to its isoflavone content. Messina and Messina (2000) observed that isoflavones in soybean increased the bone mineral content of postmenopausal women and thus decreased the possibility of osteoporosis. Tempeh also contains high calcium which is also good for bone health (Nakamichi *et al*., 2008).

Berghofer *et al*. (2001) revealed that tempeh fermentation increased the antioxidative effects in the raw materials which is helpful in preventing many degenerative diseases. Badger *et al.* (2005) stated that soy protein may have a role in reducing the risk of cancers, particularly breast, colon and prostate cancer. According to Jens (2008), tempeh contains genistein, which plays a significant role in lowering the risk of prostate cancer in men.

According to Sudermadji and Suparmo (1997), tempeh has good antidiarrhoeal properties. Kiers (2001) reported that soybeans fermented with *Rhizopus* had better protection against enterotoxigenic *E. coli* (ETEC) induced diarrhoea. Furthermore, fermentation of cooked soybeans, especially with *Bacillus subtilis*, resulted in increased feed efficiency probably as a result of increased digestibility. These characteristics imply the potential of using fermented soybeans in individuals suffering from diarrhoea and malnutrition.

The protein in tempeh is excellent for diabetic patients who tend to have problems with animal sources of proteins. The protein and fiber in tempeh can also prevent high blood sugar levels and help in keeping blood sugar level under control (Kennedy, 1995). Chaverrí (2004) reported that people with reduced kidney function such as diabetics who have nephropathy, can benefit by replacing animal protein with tempeh protein or with any soy protein.

Rhizopus oligosporus can inhibit the growth and aflatoxin B_1 accumulation of *Aspergillus flavus* and *Aspergillus parasiticus* (Nout, 1989). *Rhizopus oligosporus* has been reported to produce four to five anti-bacterial compounds during soybean tempeh fermentation (Berghofer *et al.*, 2001).

An antibacterial protein has been purified from *R. oligosporus*, with activities against *Bacillus* spp. (especially against *Bacillus subtilis*), *Staphylococcus aureus* and *Streptococcus cremoris* (Kobayasi *et al.*, 1992). *R. oligosporus* can also produce certain compounds that interfere with the adhesion of *E. coli* to small intestinal brush-border membranes (Kiers *et al.*, 2002). The fungus also produces phenolic compounds that inhibit the growth of pathogenic bacteria such as *Helicobacter pylori* (Correia *et al.*, 2004; McCue *et al.*, 2004; Vattem *et al.*, 2004). Feng (2006) also reported that tempeh fungus *Rhizopus oligosporus* possesses antimicrobial properties.

2.7. TEMPEH PRODUCTS

A variety of indigenous fermented foods exist today; however, tempeh has been one of the most widely accepted and researched mold-modified fermented products (Hachmeister and Fung, 1993).

Because tempeh is firm and it can be formed into a patty, it is used as a substitute for animal products which are called as "mock" burgers or sandwiches (Nout and Rombouts, 1990). In China, soybean tempeh is milled and mixed with meat to make sausages in order to reduce the use of meat, but still maintain the protein quality (Jujin *et al.*, 2000). Tempeh has a complex flavour that has been described as nutty, meaty, and mushroom-like and is very suitable for incorporating into crackers, cakes, and sandwiches.

The most popular and simplest way of serving is shallow-fried or deep–fried tempeh (Shurtleff and Aoyagi, 2001). Fresh tempeh is often prepared by cutting it into pieces, soaking in brine or salty sauce, and then frying. Cooked tempeh can be eaten alone, or used in stir fries, soups, salads, sandwiches, and stews (Mejias *et al*., 2006).

Tempeh has also been processed into powder and used as ready-to-prepare food mixes (Vaidehi *et al.*, 1996), ice-cream (JuQin *et al.*, 2001), an additive to infant weaning food (Egounlety *et al.*, 2002) or as an ingredient in biscuits (Matsuo, 2006).

The basic soybean tempeh can be fried, deep-fried, mixed into sauces and salads, spread over pizzas, added to soups, noodle dishes, and stews, and eaten in many other ways (Mittal and Garg, 1990). Cracked wheat, barley, rye, oats, and rice can all be used alone or half and half with soybeans to create luscious new tempeh flavors and textures (Hachmeister and Fung, 1993).

In Indonesia, tempeh is sold fresh, to be cooked by slicing and frying or as processed biscuits or crisps. (Djien and Hesseltine, 1990). Masako (2006) suggested that tempeh powder is more suitable than quinoa powder as an ingredient of biscuit, and may be added to flour in amounts of up to 20 per cent.

Tempeh boiled with spices and palm sugar, and then fried for a few minutes to enhance the taste produces a damp, spicy, sweet and dark-colored tempeh which is known as *tempe bacem* (Shurtleff and Aoyagi, 2001).

Tempe kering is raw tempeh cut into little sticks, deep fried then mixed with spices and sugar, often mixed with separately fried peanuts and anchovies, which can be stored up to a month if cooked properly (Brissenden, 2003).

Chips made from tempeh has very high acceptability and the deep frying of tempeh yield chips that are delicate, light and crisp (Shurtleff and Aoyagi, 2001). Some tempeh chips are also made by dipping in batter that contains spices and then deep fried (Clark, 2007).

Fried snacks made from cereal material and tempeh flour (2-25 per cent on dry weight basis) are mixed to form a shapable dough which is then formed into the desired shaped product prior to being fried and the finished snacks are crisp or friable with increased protein content and reduced fat content over similar chips made without tempeh flour (Yueh *et al*., 1972).

In Indoneasia, over ripen tempeh powder is used in soup mixes as flavouring which imparts a nutty and meaty flavour to the soup (Applewhite, 1988). Vaidehi *et al*. (1996) developed ready-to-prepare mixes for soup by incorporating dried tempeh made from legume, cereal and vegetable combination and reported that tempeh soups were acceptable and all were rated between fair and good.

2.8. SAFETY OF FERMENTED FOODS

In every part of the world, people wage a constant battle against food contamination and the resulting food borne diseases and food wastage. Fermentation is one of the oldest technologies used for food preservation and today a large variety of fermented foods are derived from this technology which is used in households, small scale as well as large scale food industries (Sugihara, 1985). Even though fermented foods are beneficial to our diet, their safety is of concern every where (Kingsley *et al*., 2009).

One of the obvious considerations in the development of fermented foods is that the organisms we use are non toxic and non pathogenic (Burdock, 2000). The safety of fermented foods was established through experience and primarily reflects a lack of pathogenicity and acute toxicity and little information is available concerning the long term effects of consuming fermented foods (Buchanan, 2007).

Bacteria such as *Lactobacillus*, *Leuconostoc*, and *Pediococcus* species frequently found in fermented foods, have been used extensively in food processing throughout human history, and ingestion of foods containing live bacteria, dead bacteria, and metabolites of these microorganisms has taken place for a long time (Mayra-Makein and Bigret, 1993).

Reports have shown that some fermented foods containing lactic acid bacteria (LAB) are involved in clinical infections, particularly endocarditis (Aguirre and Collins, 1993). According to Oakey *et al.* (1995) some lactobacilli in the fermented foods produce enzymes that would enable the breakdown of human glycoproteins and the synthesis and lysis of human fibrin clots, characteristics which aid the colonization and survival of bacteria against endocarditis causing organisms. However Saxelin *et al.* (1996) had reported that cases of infection due to *Lactobacilli* and *Bifidobacterium* through fermented foods are extremely rare and are estimated to represent 0.05 -0.4 per cent of cases of infective endocarditis and bacteraemia.

Available data indicate that no harmful effects have been observed in controlled clinical studies with *Lactobacilli* and *Bifidobacteria* which is found in fermented foods (Donohue *et al*., 1998).

According to Thompson and Marth (1986), *Enterococci* occur and grow in a variety of cheeses, especially artisanal cheeses produced from raw or pasteurised goat, ewe's, water-buffalo or bovine milk. High levels of contaminating *Enterococci* usually result from poor hygienic practices during cheese manufacture. *Enterococcus faccium* and *Enterococcus faecalis* are frequently seen in fermented foods involved in clinical infections and there is concern over the emergence of vancomycin resistant strains (Gamer, 1994).

According to Nout (1994), cases of microbial food-borne infection have been reported in association with fresh cheese, sausages, fermented fish and fermented cereals. Another risk involves microbial food intoxications due to mycotoxin contaminated raw materials, production of bacterial toxins or possible mycotoxin production by fungal inoculants (Jay, 1996).

Even though no cases of food poisoning have ever been reported after consuming tempeh, there is still a risk due to the potential growth of moulds and pathogenic bacteria and the production of mycotoxins and bacterial toxins (Swan and Hesseltine, 1979).

In practice, even in Indonesian cottage industries, soybean tempeh is rarely reported to contain any mycotoxin (Hesseltine, 1995).

Co-inoculation with lactic acid bacteria (LAB) improves hygienic safety and possibly also the flavour of soybean tempeh (Nout and Rombouts, 1990). Pathogenic bacteria do not grow well in acidified substrates and even if they can grow, they are normally controlled by Lactic acid bacteria in tempeh. Some fungal species closely related to *R. oligosporus* produce toxins (Jennessen *et al.*, 2005), and misidentification could potentially lead to the use of a toxin-producing strain for tempeh fermentation.

Tempe bongkrek is an Indonesian food made by fermentation of coconut presscake or coconut milk residue by *Rhizopus oligosporus* (Garcia *et al.,* 1999). Consumption of *tempe bongkrek* is associated with a food borne human intoxication and significant numbers of deaths annually (Buckle and Kartadarma, 2008).

According to Jiao *et al*. (2003), the bacterium *Burkholderia cocovenenans*, which is the causative organism, produces two toxins, toxoflavin and bongkrekic acid (also commonly referred to as bongkrek acid).

Bongkrek acid is highly toxic as it inhibits the conversion of ADP to ATP in the mitochondria and this result in a high fatality rate among the victims of bongkrek acid poisoning (Henderson and Lardy, 1970).

From a food processing point of view, major risk enhancing factors are the use of contaminated raw materials, lack of pasteurization, and use of poorly controlled natural fermentations (Pariza and Johnson, 2001). Also sub-optimal fermentation starters and inadequate storage and maturation conditions as well as consumption without prior cooking may reduce the safety of fermented foods (Chmielewski and

Frank, 2006). In addition to ensuring adequate processing conditions, the development of non toxigenic starters with ability to antagonize pathogenic microorganisms and to degrade toxic substances needs continued attention (Hotz and Gibson, 2007).

Materials and Methods

3. MATERIALS AND METHODS

The methods followed and the materials used in the study "Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes" are given under the following heads.

3.1. Collection of raw materials

3.2. Standardisation of tempeh with legumes and cereals.

3.2.1. Preparation of starter culture

3.2.2. Preparation of tempeh

3.3. Acceptability of primary products with fresh tempeh types

3.3.1. Preparation of tempeh chips

3.3.2. Preparation of tempeh roast

3.3.3. Acceptability of tempeh chips and tempeh roast

3.3.3.1. Selection of judges for acceptability studies

3.3.3.2. Preparation of score card

3.3.3.3. Organoleptic evaluation

3.4. *in vitro* starch and protein digestibility of fresh tempeh types

3.5. Selection of fresh tempeh types

3.6. Nutritional and shelf life studies in selected fresh tempeh types

3.6.1. Analysis of chemical constituents in selected fresh tempeh types

3.6.1.1. Moisture

3.6.1.2. Protein

3.6.1.3. Starch

3.6.1.4. Reducing and total sugars

3.6.1.5. Fiber

3.6.1.6. Total fats

3.6.1.7. Thiamine

3.6.1.8. Riboflavin

3.6.1.9. β carotene

3.6.1.10. Vitamin C

3.6.1.11. Calcium

3.6.1.12. Iron

3.6.1.13. Phosphorus

3.6.1.14. Potassium

3.6.1.15. Zinc

3.6.2. *in vitro* availability of minerals from selected fresh tempeh types

3.6.3. Probiotic activity of the selected fresh tempeh types by standard *in vitro* procedures for

3.6.3.1. Acid tolerance of the organisms in tempeh

3.6.3.2. Bile acid tolerance of the organisms in tempeh

3.6.3.3. Antimicrobial activities of tempeh against enteropathogenic bacteria

3.6.4. Shelf life studies of selected tempeh types

3.6.4.1. Sensory evaluation of the fresh and stored tempeh types

3.6.4.2. Enumeration of total micro flora

3.6.4.3. Acceptability of the products prepared from the fresh and stored samples

3.7. Quality evaluation and shelf life study in tempeh flours

3.7.1. Preparation and storage of tempeh flours

3.7.2. Changes in chemical constituents in tempeh flours during storage

3.7.3. Changes in *in vitro* starch and protein digestibility of tempeh flours

3.7.4. Enumeration of total micro flora in tempeh flours

3.7.5. Insect infestation of tempeh flours during storage

3.8. Standardisation of tempeh based instant soup mixes

3.8.1. Preparation of vegetable mix

3.8.2. Preparation of spice mix

3.8.3. Developing soup mixes

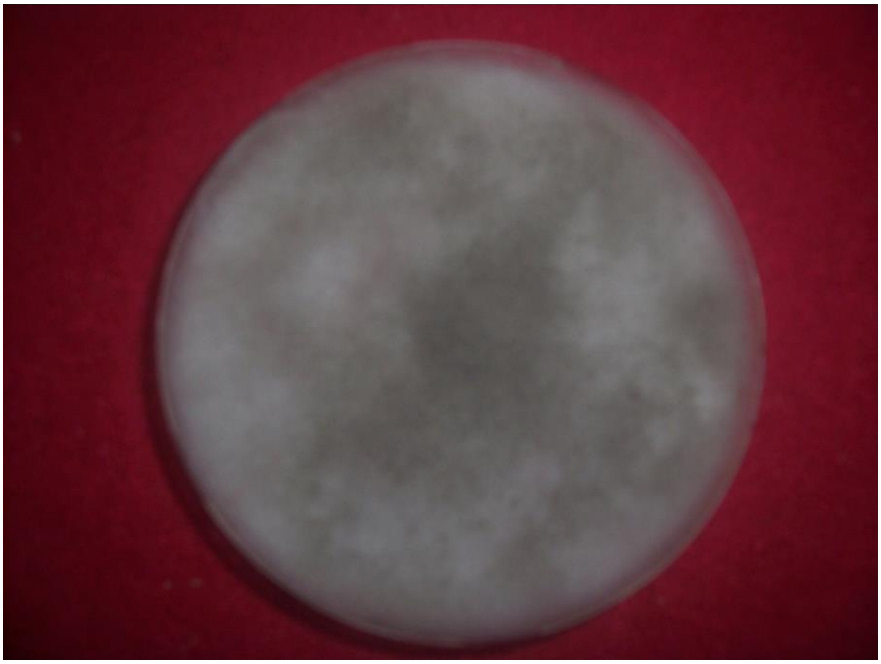
3.1. COLLECTION OF RAW MATERIALS

Tempeh was prepared with cereals like rice and wheat, and with legumes like green gram, cowpea and soybean. The cereals and legumes were purchased in one lot from the local market. Vegetables like carrots and beans, spices and condiments such as garlic, ginger, onion and pepper powder and corn flour for standardising soup mixes were also purchased from the market.

Tempeh is an Indonesian fermented food consisting of soybeans partially digested and bound together by mycelium of *Rhizopus*. *Rhizopus oligosporus* is the most frequently isolated organism from natural tempeh samples (Jurus and Sundberg, 1976). Hence, a pure culture of *Rhizopus oligosporus* (Plate 1) was used for tempeh fermentation.

Pure culture of *Rhizopus oligosporus* – MTCC 556, was obtained from Institute of Microbial Technology (IMTECH), Chandigrah. Pure cultures of enteropathogens like *Escherichia coli* (MTCC 40), *Salmonella enteritidis* (MTCC 3219), *Bacillus cereus* (MTCC 430) and *Staphylococcus aureus* (MTCC 87) were also obtained from IMTECH to study the antimicrobial activity of tempeh.

Plate 1. ***Rhizopus oligosporus***



3.2. STANDARDISATION OF TEMPEH WITH LEGUMES AND CEREALS

Regular tempeh without mentioning any pulse is prepared with soybeans. Tempeh fermentation was carried out with pure cultures of *Rhizopus oligosporus*. The standard procedure adopted by Jurus and Sundberg (1976) was followed for preparing tempeh with soybeans as control, with the selected legumes like green gram and cowpea and also with the selected legumes in combination with different proportions of cereals like rice and wheat.

3.2.1. Preparation of starter culture

Pure cultures of *Rhizopus oligosporus* – MTCC 556 obtained from IMTECH was sub cultured on three per cent malt agar medium. Starter culture was prepared (Plate 2) by the modified procedure of Jurus and Sundberg (1976). To the sub cultured malt agar medium, added 10 ml of distilled water and vortexed for 3 minutes. The spore suspension thus obtained was further used for inoculation of the soybeans. The soybeans (1 kg) were cleaned, washed, and boiled for 30 minutes, just making it soft. This was again washed and soaked overnight (12 hours). The legumes were dehulled by floatation in water and were surface dried. This was mixed with vinegar to adjust the pH to 4.5 (100 ml of vinegar for 1Kg of soybean). To this pH adjusted soybeans, added the spore suspension (7 ml of spore suspension for 100 g of soybean) and mixed well. The inoculated soybeans were packed in perforated polyethylene pouches by pressing them flat to a thickness of three centimeter and were sealed. Packed pouches were incubated at 32 ^{o}C for 48 hours. After 48 hours, thick firm cake of soybean tempeh was obtained. This soybean tempeh was used as the starter culture. Two grams of fresh soybean tempeh was used for inoculating one kilogram of the substrate for tempeh preparation.

3.2.2. Preparation of tempeh

Tempeh was prepared using green gram, cowpea, soybean, rice and wheat in different combinations. The following treatments were tried in two replications.

Plate 2. Flow chart of the preparation of starter culture

Soybean

Washing of soybeans

Boiling for 30 minutes

Washing of boiled soybean

Plate 2 continued……..

Overnight soaking

Dehulling

Surface drying

Addition of vinegar

Plate 2 continued……..

Inoculation with spore suspension

⬇

Incubation at 32^{o}C for 48 hours

⬇

Soybean tempeh

T_1 Soybean (100%) -control

T_2 Green gram (100%)

T_3 Cowpea (100%)

T_4 Green gram (75%) + soybean (25%)

T_5 Green gram (50%) + soybean (50%)

T_6 Cowpea (75%) + soybean (25%)

T_7 Cowpea (50%) + soybean (50%)

T_8 Green gram (75%) + rice (25%)

T_9 Green gram (50%) + rice (50%)

T_{10} Cowpea (75%) + rice (25%)

T_{11} Cowpea (50%) + rice (50%)

T_{12} Green gram (75%) + wheat (25%)

T_{13} Green gram (50%) + wheat (50%)

T_{14} Cowpea (75%) + wheat (25%)

T_{15} Cowpea (50%) + wheat (50%)

T_{16} Green gram (50%) + cowpea (50%)

T_{17} Soybean (75%) + rice (25%)

T_{18} Soybean (50%) + rice (50%)

T_{19} Soybean (75%) + wheat (25%)

T_{20} Soybean (50%) + wheat (50%)

Selected legumes were cleaned, washed, and boiled for 30 minutes, just making it soft. This was again washed and soaked overnight (12 hours). The legumes were dehulled by floatation in water and were surface dried. In treatments with cereals, wheat was washed and boiled separately for 30 minutes, drained and surface dried and was mixed with the surface dried legumes. In treatments with rice, raw rice was washed and boiled for 5 minutes, drained, surface dried and mixed with the treated surface dried legumes. The substrates were then mixed with vinegar to adjust the pH to 4.5 (100 ml of vinegar for 1 kg of substrate). This substrate (1 kg) was inoculated with two grams of tempeh starter (prepared as in 3.2.1) and mixed well. This was then packed in perforated polythene pouches by pressing them flat to a thickness of three centimeter and was sealed. Packed pouches were incubated at 32^0C-36^0C for 48 hours, during which the tempeh fermentation took place, after which the

Tempeh types

Plate 3. T_1 (100% soybean)

Plate 4. T_2 (100% green gram)

Plate 5. T_3 (100% cowpea)

Plate 6. T_4 (green gram 75% + soy 25%)

Plate 7. T_5 (green gram 50% + soy 50%)

Plate 8. T_6 (cowpea 75% + soy 25%)

Tempeh types

Plate 9. T_7 (cowpea 50% + soy 50%)

Plate 10. T_8 (green gram 75% + rice 25%)

Plate 11. T_9 (green gram 50% + rice 50%)

Plate 12. T_{10} (cowpea 75% + rice 25%)

Plate 13. T_{11} (cowpea 50% + rice 50%)

Plate 14. T_{12} (green gram 75% + wheat 25%)

Tempeh types

Plate 15. T_{13} (green gram 50% + wheat 50%)

Plate 16. T_{14} (cowpea 75% + wheat 25%)

Plate 17. T_{15} (cowpea 50% + wheat 50%)

Plate 18. T_{16} (green gram 50% + cowpea 50%)

Tempeh types

Plate 19. T_{17} (soybean 75% + rice 25%)

Plate 20. T_{18} (soybean 50% + rice 50%)

Plate 21. T_{19} (soybean 75% + wheat 25%)

Plate 22. T_{20} (soybean 50% + wheat 50%)

mycelium appeared to be more or less uniformly distributed throughout to form a firm cake with a good flavour (Plates 3 - 22).

3.3. ACCEPTABILITY OF PRIMARY PRODUCTS WITH FRESH TEMPEH

In Indonesia, tempeh is used as a meat substitute. Tempeh cakes are cut and added to stir fries and vegetable dishes. Tempeh has a tender chewy consistency that makes it an excellent meat substitute. Hence, in the present study, tempeh chips and roast were prepared for evaluating the organoleptic qualities of fresh tempeh types.

3.3.1. Preparation of tempeh chips

Fresh tempeh types were cut to thin uniform slices (4x1 cm) and fried in coconut oil with the addition of salt (Plates 23 and 24).

3.2.2. Preparation of tempeh roast

Standard procedure suggested by Philip (1993) for the preparation of meat roast was followed for the preparation of tempeh roast using fresh tempeh, by replacing meat (Plate 25). The recipe used is given in Appendix I.

3.3.3. Acceptability of tempeh chips and tempeh roasts

3.3.3.1. Selection of judges for acceptability studies

A series of acceptability trials were carried out using simple triangle test at the laboratory level and selected a panel of ten judges between the age group of 18-35 years as suggested by Jellinek (1985).

3.3.3.2. Preparation of score card

Score cards were prepared based on a nine point hedonic scale for the organoleptic evaluation of tempeh chips and tempeh roast and these are given in Appendix II and III.

Plate 23. Sliced tempeh **Plate 24. Tempeh chips**

Plate 25. Tempeh roast

3.3.3.3. Organoleptic evaluation

Sensory evaluation of the tempeh chips and roasts were carried out in the morning using score cards by the panel of 10 selected judges. The quality attributes namely appearance, colour, flavour, texture, taste, and overall acceptability were evaluated.

3.4. *IN VITRO* STARCH AND PROTEIN DIGESTIBILITY OF FRESH TEMPEH TYPES

In vitro starch digestibility (IVSD) was estimated as suggested by Satterlee *et al.* (1979). One gram of the sample in 100 ml water was gelatinised and boiled for one hour and filtered. One ml of the gelatinised solution was taken and one ml of the enzyme solution (saliva diluted with equal quantity of water) was added. The mixture was incubated at 37°C for 1- 2 hours. The reaction was stopped by adding one ml of sodium hydroxide. Later glucose was estimated by the method of Somoygi (1952).

The method proposed by Sadasivam and Manikam (1992) was followed to determine *in vitro* protein digestibility (IVPD). A multi-enzyme system, consisting of a mixture of porcine pancreatic trypsin type IX, bovine pancreatic chymotrypsin type II and porcine intestinal peptidase grade III, were used. Tempeh and distilled water were used to prepare 50 ml of an aqueous protein suspension (6.25 g protein/l) with pH adjusted to 8.0, while stirring in a water bath at 37 °C. The multi-enzyme solution was maintained in an ice bath. Five ml aliquots of the multi-enzyme solution were added with stirring to the protein suspension at 37 °C. The rapid pH drop was recorded automatically over a 10 minutes period using a pH meter. IVPD was calculated from the equation $IVPD = 210.46 - 18.10X$, where $X = pH$ after 10 minutes.

3.5. SELECTION OF FRESH TEMPEH TYPES

From the different treatments of tempeh, three fresh tempeh types which were most acceptable with high overall acceptability and with a high protein and starch digestibility were selected along with the control (T_1). These selected four tempeh types were used for further nutritional and shelf life studies with fresh tempeh and also for preparing tempeh flour and soup mix. The three treatments were selected based on the acceptability scores of tempeh chips and tempeh roast by applying Kendall's coefficient of concordance and IVSD and IVPD were analysed statistically by applying DMRT.

3.6. NUTRITIONAL AND SHELF LIFE STUDIES IN SELECTED FRESH TEMPEH TYPES

Quality aspects such as chemical composition, *in vitro* availability of minerals, probiotic activity and shelf life studies of the selected fresh tempeh types were conducted.

3.6.1. Analysis of chemical constituents in selected fresh tempeh types

Analysis was carried out with three replications of each treatment.

3.6.1.1. Moisture

Moisture content of selected fresh tempeh was estimated by the method of A.O.A.C. (1980).

To determine the moisture content, five gram of the sample was taken in a petridish and dried at 60 -70°C in a hot air oven, cooled in a desiccator and weighed. The process of heating and cooling was repeated till constant weight was achieved. The moisture content of the sample was calculated from the loss in weight during drying.

3.6.1.2. Protein

Protein was estimated by the method of A.O.A.C. (1980).

Dried and powdered tempeh (0.3 g) was digested with 6 ml conc. H_2SO_4 after adding 0.4 g of $CuSO_4$ and 3.5 g K_2SO_4 in a digestion flask until the colour of sample was converted to green. After digestion it was diluted with water and 25 ml of 40 per cent NaOH was pumped. The distillate was collected in 2 per cent boric acid containing mixed indicators and then titrated with 0.2 N HCl. The nitrogen content thus estimated was then multiplied with a factor of 6.25 to get the protein content.

3.6.1.3. Starch

Starch was estimated colorimetrically using anthrone reagent, as suggested by Sadasivam and Manikam (1992).

Weighed 0.5 g of the sample and extracted with 80 per cent ethanol to remove sugars. Residue was repeatedly extracted with hot 80 per cent ethanol to remove the sugars completely. The residue was dried over a water bath and added 5 ml water and 6.5 ml of 52 per cent perchloric acid and extracted in the cold for 20 minutes. Centrifuged the sample and re-extracted with fresh perchloric acid. The supernatant was pooled and made up to 100 ml. Pipetted out 0.2 ml of the supernatant and made up to one ml with water and added 4 ml of anthrone reagent, heated for 8 minutes, cooled and read the OD at 630 nm.

A standard graph was prepared using serial dilutions of standard glucose solution. From the graph, glucose content of the sample was obtained. Multiplied the value by a factor of 0.9 to arrive at the starch content.

3.6.1.4. Reducing sugar and total sugars

Reducing sugar was estimated by the method given by Lane and Eyon (Ranganna, 1986). Ten gram of the sample was ground thoroughly in a motor and to that, 100 ml distilled water was added and then clarified with neutral lead acetate. Excess lead was removed by adding potassium oxalate. The volume was then made up to 250 ml. An aliquot of this solution was titrated against a mixture of Fehlings solution A and B using methylene blue indicator. The reducing sugar was estimated as percentage.

From the clarified solution used for the estimation of reducing sugars, 50 ml was taken and boiled gently after adding citric acid and water. It was later neutralized with sodium hydroxide and the volume was made up to 250 ml. An aliquot of this solution was titrated against Fehlings A and B. The total sugar content was expressed as percentage.

3.6.1.5. Fiber

Crude fibre content was estimated by acid-alkali digestion method as suggested by Chopra and Kanwar (1978).

Two gram of sample was boiled with 200 ml of 1.25 per cent sulphuric acid for 30 minutes. It was filtered through a muslin cloth and washed with boiling water and again boiled with 200 ml of 1.25 per cent sodium hydroxide for 30 minutes. Repeated the filteration through muslin cloth and washed with sulphuric acid, water and alcohol in a sequential manner. Transferred the residue to a pre-weighed ashing dish. The residue was ignited for 30 minutes in a muffle furnace at 250°C, cooled in a desicator and weighed. The fibre content of the sample was calculated from loss in weight on ignition.

3.6.1.6. Total fats

The fat content of tempeh was estimated by the method of A.O.A.C. (1955).

Five gram of dried and powdered tempeh was taken in a thimble and plugged with cotton. The material was extracted with petroleum ether for six hours without interruption by gentle heating in a soxhlet apparatus. Extraction flask was then cooled, and ether was removed by heating and weight was taken. The fat content was expressed as g per 100g of the sample.

3.6.1.7. Thiamine

Thiamine content was estimated as suggested by Sadasivam and Manikam (1992). Dissolved 50 mg of thiamine hydrochloride in 500 ml of 0.1N sulphuric acid containing 25 per cent alcohol to get stock solution and from this working standard was prepared.

Five gram of finely ground sample was taken in a 250 ml conical flask in duplicate. Slowly added 100 ml 0.1N sulphuric acid without shaking, and was kept overnight. After shaking vigorously, filtered through Whatman No.1 filter paper and discarded the first 10-15 ml of the filtrate. Pipetted out 10 ml of the extract in duplicate into 100 ml separating funnels. Pipetted out 10 ml of working standard and added 3 ml of 15 per cent sodium hydroxide into each separating funnel immediately followed by four drops (0.2 ml) of ferricyanide solution. 10 ml of the extract without the addition of ferricyanide solution was set as the sample blank. After shaking gently for exactly 30 seconds, added 15 ml of isobutanol rapidly from a quick delivery burette. Stoppered immediately, shook vigorously for 60 seconds and allowed the layers to separate. Drained off the bottom layer carefully and discarded it and added one spatula full of sodium sulphate directly into the separating funnel, stoppered and swirled gently to clarify the extract. The clear extract was collected from the top into a clean dry test tube and read at an excitation wave length of 365 nm and emission wave length 435 nm, excitation band pass and emission band pass of 10 nm and sensitivity set at 500 V in a spectroflurimeter. The fluorescence of the sample was noted. The thiamine content was expressed as mg per 100g of the sample.

3.6.1.8. Riboflavin

Riboflavin content was estimated as suggested by Sadasivam and Manikam (1992).

Dissolved five milligram of riboflavin standard in 100 ml standard flask with five per cent acetic acid. The flask was then covered with aluminum foil to prevent decomposition of riboflavin. Further diluted to give 10 ppm with five per cent acetic acid. Blank was set at 5 per cent acetic acid.

Weighed two gram of the sample into 250 ml conical flask and added 75 ml 0.1N H_2SO_4, and autoclaved the mixtures for 30 minutes. Cooled and added five ml of 2.5 molar sodium acetate solution and kept for one hour. Transferred to volumetric flask and made up to 100 ml. Filtered and discarded the first 10-15 ml. Ten ml of the sample solution was taken and added two ml of water and one ml of potassium permanganate (4%) solution, kept for two minutes and then added one ml of hydrogen peroxide solution and was read immediately in the spectroflurimeter with an excitation wave length of 390nm, emission wave length of 520 nm, excitation band pass and emission band pass at 10 nm and with an EHT of 550 Volt. The riboflavin content was expressed as mg per 100g of the sample.

3.6.1.9. β carotene

β carotene was estimated by the method of A.O.A.C. (1970) using saturated n-butanol. To five gram of the sample, 50 ml of saturated butanol was added and shaken for a minute and kept overnight. Decanted the supernatant and read the colour intensity at 435 nm in a spectrophotometer. The β carotene was expressed in µg per 100 g.

3.6.1.10. Vitamin C

Vitamin C content of the fresh tempeh was estimated by the method suggested by A.O.A.C. (1980) using 2, 6 dichlorophenol indophenol dye and the content was expressed in mg per 100g of the sample.

3.6.1.11. Calcium

The calcium content was estimated by atomic absorption spectrophotometric method using the diacid extract prepared from the sample (Perkin-Elmer, 1982).

The diacid extract was made up to 100 ml and 10 ml of the made up solution was again diluted to 50 ml. This solution was read directly in atomic absorption spectro photometer and calcium content was expressed in mg per 100g.

3.6.1.12. Iron

Iron content was estimated by atomic absorption spectrophotometric method using the diacid extract prepared from the sample (Perkin-Elmer, 1982).

The diacid extract was made up to 100 ml and 10 ml of the made up solution was again diluted to 50 ml. This solution was read directly in atomic absorption spectro photometer and iron content was expressed in mg per 100 g.

3.6.1.13. Phosphorus

The phosphorus content was analyzed colorimetrically as suggested by Jackson (1973), which gives yellow colour with nitric acid vandate molybdate reagent.

To five ml of pre-digested aliquot, five ml of nitric acid vandate molybdate reagent was added and made up to 50 ml with distilled water. After 10 minutes, the OD was read at 420 nm.

A standard graph was prepared using serial dilution of standard phosphorus solution. The phosphorus content of the sample was estimated from the standard graph and expressed in mg per 100 g.

3.6.1.14. Potassium

The method suggested by Jackson (1973) was followed for the estimation of potassium using a flame photometer

One gram of the digested solution was made up to 25 ml and read directly in a flame photometer. The potassium content was expressed in mg per 100 g of the sample.

3.6.1.15. Zinc

The zinc content was estimated using atomic absorption spectrophotometer as suggested by (Perkin-Elmer, 1982).

The diacid extract was made up to 100 ml and was read directly in atomic absorption spectro photometer and zinc content was expressed in mg per 100 g.

3.6.2. *in vitro* availability of minerals from selected fresh tempeh types

HCl extractability of minerals in foods is an index of their bioavailability from foods (Chompreeda and Fields, 1984). Thus the solubility of minerals in foods, subject to *in vitro* gastric intestinal digestion is a useful indicator of mineral bioavailability (Kim and Zemel, 1986).

in vitro availability of calcium, iron, phosphorus, potassium, and zinc of the selected tempeh types were assessed. For this the HCl extractability of the minerals were analysed. The samples were extracted with 0.03 N Hydrochloric acid by shaking the contents at 37^0 C for three hours. The clear extract obtained after filtration with Whatman No: 42 filter paper was oven dried at 100^0 C and wet acid digested. The amount of the HCl extractable calcium, phosphorus, iron, potassium and zinc in the digested samples were determined by the methods as described above for the estimation of total minerals. HCl extractability of each mineral was derived by using the following formula (Duhan *et al.*, 2001).

$$\textbf{Mineral extractability, \%} = \frac{\textbf{Mineral extractability in 0.03N HCL}}{\textbf{Total mineral}} \times \textbf{100}$$

3.6.3. Probiotic activity of the selected fresh tempeh

Fermented foods are thought to have probiotic micro organisms. The probiotic effects of fermented foods depend on a number of factors including the strain chosen, level of consumption, duration and frequency of exposure, and the physiological condition of the individual (Koop, 2001). Factors such as gastric juice with its antibacterial effect, low pH and bile salt concentration are factors which inhibit the growth or kill the probiotic organisms in the gut. (Marteau *et al.*, 1993). Hence, in this study, probiotic characteristics such as acid and bile acid tolerance and antimicrobial activities of tempeh types against enteropathogenic bacteria were studied *in vitro*.

3.6.3.1. Acid tolerance of the micro organisms found in tempeh types

The survival of any organism in the stomach should be pH - HCl dependent (Giannella *et al.*, 1972). In this study, organisms in fresh temph were tested *in vitro* for their ability to resist the action of gastric acidity.

Nutrient broth was prepared and 10 ml was dispensed into each boiling tube. Medium was adjusted to pH 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 using pH meter. Medium was sterilised and inoculated with one gram of fresh tempeh and subsequently incubated the tubes for a period of three hours at 37˚C. After incubation, 1000 µl of the sample from each flask was serially diluted in sterile peptone water and sensible dilutions were plated by spread plate method on nutrient agar media and Sabouraud's dextrose agar media for the enumeration of bacteria and yeast respectively. The plates were incubated at 37 ˚C for 24 h and 72 h respectively for bacteria and yeast.

Rhizopus oligosporus, the fungus added to the substrate for the preparation of tempeh was tested for their acid tolerance by assessing their biomass at different pH levels. Liquid malt extract medium was prepared and 100 ml was dispensed into each conical flask. Medium was adjusted to pH 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 using pH meter. *Rhizopus oligosporus* was inoculated in each pH adjusted conical flask and incubated at 37 ˚C for 96 h. After incubation, the media was filtered with Whatman No.1 filter paper. The initial weight of the filtrate was taken and the filtrate was dried at 60-70°C in a hot air oven till constant weight was achieved and the dry weight was also noted.

3.6.3.2. Bile acid tolerance of the organisms found in tempeh types

Bile acid tolerance is an essential criterion for a probiotic organism for colonization in the colon (Huis and Havenaar, 1992). The bile acid concentration in human intestine varies in different regions (jejunum - 4 per cent bile, ileum - 2 per cent bile, large intestine - 1.5 per cent bile) (Chou and Weimer, 1999). Nutrient broth was prepared and 10 ml of the broth was distributed to each conical flask. Bile salt was adjusted to 1 to 4 per cent levels in the media. A control was maintained without bile salt. The media were sterilised at 121^{o}C for 15 minutes.

One gram of tempeh was incubated at 37^{0}C for 3 hours in 10 ml nutrient broth in which bile salt concentration was adjusted. After each hour of incubation, 1000µl of the sample was serially diluted up to 10^{-8} dilution in sterile peptone water and sensible dilutions were plated on nutrient agar, Sabouraud's dextrose agar and potato dextrose agar medium by spread plate method for the respective enumeration of bacteria, fungi and yeast and incubated at 37^{o}C under absolute anaerobic conditions for 48 to 96 h. The number of colonies were counted and recorded.

3.6.3.3. Antimicrobial activities of tempeh against enteropathogenic bacteria

Fermented foods contain probiotic organisms and these exhibit antagonistic action towards enteropathogens such as *Escherichia coli*, *Shigella*, *Salmonella*, *Staphylococcus*, *Bacillus*, *Proteus* etc (Khedkar *et al.*, 1998). Hence, in this experiment an attempt was made to study the antibacterial activity of tempeh. The enteropathogens tested were strains of *E.coli* (MTCC 40), *Salmonella enteritidis* (MTCC 3219), *Bacillus cereus* (MTCC 430) and *Staphylococcus aureus* (MTCC 87). All these cultures obtained from IMTECH, were subcultured in nutrient agar and maintained at 4 to 8 ˚C.

The mode of inhibition of tempeh organisms were determined by agar well assay (Singh and Sharma, 1999). Saline suspensions (0.85%) of the pathogens were made using sterile cotton swab; lawn culture of the pathogen was made in nutrient agar in sterile plates by streaking the entire agar surface. Plates were allowed to set and dry. Wells of 5 mm diameter were cut with sterile well borer in each plate.

Tempeh samples were incubated in nutrient broth for a period of three hours. Added 0.1 ml of the incubated sample to the well and incubated at 37^0C for 48 hours. After incubation, the formation of the clear zone around the wells was checked and compared with the control.

3.6.4. Shelf life studies of selected fresh tempeh types

The selected three fresh tempeh types along with the control (T_1) were packed in polythene bags of 250 gauge thickness and were kept under refrigerated condition (3 to 7^0C) for 12 days and in a deep freezer (-15^0 to -18^0 C) for one month.

Quality evaluation was done initially and during 3rd, 6th, 9th and 12th day in refrigerated samples. In frozen samples, evaluation was done initially, during the 15th day and 30th day of storage.

The following quality aspects were studied in the stored samples

3.6.4.1. Sensory evaluation of the stored tempeh

Changes in appearance, colour, flavour, and texture of the samples were assessed by direct observation in the uncooked samples.

3.6.4.2. Enumeration of total micro flora

The totals microbial counts in the fresh tempeh types and also in the stored samples were enumerated by serial dilution and plate count method as described by Agarwal and Hasija (1986). Ten gram of sample was added to 90 ml sterile water and agitated for 20 minutes. One ml of this solution was transferred to a test tube containing 9 ml of sterile water to get 10^{-2} dilution and similarly 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} , 10^{-7} and 10^{-8} dilutions were also prepared.

Enumeration of total microbial count was carried out using nutrient agar media for bacteria, Potato dextrose agar media for fungus and Sabouraud's dextrose agar media for yeast. The dilution used for assessing the bacterial count was 10^{-8}. The dilutions used for assessing fungus and yeast was 10^{-6} and 10^{-5} respectively.

3.6.4.3. Acceptability of the products prepared from stored samples

Tempeh chips and roast were prepared as explained in 3.3.1. and 3.2.2. respectively with the stored samples and sensory evaluation of the tempeh chips and roast were carried out in the morning using score cards based on a nine point hedonic scale (Appendix II and III) by the panel of 10 selected judges. The quality attributes namely appearance, colour, flavour, texture, taste, and overall acceptability were evaluated and acceptability of the products was studied.

3.7. QUALITY EVALUATION AND SHELF LIFE STUDY IN TEMPEH FLOURS

3.7.1. Preparation and storage of tempeh flours

The selected three fresh tempeh types along with the control were sliced, steam blanched for 15 minutes and dried in hot air oven at 70°C. The dried tempeh types were ground and sieved through a mesh of 40 size to get a uniform flour. The flours were packed in metalised polyester laminate pouches and were stored in room temperature for six months.

3.7.2. Changes in chemical constituents in tempeh flours during storage

Tempeh flours were analysed for moisture, protein, starch, fiber, total fats, thiamine, riboflavin, calcium, iron, phosphorus, potassium and zinc initially and after 6^{th} month of storage by the methods given in 3.6.1.1, 3.6.1.2, 3.6.1.3, 3.6.1.5, 3.6.1.6, 3.6.1.7, 3.6.1.8, 3.6.1.11, 3.6.1.12, 3.6.1.13, 3.6.1.14 and 3.6.1.15 respectively.

3.7.3. Changes in in vitro starch and protein digestibility of tempeh flours during storage

IVSD and IVPD of the tempeh flours were evaluated initially and during 6^{th} month of storage by the methods given in 3.4.

3.7.4. Enumeration of total microflora in tempeh flours

The total microbial count in tempeh flours were enumerated initially and during the 6^{th} month by serial dilution and plate count method as described in 3.6.4.2. The dilution used for the assessment of bacteria was 10^{-5} whereas for fungus and yeast, 10^{-3} dilution was used.

3.7.5. Insect infestation of tempeh flour

The insect infestation of the stored tempeh flours were assessed initially and also during the sixth month of storage. The insect infestation was checked by visual observation of tempeh flours and also by examining the flours under the microscope. The flours were sieved and observed under microscope.

3.8. STANDARDISATION OF TEMPEH BASED INSTANT SOUP MIXES

Because of its high nutritional value and acceptability, tempeh and its second generation products will have an impact on the consumers. Tempeh and its second generation products can solve the problem of under nutrition. Hence, to develop a second generation tempeh product with high acceptability, tempeh flours (4 treatments) prepared from the selected fresh tempeh types were used for standardising soup mixes with suitable blending materials. All the analysis was done in three replications.

3.8.1. Preparation of vegetable mix

Carrot (50 g) and beans (50 g) were shredded and blanched in boiling water containing potassium metabisulphite (0.2%) for 3 minutes. This was dried in a vacuum oven. The dried vegetable mix was used in soup mixes (Plate 26).

3.8.2. Preparation of spice mix

Onion (50 g), garlic (50 g) and ginger (20g) were peeled, sliced and blanched in boiling water containing potassium metabisulphite (0.2%) for 3 minutes. This was then ground to a paste and dried in a vacuum oven. The dehydrated spices were ground to a fine powder and were used in soup mixes (Plate 27).

3.8.3. Developing soup mixes

The selected four treatments of tempeh flour were used for making soup mixes. The composition of each treatment is given in Table 1.

Table 1. Compositions of soup mixes with tempeh flour

Ingredients	Composition for each treatment			
	$S_{1(50)}$	$S_{2(55)}$	$S_{3(60)}$	$S_{4(65)}$
Tempeh flour	50	55	60	65
Corn flour	20	15	10	5
	70	**70**	**70**	**70**
Vegetable mix	10	10	10	10
Spice mix	5	5	5	5
Pepper powder	5	5	5	5
Salt	5	5	5	5
Sugar	3	3	3	3
Citric acid	2	2	2	2
	30	**30**	**30**	**30**
	100	100	100	100

All the ingredients were well blended to get a uniform soup mix

Plate 26. Vegetable mix

Plate 27. Spice mix

3.8.4. Storage studies of soup mixes

The soup mixes (16 no's) were packed in metalised polyester laminate pouches and were stored for six months under ambient conditions. The quality aspects of the soup mixes were studied by preparing soups initially, and during the 6th month of storage.

3.8.4.1. Evaluation of chemical composition of soup mixes

Soup mixes were analysed for moisture, protein, starch, fiber, total fats, thiamine, riboflavin, calcium, iron, phosphorus, potassium and zinc by the methods given in 3.6.1.1, 3.6.1.2, 3.6.1.3, 3.6.1.5, 3.6.1.6, 3.6.1.7, 3.6.1.8, 3.6.1.11, 3.6.1.12, 3.6.1.13, 3.6.1.14 and 3.6.1.15 respectively.

3.8.4.2. Microbial enumeration and insect infestation of soup mixes

The total microbial count in soup mixes were enumerated by serial dilution and plate count method as described in 3.6.4.2. The insect infestation of the soup mixes were observed as described in 3.7.5.

3.8.4.3. Acceptability studies and organoleptic evaluation of soups prepared with the soup mixes

The quantity of water required to make a soup of acceptable consistency with a known quantity of the soup mix was standardised by repeated trials. The soup mix was cooked in water and the time taken was noted. The sensory evaluation of the soups mixes were carried out in the morning using score cards based on a nine point hedonic scale (Appendix IV) by the panel of 10 selected judges. The quality attributes namely appearance, colour, flavour, consistency, taste, and overall acceptability were evaluated and acceptability of the products was studied.

3.8.4.4. Selection of soup mixes

From the 16 soup mixes kept for shelf life studies (4 treatments and for each treatment, 4 compositions), one soup mix composition was identified from each treatment (total 4 soup mixes) with good overall acceptability and storage stability with respect to microbial load and nutritional constituents after six months of storage.

3.9. COST OF PRODUCTION

Cost analysis of the selected fresh tempeh types, tempeh flours and the selected soup mix with each tempeh flour was done to assess the extent of expense incurred to prepare the soup mixes.

The total cost incurred was analysed taking into account the fixed and variable cost. The cost of gadgets and vessels were accounted in fixed cost. The variable cost or operational cost involved the amount incurred on food materials in the preparation of the product, packaging cost, labour charge, and fuel charges. Interest @ 12 per cent and depreciation @ 12 per cent on the fixed cost was also taken into account. From the total cost, the cost of production of unit pack of each product was calculated. The market prices of similar popularly available products were compared with the developed products and the benefit cost ratio was calculated based on this cost.

3.10. STATISTICAL ANALYSIS

The data was analysed by applying statistical techniques such as Kendall's coefficient of concordance, ANOVA and paired't' test.

4. RESULTS

The results pertaining to the study entitled "Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes" are presented in this chapter.

4.1. Standardisation of tempeh with legumes and cereals.

In the present study, tempeh fermentations were carried out with pure cultures of *Rhizopus oligosporus*. Twenty different combinations [T_1- 100% soybean, T_2- 100% green gram, T_3 – 100% cowpea, T_4 – green gram (75%) + soybean (25%), T_5 – green gram (50%) + soybean (50%), T_6 – cowpea (75%) + soybean (75%), T_7 – cowpea (50%) + soybean (50%), T_8 - green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%), T_{10} - cowpea (75%) + rice (25%), T_{11} - cowpea (50%) + rice (50%), T_{12} - green gram (75%) + wheat (25%), T_{13} - green gram (50%) + wheat (50%), T_{14} - cowpea (75%) + wheat (25%), T_{15} - cowpea (50%) + wheat (50%), T_{16} - green gram (50%) + cowpea (50%), T_{17} - soybean (75%) + rice (25%), T_{18} - soybean (50%) + rice (50%), T_{19} - soybean (75%) + wheat (25%), T_{20} - soybean (50%) + wheat (50%)] of tempeh were prepared with selected legumes and cereals. The 100 per cent soybean tempeh was the control (T_1). Soybean fermented with pure cultures of *Rhizopus oligosporus* - MTCC – 556 was used as tempeh starter.

The tempeh prepared with 100 per cent soybeans (T_1) was found to be a thick firm cake with the white cottony mycelium distributed uniformly. All the other treatments had less dense mycelium than the control. However, the combinations with soybeans had a more firm texture and more mycelial coverage. The entire contents could be lifted out as a whole piece. Binding of cotyledons with the fungal hyphae was found to be less in treatments having cowpea. The treatments with green gram had a comparable firmness with the control (T_1). Among the cereals, the combinations with rice had a more firm texture than that of wheat. All the treatments had a very nutty and acceptable flavour (Plates 28 - 47).

Tempeh types with cut surface

Plate 28. T_1 (100% soybean)

Plate 29. T_2 (100% green gram)

Plate 30. T_3 (100% cowpea)

Plate 31. T_4 (green gram 75% + soybean 25%)

Tempeh types with cut surface


Plate 32. T_5 (green gram 50% + soybean 50%)


Plate 33. T_6 (cowpea 75% + soybean 25%)


Plate 34. T_7 (cowpea 50% + soybean 50%)


Plate 35. T_8 (green gram 75% + rice 25%)

Tempeh types with cut surface

Plate 36. T_9 (green gram 50% + rice 50%)

Plate 37. T_{10} (cowpea 75% + rice 25%)

Plate 38. T_{11} (cowpea 50% + rice 50%)

Plate 39. T_{12} (green gram 75% + wheat 25%

Tempeh types with cut surface

Plate 40. T_{13} (green gram 50% + wheat 50%)

Plate 41. T_{14} (cowpea 75% + wheat 25%)

Plate 42. T_{15} (cowpea 50% + wheat 50%)

Plate 43. T_{16} (green gram 50% + cowpea 50%)

Tempeh types with cut surface

Plate 44. T_{17} (soybean 75% + rice 25%)

Plate 45. T_{18} (soybean 50% + rice 50%)

Plate 46. T_{19} (soybean 75% + wheat 25%)

Plate 47. T_{20} (soybean 50% + wheat 50%)

4.1.1. Acceptability of primary products with fresh tempeh

From fresh tempeh types, tempeh chips and tempeh roast were prepared for evaluating the organoleptic qualities. All the twenty tempeh types with different combinations of pulses and cereals were evaluated for their acceptability.

4.1.1.1 Organoleptic qualities of tempeh chips

Tempeh chips were prepared with the twenty different combinations of fresh tempeh including the control (T_1) and each was evaluated for their organoleptic qualities such as appearance, colour, flavour, texture, taste and overall acceptability by a selected panel of ten judges using a nine point hedonic scale. Mean score obtained for the organoleptic qualities of tempeh chips are presented in Table 2.

Table 2. Mean score for the organoleptic qualities of tempeh chips

Treatments	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability
T_1	8.9 (17.22)	8.9 (16.83)	7.8 (10.28)	8.4 (15.17)	7.4 (7.33)	8.3 (15.39)
T_2	9.0 (18.11)	9.0 (17.72)	9.0 (18.44)	8.9 (17.83)	8.8 (17.22)	8.9 (19.94)
T_3	8.1(10.78)	7.8 (7.89)	8.7 (17.11)	7.3 (7.83)	8.5 (16.11)	8.1 (12.89)
T_4	7.5 (6.39)	8.0 (9.44)	8.7 (17.11)	9.0 (18.39)	8.4 (15.17)	8.4 (17.39)
T_5	7.5 (6.39)	7.8 (7.89)	7.4 (7.56)	8.2 (13.94)	7.6 (10.00)	7.6 (7.17)
T_6	8.7 (7.22)	7.6 (6.22)	7.4 (7.56)	7.5 (8.78)	7.2 (5.67)	7.5 (4.39)
T_7	8.6 (15.44)	8.7 (15.06)	7.6 (8.44)	8.8 (17.33)	8.0 (12.44)	8.5 (17.22)
T_8	8.5 (15.06)	8.6 (14.17)	8.4 (14.83)	8.0 (12.39)	8.0 (12.44)	8.3 (15.39)
T_9	7.5 (6.39)	7.8 (7.89)	7.8 (10.28)	8.4 (14.39)	8.4 (15.17)	8.1(12.78)
T_{10}	7.7 (8.17)	7.7 (7.83)	7.7 (9.56)	7.1 (6.56)	8.2 (13.06)	7.7 (9.06)
T_{11}	7.5 (6.39)	7.2 (3.83)	8.3 (14.17)	7.3 (7.83)	8.7 (16.67)	7.8 (9.50)
T_{12}	7.5 (6.39)	7.8 (7.89)	7.9 (10.89)	7.7 (10.44)	7.5 (8.11)	7.7 (9.06)
T_{13}	7.3 (4.67)	8 (9.44)	7.5 (8.56)	6.2 (2.94)	7.5 (8.11)	7.4 (3.61)
T_{14}	7.4 (5.61)	8.4 (13.06)	8.5 (15.78)	7.5 (8.78)	7.7 (10.94)	7.9 (11.56)
T_{15}	7.7 (8.17)	7.9 (8.56)	7.6 (8.33)	7.3 (7.83)	7.5 (8.11)	7.6 (7.17)
T_{16}	7.5 (6.39)	7.2 (3.83)	6.7 (3.06)	6.4 (3.61)	6.4 (2.22)	6.9 (1.44)
T_{17}	8.2 (12.28)	8.1 (10.44)	6.7 (3.06)	6.8 (4.72)	6.7 (3.33)	7.3 (3.89)
T_{18}	7.8 (8.89)	8.1(10.44)	7.1 (6.00)	7.9 (11.72)	7.9 (11.33)	7.9 (11.56)
T_{19}	9.0 (18.11)	8.5 (14.22)	7.6 (8.56)	8.0 (12.61)	7.6 (10.00)	8.2 (14.94)
T_{20}	7.9 (9.83)	8.6 (14.17)	7.9 (10.89)	7.1 (6.56)	7.3 (7.28)	7.7 (9.06)
Kendall's (W) value	0.627**	0.570**	0.655**	0.696**	0.620**	0.765**

Figures in parenthesis indicate mean rank scores
** - Significant at 1% level

As revealed in Table 2, the treatments T_2 and T_{19} had the highest mean score (9.0) for appearance and the lowest mean score was for T_{13} (7.3). Colour of the tempeh chips was best with a maximum score for T_2 (9.00) and the lowest score was for T_{16} (7.2). Regarding the flavour of the tempeh chips, the highest mean score was for T_2 (9.00) and the lowest score for the treatments T_{16} and T_{17} (6.7). The treatment T_4 had the highest mean score of 9.00 for texture and the lowest score was for T_{13} (6.2). Taste of the tempeh chips was found to be the best for the treatment T_2 (8.8) and lowest score was for T_{16} (6.4). Overall acceptability of the tempeh chips was highest for the treatment T_2 with a mean score of 8.9 and the lowest overall acceptability score was for T_{16} (6.9).

Statistical analysis by applying Kendall's (W) test showed that Kendall's (W) value was highly significant with regards to all the quality attributes. The treatment T_2 had the highest mean rank score for all the organoleptic parameters except for texture. Mean rank score for overall acceptability of the tempeh chips was also highest for the treatment $T_{2.}$ The treatment T_{16} had the lowest mean rank score for all the organoleptic parameters except in appearance and also had the lowest rank score for overall acceptability.

4.1.1.2. Organoleptic qualities of tempeh roast

Tempeh roasts were prepared with the twenty treatments of fresh tempeh including the control. Sensory evaluation of tempeh roast were carried out using score cards based on a nine point hedonic scale by the panel of ten judges. The quality attributes of tempeh roast such as appearance, colour, flavour, texture, taste and overall acceptability were evaluated and is presented in Table 3.

Table 3. Mean score for the organoleptic qualities of tempeh roast

Treatments	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability
T_1	9.0 (14.5)	9.0 (14.72)	7.1 (4.67)	8.6 (12.83)	7.5 (7.28)	8.3 (10.44)
T_2	8.8 (13.44)	8.8 (13.61)	8.7 (15.89)	8.7 (14.56)	8.3 (12.33)	8.7 (16.33)
T_3	8.5 (9.11)	8.4 (9.28)	8.1 (11.06)	7.3 (3.44)	8.8 (16.22)	8.2 (10.28)
T_4	8.5 (9.11)	8.4 (9.28)	7.4 (6.17)	7.7 (6.28)	7.1 (4.61)	7.9 (4.61)
T_5	8.3 (8.00)	8.6 (10.33)	7.7 (8.22)	9.0 (16.39)	7.6 (7.83)	8.3 (10.44)
T_6	7.6 (3.11)	7.5 (3.22)	8.1 (10.94)	7.4 (4.33)	7.5 (7.28)	7.5 (2.50)
T_7	8.5 (10.17)	8.4 (9.28)	7.6 (7.33)	8.6 (13.00)	7.4 (7.00)	8.1 (7.44)
T_8	8.9 (13.44)	8.7 (12.56)	8.8 (16.00)	8.5 (12.78)	8.7 (15.39)	8.5 (13.11)
T_9	9.0 (14.45)	8.6 (11.50)	8.7 (15.89)	8.4 (11.83)	8.3 (12.33)	8.5 (13.39)
T_{10}	8.6 (11.28)	8.4 (9.28)	8.4 (13.78)	8.8 (15.39)	8.7 (16.11)	8.6 (16.00)
T_{11}	8.6 (11.28)	8.8 (12.56)	8.8 (16.00)	8.7 (14.56)	8.9 (16.94)	8.9 (17.94)
T_{12}	8.5 (10.17)	8.6 (11.50)	8.1 (11.06)	8.5 (12.06)	8.7 (16.11)	8.5 (13.44)
T_{13}	8.6 (11.28)	8.5 (9.33)	8.1 (11.06)	8.7 (14.56)	8.3 (12.33)	8.6 (14.67)
T_{14}	8.6 (11.28)	8.3 (7.17)	8.5 (14.44)	7.3 (3.44)	8.4 (13.17)	8.2 (10.11)
T_{15}	8.5 (10.17)	8.2 (6.11)	8.3 (12.78)	8.0 (8.94)	8.3 (12.33)	8.4 (11.78)
T_{16}	8.3 (8.00)	8.3 (7.17)	8.0 (10.22)	7.8 (7.44)	7.8 (10.00)	8.0 (8.06)
T_{17}	8.2 (5.94)	8.3 (7.17)	6.5 (3.56)	8.0 (7.83)	6.4 (3.72)	7.7 (4.11)
T_{18}	8.7 (11.28)	8.2 (6.11)	6.9 (4.33)	8.0 (8.67)	6.8 (4,56)	7.9 (5.89)
T_{19}	8.9 (13.44)	8.3 (7.17)	7.9 (9.50)	8.0 (8.17)	7.6 (7.89)	8.3 (9.78)
T_{20}	8.5 (10.17)	8.3 (7.17)	7.4 (6.17)	8.8 (16.22)	7.3 (6.17)	8.3 (10.22)
Kendall's (W) value	0.333**	0.401**	0.609**	0.553**	0.636**	0.529**

Figures in parenthesis indicate mean rank scores
** - Significant at 1% level

Organoleptic evaluation of the tempeh roast revealed that, the mean score for the overall acceptability were found to be high for all the treatments except for T_{17} and T_{18}. The highest mean score for appearance was for the treatments T_1 and T_9 (9.0) and the lowest score was for the treatment T_6 with a mean score of 7.6. The mean score for colour was highest in T_1 (9.0) and lowest in T_6 (7.5). The lowest score for flavour of the tempeh roasts was for the treatment T_{17} (6.5) and the highest in T_8 and T_{11} (8.8). The treatments T_3 and T_{14} had the lowest score of 7.3 for texture and the highest score was for T_5 (9.0). The mean score for taste varied from 6.4 to 8.9 with T_{17} and T_{11} having the lowest and highest scores respectively. The lowest overall acceptability score in tempeh roasts was for T_6 (7.5) and the highest score was for T_{11} (8.9). The Kendall's (W) test showed that the mean rank score for all the parameters were highly significant.

4.2. *In vitro* starch and protein digestibility of fresh tempeh

The *in vitro* starch digestibility (IVSD) and *in vitro* protein digestibility (IVPD) of twenty treatments of fresh tempeh types were evaluated and is presented in Table 4.

Table 4. *In vitro* starch and protein digestibility of fresh tempeh

Treatments	IVSD (%)	IVPD (%)
T_1	69.18^{j}	72.03^{gh}
T_2	80.32^{bc}	88.57^{ab}
T_3	72.43^{i}	77.33^{f}
T_4	80.40^{bc}	81.23^{cd}
T_5	80.79^{ab}	78.04^{ef}
T_6	79.22^{bcde}	73.95^{g}
T_7	78.32^{cdef}	72.31^{gh}
T_8	80.00^{bc}	88.98^{a}
T_9	82.83^{a}	86.72^{b}
T_{10}	76.23^{fgh}	80.34^{cd}
T_{11}	78.08^{cdef}	79.75^{de}
T_{12}	77.53^{defg}	81.32^{cd}
T_{13}	78.22^{cdef}	82.08^{c}
T_{14}	75.06^{h}	76.12^{f}
T_{15}	75.67^{gh}	73.20^{gh}
T_{16}	77.18^{efgh}	76.82^{f}
T_{17}	77.62^{defg}	72.66^{gh}
T_{18}	76.31^{fgh}	72.61^{gh}
T_{19}	77.64^{defg}	71.94^{gh}
T_{20}	74.93^{h}	71.43^{h}

DMRT coloumn wise comparison
Values with same superscripts do not have significant difference

The IVSD of the fresh tempeh types were found to be high in all the treatments except for T_1 (control). IVSD ranged from 69.18 to 82.83 per cent with the lowest IVSD in T_1 (control) and the highest in T_9.

According to the DMRT, the fresh tempeh prepared with different combinations of pulses and cereals were categorized into ten subsets on the basis of IVSD. IVSD of all the treatments were significantly high when compared to the control T_1. T_9 (82.83) had the highest IVSD. The treatment T_5 was found to be on par with T_9.

As revealed in Table 4, the IVPD of different treatments ranged from 71.43 to 88.98 per cent. The highest IVPD was for T_8 (88.98 %) and the lowest for T_{20} (71.43%).

On the basis of DMRT, fresh tempeh prepared with different combinations were categorised into were catagorised into eight subsets with regards to their IVPD. Significant difference with regards to the IVPD was found among most of the treatments. The IVPD for the treatment T_2 (88.57 %) was on par with T_8 which had the highest IVPD (88.98 %). The IVPD of T_1, T_7, T_{15}, T_{17}, T_{18}, and T_{19} were significantly low, which were on par with the treatment T_{20} which had the lowest IVPD.

4.3. Selection of tempeh types

From the different treatments of tempeh, three fresh tempeh types which were most acceptable with a high overall acceptability score and with a high protein and starch digestibility, were selected along with the control (T_1), for further nutritional and shelf life studies with fresh tempeh and also for preparing tempeh flour and soup mixes. The three treatments were selected based on the acceptability scores of tempeh chips and tempeh roast by applying Kendall's coefficient of concordance (Table 2 and 3), and the percentage of IVSD and IVPD by DMRT (Table 4).

According to the acceptability scores obtained for the tempeh chips (Table 2), T_2 had the highest mean rank score for all the organoleptic parameters except for texture which nevertheless had a high mean rank score. The mean rank score for overall acceptability of the tempeh chips was also highest for the treatment T_2 (19.94) followed by T_4 (17.39). The treatments T_1, T_3, T_7, T_8, T_9 and T_{19} also had relatively high mean rank scores (> 12.0).

Regarding the product tempeh roast , as indicated in Table 3, the highest mean rank score for overall acceptability was for the treatment T_{11} (17.94) followed by the treatment T_2 (16.33). The treatments T_8, T_9, T_{10}, T_{12} and T_{13} also had high mean rank scores (> 13.0).

As regards the IVSD (Table 4), the highest IVSD was for the treatment T_9. The treatments T_2, T_4, and T_8 had a relatively higher IVSD, though not on par with the highest IVSD obtained for T_9.

The treatment with the highest IVPD was T_8 and T_2 was on par with T_8. The treatment T_9 had the next best IVPD which was found to be on par with the treatment T_2.

From the statistical analysis furnished in tables 2, 3 and 4, the three treatments selected considering the overall acceptability, IVSD and IVPD were T_2, T_8 and T_9. T_1 was also selected as control.

4.4. Nutritional and shelf life studies in selected fresh tempeh types

The selected three treatments T_2 (green gram 100%), T_8 (green gram 75% + rice 25%), T_9 (green gram 50% + rice 50%) along with control T_1 (soybean 100%), were used for further studies.

4.4.1 Chemical constituents in selected fresh tempeh types

4.4.1.1. Moisture, protein, fiber and total fat content in selected fresh tempeh types

The moisture, protein, fiber and total fat content of selected fresh tempeh types are presented in Table 5 and illustrated in Figure 1, 2, 3 and 4 respectively.

Table 5. Moisture, protein, fiber and total fat in selected fresh tempeh types (per 100g)

Treatments	Moisture (g)	Protein (g)	Fiber (g)	Total fat (g)
T_1	55.85^a	21.09^a	1.32^c	10.12^a
T_2	46.54^b	12.04^b	2.52^a	0.61^b
T_8	44.20^c	10.16^c	1.57^b	0.49^b
T_9	40.79^d	8.36^d	1.03^d	0.39^b

T_1 - 100% soybean, T_2 – 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Values with same super script do not have significant difference
Values are mean of three independent determinations

As revealed in Table 5, the moisture content of different treatments ranged from 40.79 to 55.85 g/100g (Fig.1). The moisture content was found to be highest in T_1 (control), and the lowest moisture content was in T_9. On the basis of DMRT, all the treatments differed significantly with respect to their moisture content.

The protein content of the fresh tempeh types varied between 8.36 and 21.09 g/100g with the lowest protein in T_9 and the highest in T_1 (Fig.2). Significant difference in protein content was observed among all the treatments. Statistically, the protein content in all the treatments was significantly low when compared to the control (T_1).

Maximum fiber content (Fig.3) was observed in treatment T_2 (2.52 g /100g) whereas the lowest fiber content was observed in T_9 (1.03 g/100g).There was a significant variation in the fiber content of different treatments.

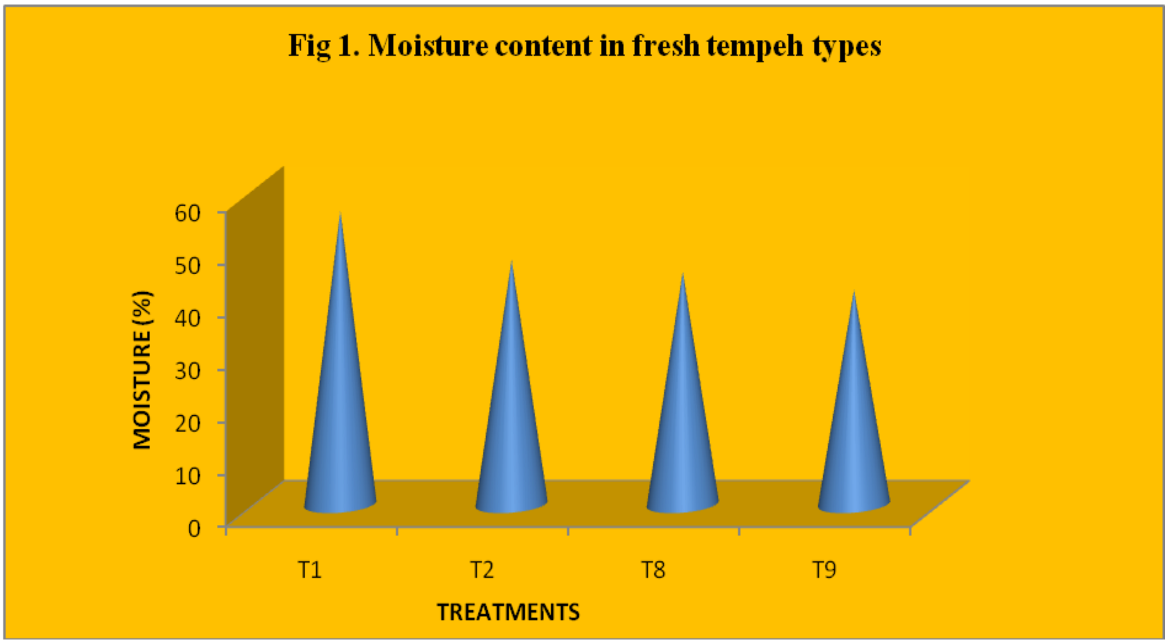


Fig 1. Moisture content in fresh tempeh types
60
50
40
30
20
10
0
MOISTURE (%)
T1
T2
T8
T9
TREATMENTS

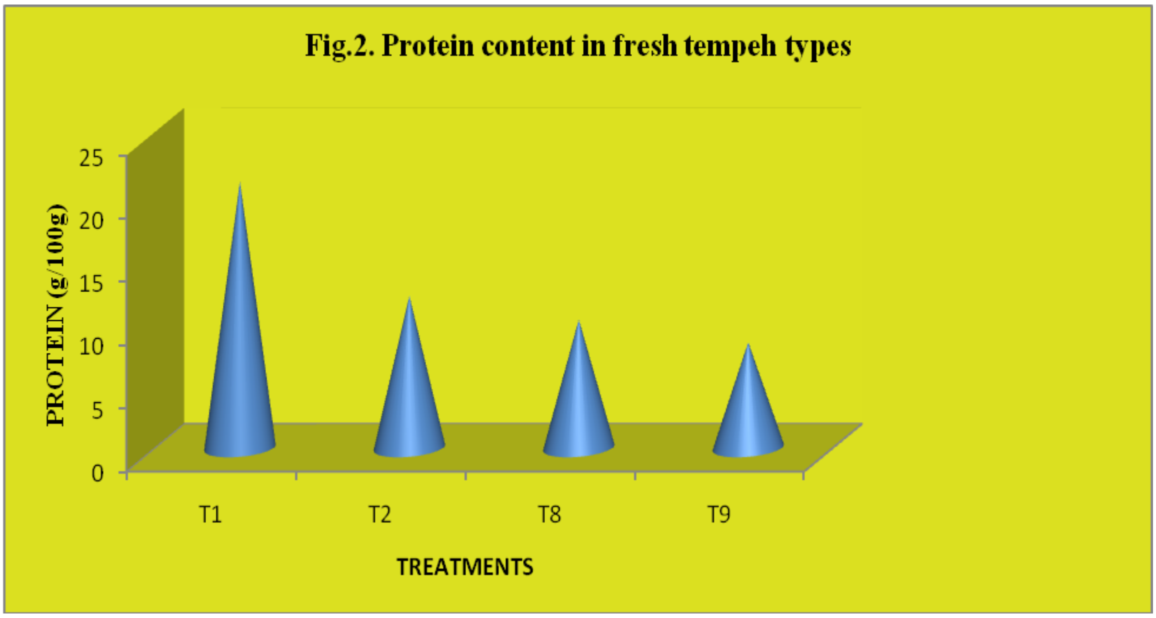


Fig.2. Protein content in fresh tempeh types
25
20
15
10
5
0
PROTEIN (g/100g)
T1
T2
T8
T9
TREATMENTS

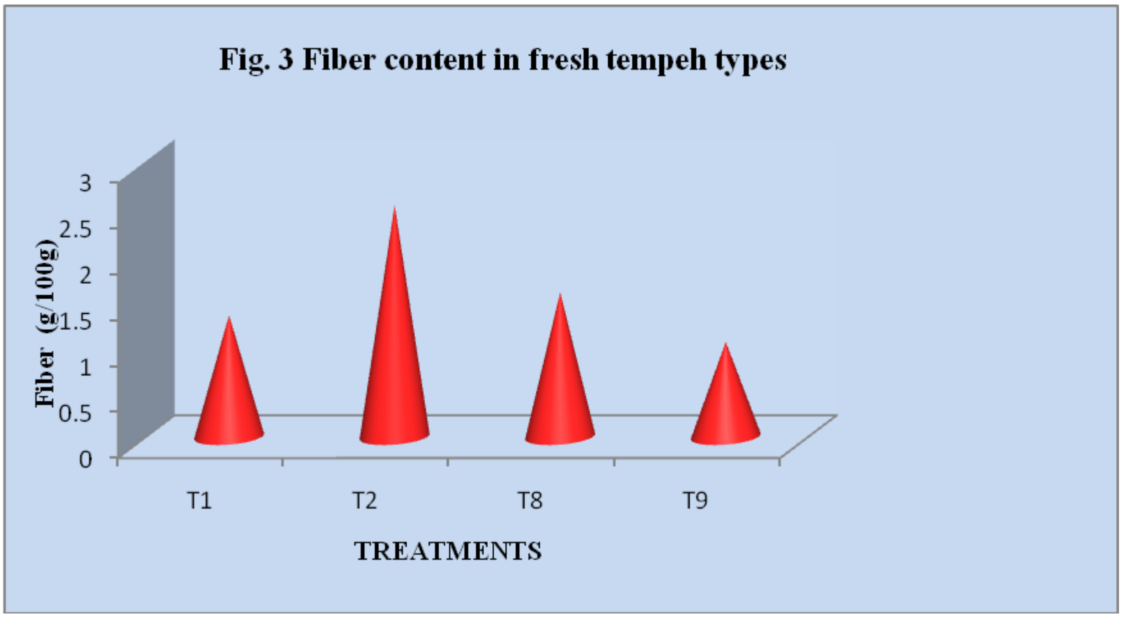


Fig. 3 Fiber content in fresh tempeh types
Fiber (g/100g)
3
2.5
2
1.5
1
0.5
0
T1
T2
T8
T9
TREATMENTS

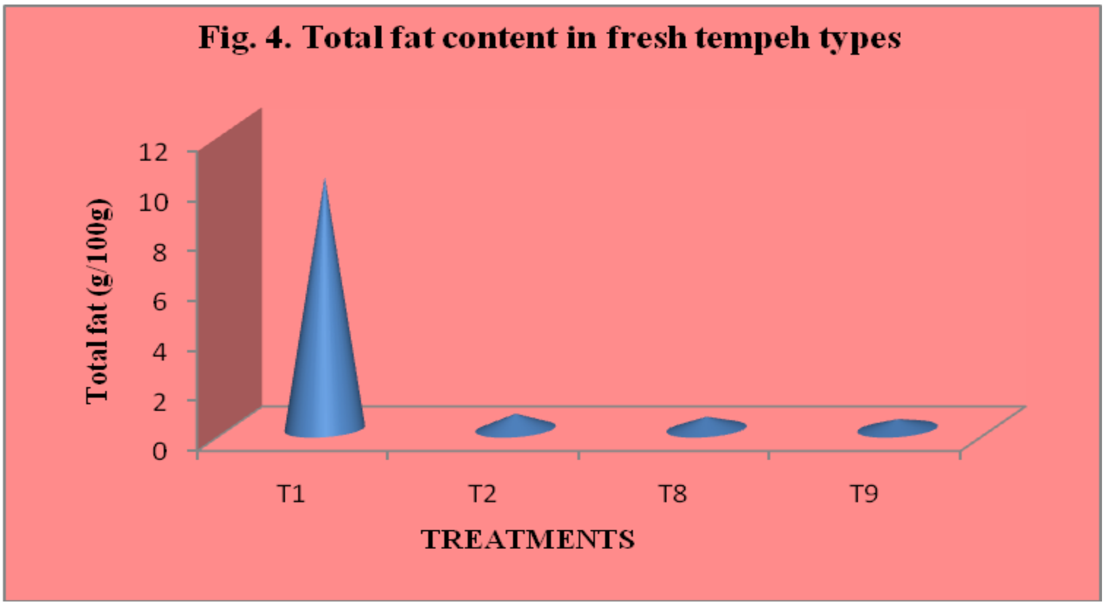


Fig. 4. Total fat content in fresh tempeh types
Total fat (g/100g)
12
10
8
6
4
2
0
T1
T2
T8
T9
TREATMENTS

The total fat content (Fig. 4) of the selected fresh tempeh types ranged from 0.39 to 10.12 g/100g with the lowest and highest total fat content in T_9 and T_1 respectively. There was no significant variation in the fat content of the treatments T_2, T_8 and T_9. Total fat content of the control (T_1) was significantly high when compared to all other treatments.

4.4.1.2. Starch, reducing sugar and total sugar in the selected fresh tempeh types

The starch, reducing sugar and total sugar in the selected fresh tempeh types are presented in Table 6 and illustrated in Figure 5, 6 and 7 respectively.

Table 6. Starch, reducing sugar and total sugar in selected fresh tempeh types (per 100g)

Treatments	Starch (g)	Reducing sugar (g)	Total sugar (g)
T_1	5.84^d	1.97^a	3.92^b
T_2	18.59^c	2.01^a	4.21^a
T_8	24.59^b	1.65^b	3.67^c
T_9	29.13^a	1.02^c	3.21^d

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Values with same super script do not have significant difference
Values are mean of three independent determinations

The starch content (Fig.5) of the selected fresh tempeh types varied from 5.84 to 29.13 g/100g with the lowest starch content in T_1 and the highest in T_9. Significant variation was observed in the starch content of all the treatments.

Reducing sugar (Fig.6) was maximum in T_2 (2.01 g/100g) and minimum in T_9 (1.02 g/100g). T_1 was on par with T_2. The treatments T_1 and T_2 were statistically superior in reducing sugar content than the other two treatments.

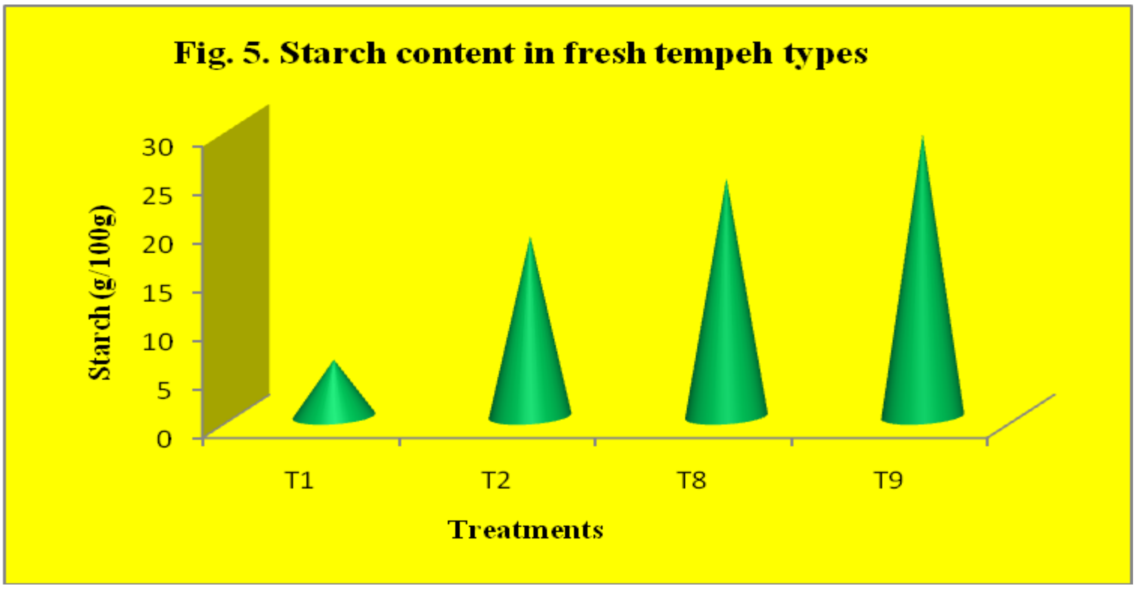


Fig. 5. Starch content in fresh tempeh types
Starch (g/100g)
30
25
20
15
10
5
0
T1
T2
T8
T9
Treatments

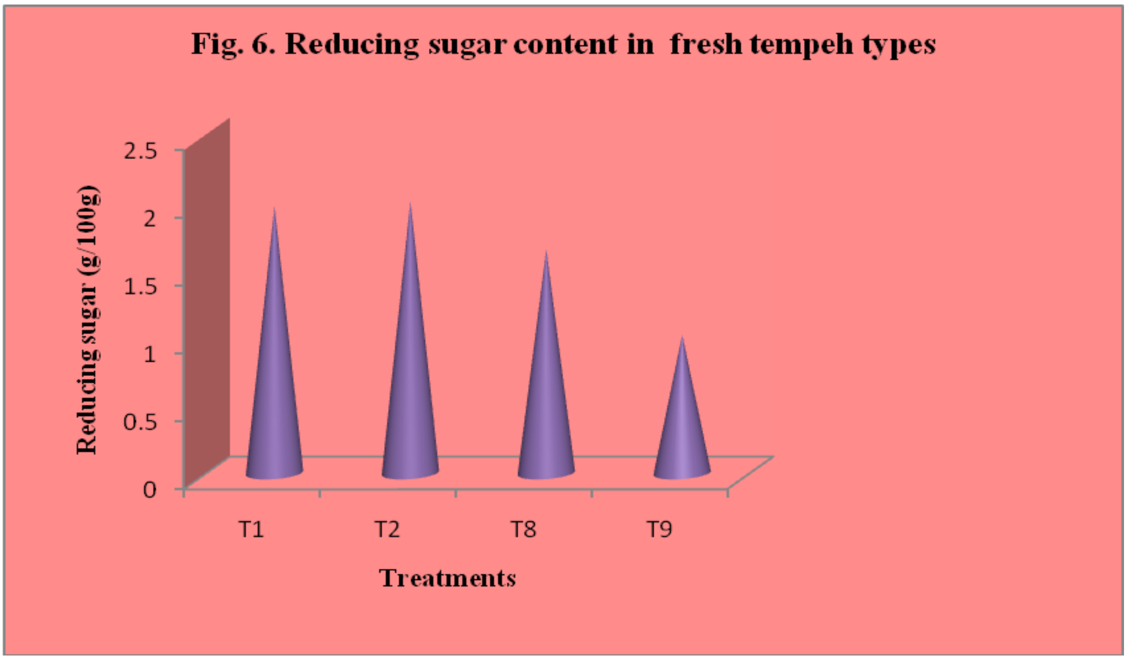


Fig. 6. Reducing sugar content in fresh tempeh types
Reducing sugar (g/100g)
2.5
2
1.5
1
0.5
0
T1
T2
T8
T9
Treatments

As revealed in the Table, the total sugar content (Fig.7) was found to be highest in the treatment T_2 (4.21 g/100g) and lowest in T_9 (3.21 g/100g). Statistically, significant differences in the total sugar content of different treatments were observed.

4.4.1.3. Thiamine, riboflavin, β carotene and vitamin C in selected fresh tempeh types

The thiamine, riboflavin, β carotene and vitamin C content in selected fresh tempeh types are presented in Table 7 and illustrated in Figure 8, 9 and 10 respectively.

Table 7. Thiamine, riboflavin, β carotene and vitamin C in selected fresh tempeh types (per 100g)

Treatments	Thiamine (mg)	Riboflavin (mg)	β carotene (μg)	Vitamin C (mg)
T_1	0.31^{a}	0.27^{ab}	331.85^{a}	NIL
T_2	0.21^{b}	0.30^{a}	63.01^{b}	NIL
T_8	0.12^{c}	0.25^{ab}	48.56^{b}	NIL
T_9	0.09^{c}	0.19^{b}	37.07^{b}	NIL

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Values with same super script do not have significant difference
Values are mean of three independent determinations

The thiamine content of the different treatments ranged between 0.09 to 0.31 mg/100g. The lowest thiamine content was observed in treatment T_9 and the highest in T_1. The control (T_1) had significantly higher thiamine content than all other treatments. There was no significant difference in the thiamine content of the treatments T_8 and T_9. Thiamine content in different treatments is illustrated in Figure 8.

Maximum riboflavin content (Fig. 9) was found in treatment T_2 (0.30 mg/100g) and the lowest in T_9 (0.19 mg/100g). DMRT showed a significant variation

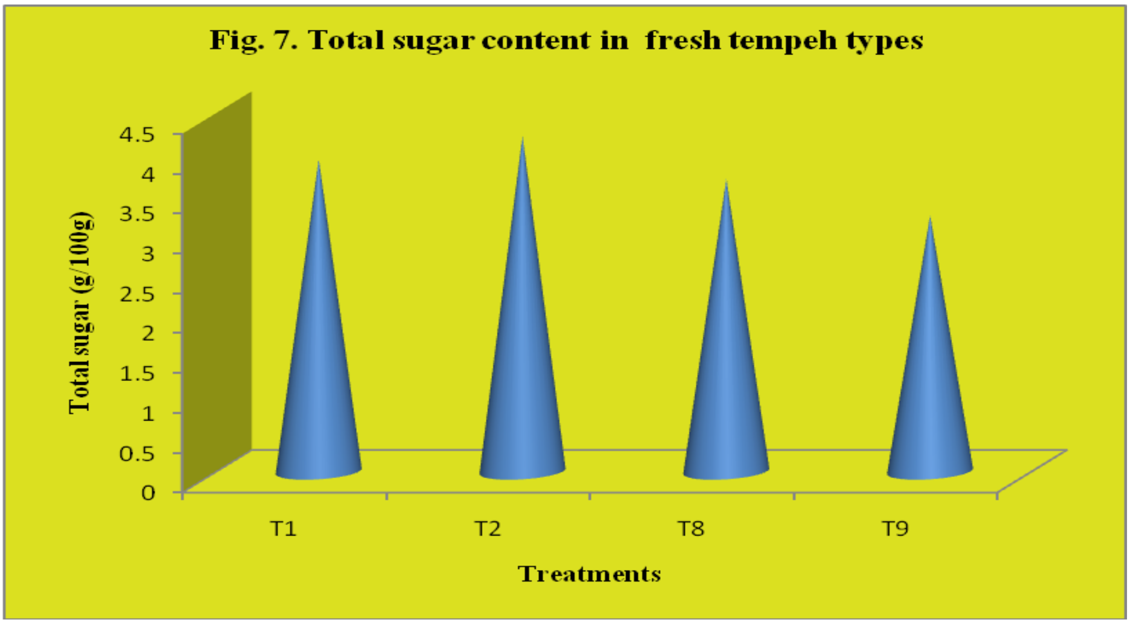


Fig. 7. Total sugar content in fresh tempeh types
Total sugar (g/100g)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
T1
T2
T8
T9
Treatments

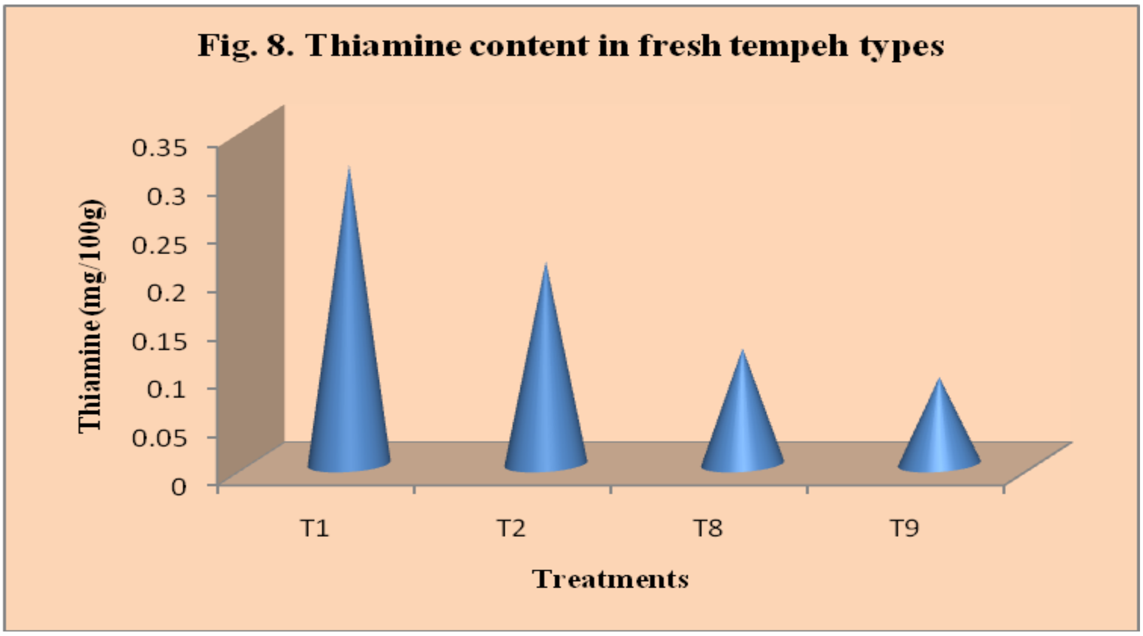


Fig. 8. Thiamine content in fresh tempeh types
Thiamine (mg/100g)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
T1
T2
T8
T9
Treatments

Fig. 9. Riboflavin content in fresh tempeh types

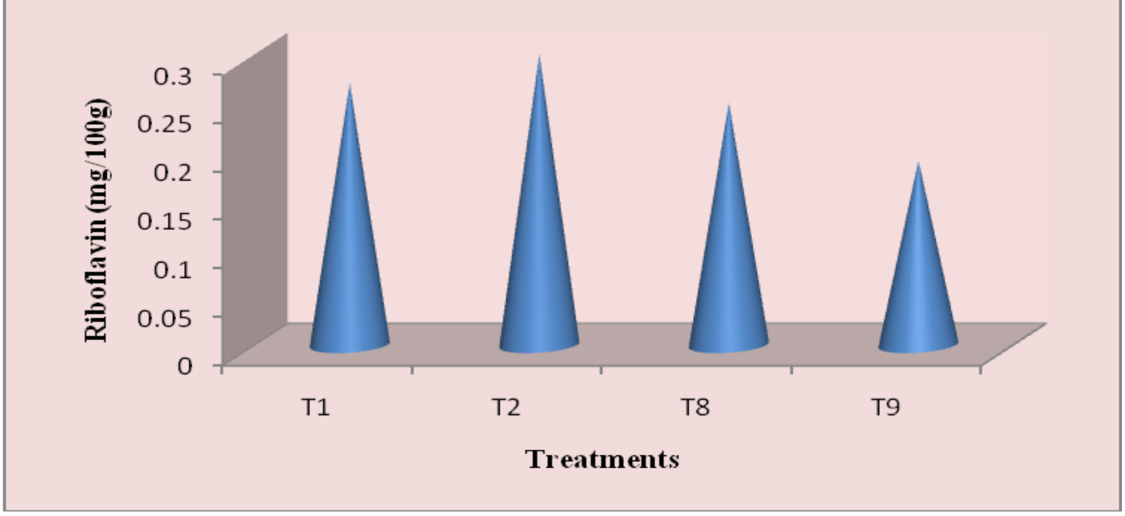
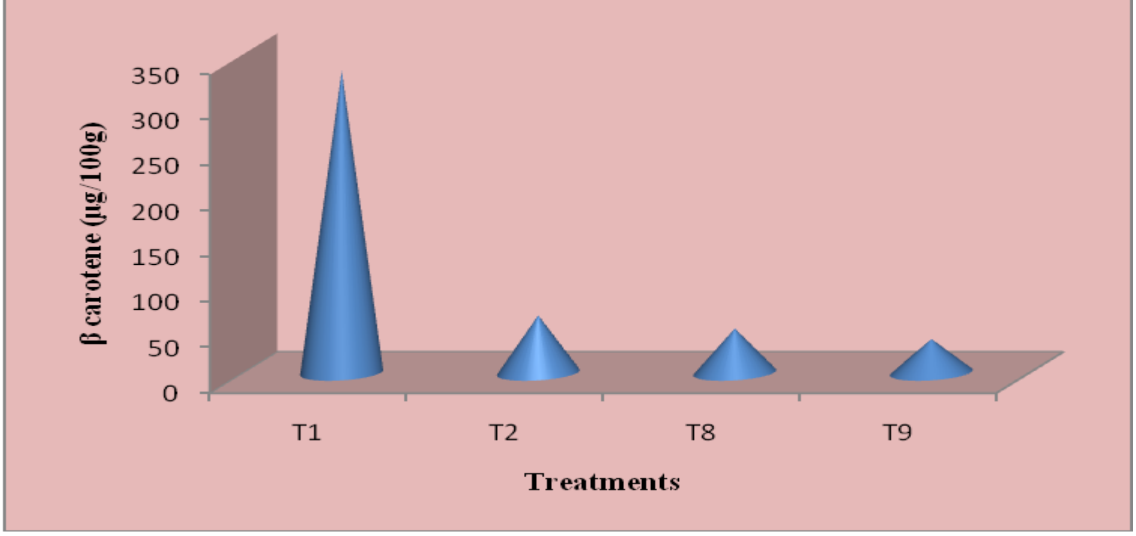


Fig. 10. β carotene content in fresh tempeh types



in the riboflavin content of these treatments. T_1 and T_8 were on par with T_2 in riboflavin content.

β carotene content (Fig.10) of the different treatments varied from 37.07 to 331.85 μg/100g with the lowest β carotene in treatment T_9 and the highest in T_1. β carotene was found to be significantly high in T_1 when compared to the other three treatments, which showed no significant difference among themselves in β carotene content.

None of the selected fresh tempeh types contained vitamin C.

4.4.1.4. Calcium, iron, phosphorus, potassium and zinc in selected fresh tempeh types

The calcium, iron, phosphorus, potassium and zinc in selected fresh tempeh types are presented in Table 8 and illustrated in Figure 11, 12, 13, 14 and 15 respectively.

Table 8. Calcium, iron, phosphorus, potassium and zinc in selected fresh tempeh types (per 100g)

Treatments	Calcium (mg)	Iron (mg)	Phosphorus (mg)	Potassium (mg)	Zinc (mg)
T_1	149.10^{a}	3.6^{a}	270.61^{a}	296.62^{d}	2.17^{a}
T_2	61.03^{b}	1.9^{b}	188.46^{b}	525.11^{a}	1.79^{b}
T_8	51.05^{bc}	1.75^{b}	185.25^{b}	419.99^{b}	1.60^{c}
T_9	40.37^{c}	1.53^{b}	158.52^{c}	330.12^{c}	1.34^{d}

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Values with same super script do not have significant difference
Values are mean of three independent determinations

As revealed in Table 8, the calcium content (Fig.11) of the selected fresh tempeh types ranged from 40.37 to 149.10 mg/100g with the lowest calcium content

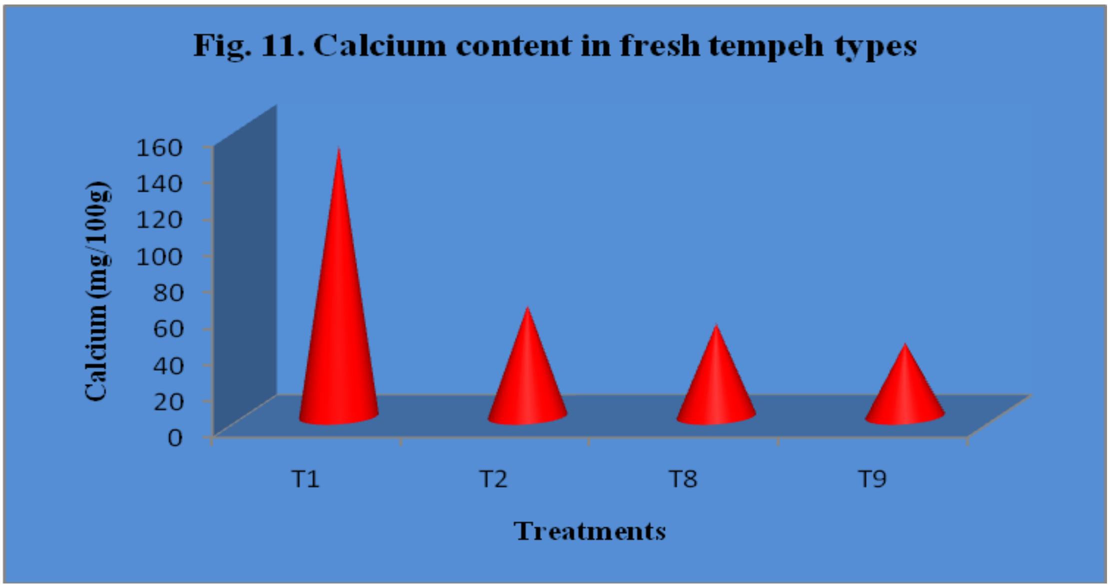


Fig. 11. Calcium content in fresh tempeh types

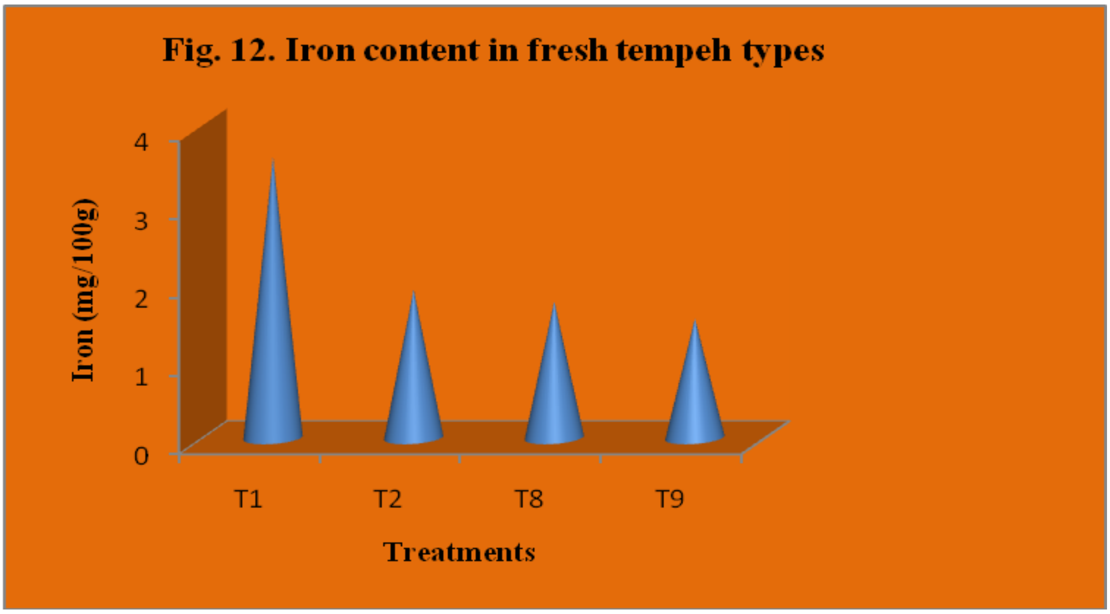


Fig. 12. Iron content in fresh tempeh types

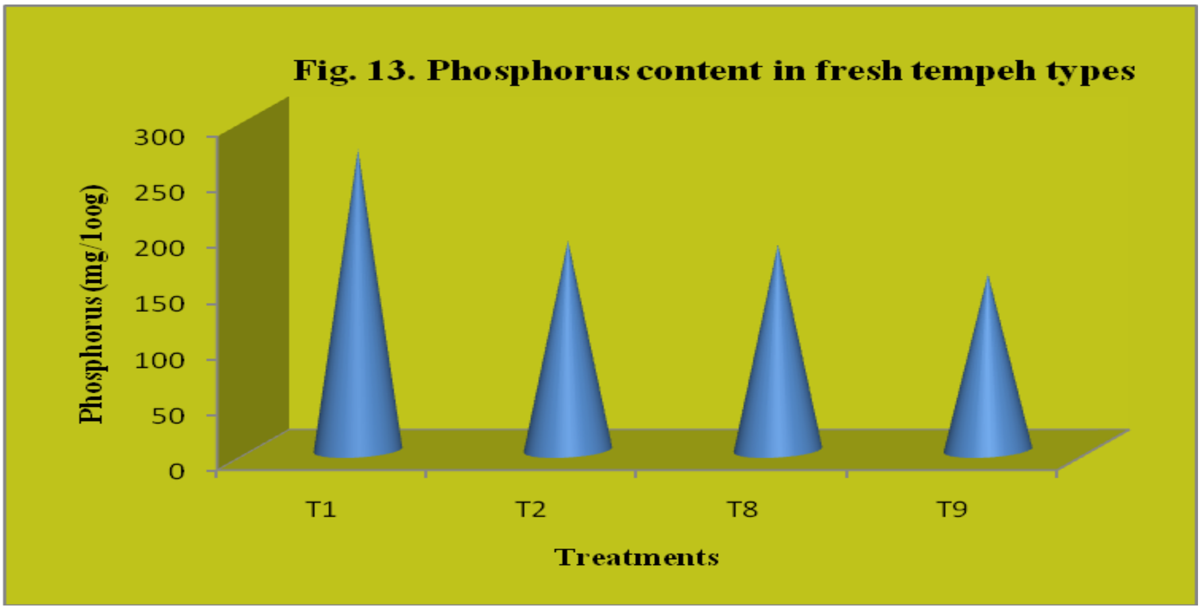


Fig. 13. Phosphorus content in fresh tempeh types

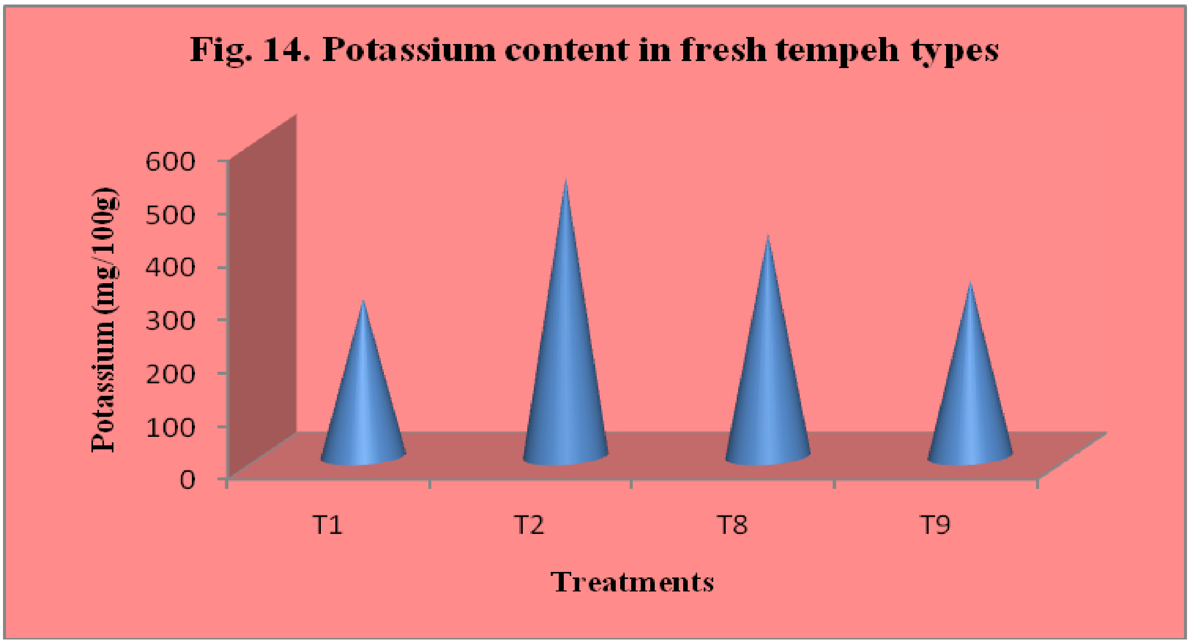
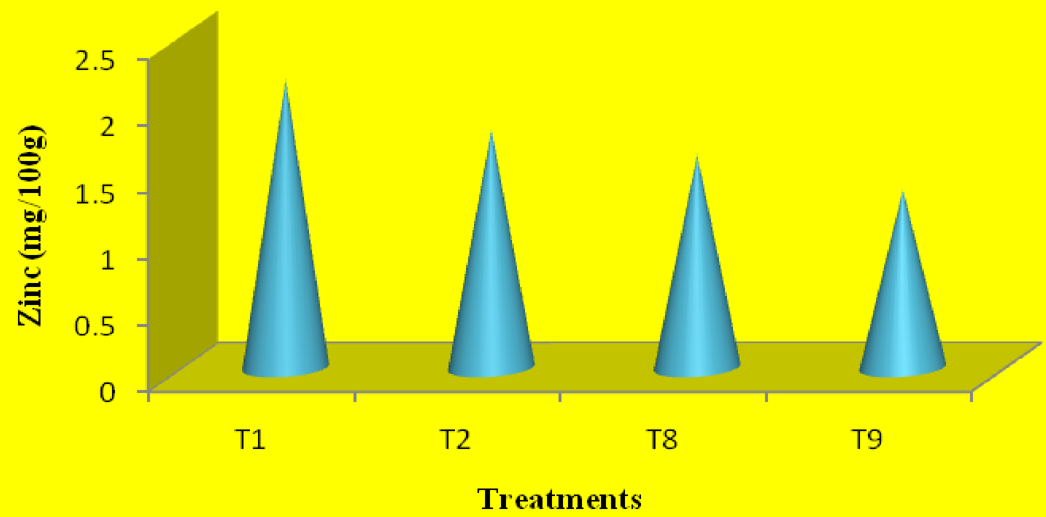


Fig. 14. Potassium content in fresh tempeh types

Fig. 15. Zinc content in fresh tempeh types



in treatment T_9 and highest in T_1. In all the treatments, calcium content was significantly low when compared to the control (T_1).

The iron content (Fig.12) was found to be maximum in T_1 (3.6 mg/100g) and minimum in T_9 (1.53 mg/100g). Iron content in T_1 was significantly high when compared to other treatments, which showed no significant variation among themselves.

The highest phosphorus content was also observed in treatment T_1 (270.61 mg/100g) and lowest in T_9 (158.52 mg/100g). Phosphorus content was significantly high in T_1 when compared to other treatments. There was no significant difference in the phosphorus content of the treatments T_2 (188.46 mg/100g) and T_8 (185.25 mg/100g). The phosphorus content in different treatments is illustrated in Figure 13.

The potassium content (Fig.14) of the selected fresh tempeh types varied from 296.62 to 525.11 mg/100g with the lowest potassium content in treatment T_1 and highest in $T_{2.}$ A significant variation in the potassium content was observed among all the treatments. Potassium content was significantly high in all the treatments when compared to control (T_1).

Among the different treatments, the lowest zinc content (Fig.15) was found in treatment T_9 (1.34mg/100g) and the highest in T_1 (2.17 mg/100g). Statistically there was a significant variation in the zinc content of different treatments.

4.4.2. *in vitro* availability of minerals from selected fresh tempeh types

The *in vitro* availability of minerals from different tempeh types are presented in Table 9 and illustrated in Figure 16.

Table 9. *In vitro* availability of calcium, iron, phosphorous, potassium and zinc from selected fresh tempeh types

Treatments	Calcium (%)	Iron (%)	Phosphorus (%)	Potassium (%)	Zinc (%)
T_1	49.00^c	64.52^a	49.68^b	46.30^d	82.23^a
T_2	56.63^b	66.07^a	61.49^a	54.15^c	86.17^a
T_8	60.70^a	64.74^a	62.15^a	60.36^b	87.17^a
T_9	61.77^a	66.82^a	65.72^a	63.60^a	88.99^a

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Values with same super script do not have significant difference
Values are mean of three independent determinations

As revealed in Table 9, the *in vitro* availability of calcium was found to be highest (61.77 %) from the treatment T_9 and lowest (49.00 %) from the control (T_1). The treatments T_8 and T_9 showed no significant difference between them and were statistically superior when compared to the other two treatments. In all the treatments, availability of calcium was significantly high when compared to the control T_1.

The *in vitro* availability of iron from different treatments ranged from 64.52 to 66.82 per cent with the lowest iron availability from the treatment T_1 and highest from T_9. There was no significant difference among the treatments with regards to their *in vitro* iron availability.

Availability of phosphorus from the selected fresh tempeh types was found to be maximum in treatment T_9 (65.72%) and minimum in T_1 (49.68%). Statistically there was no significant variation in the phosphorus availability of the treatments T_2, T_8 and T_9. In T_1, phosphorus availability was significantly low when compared to the other treatments.

The *in vitro* availability of potassium was highest in treatment T_9 (63.60%) and lowest in T_1 (46.30%). Significant variation was observed among all the treatments with regards to their *in vitro* availability of potassium.

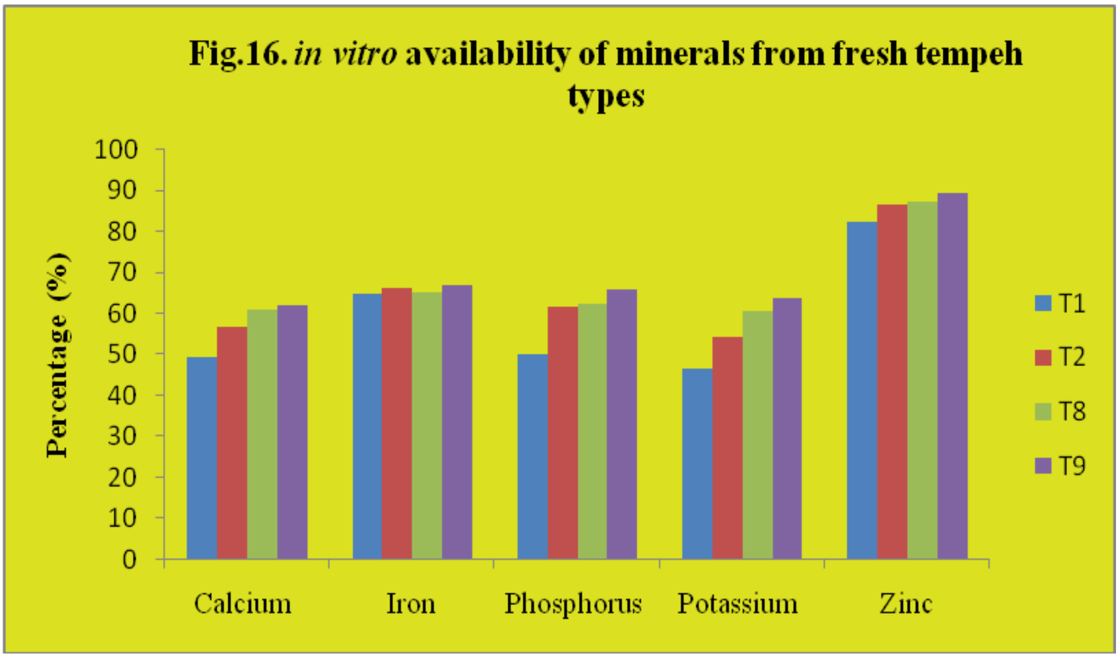


Fig.16. in vitro availability of minerals from fresh tempeh types
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
Calcium
Iron
Phosphorus
Potassium
Zinc
T1
T2
T8
T9

In vitro availability of zinc was found to be high in all the treatments and ranged from 82.23 to 88.99 per cent. The lowest *in vitro* availability of zinc was from the treatment T_1 and highest from T_9. There was no significant variation among the different treatments with regards to their *in vitro* zinc availability.

4.4.3. Probiotic activity of the selected fresh tempeh types

Probiotic characteristics such as *in vitro* acid and bile acid tolerance of the micro organisms in tempeh and antimicrobial activities of tempeh against enteropathogenic bacteria were studied in this experiment.

4.4.3.1. Acid tolerance of the microorganisms found in tempeh

The acid tolerance of the bacteria present in fresh tempeh types were tested by *in vitro* method and the results are furnished in Table 10.

Table 10. Viability of bacteria in fresh tempeh types at different pH levels ($\times 10^8$ cfu/g)

pH	T_1	T_2	T_8	T_9
1.5	NG	NG	NG	NG
2.0	NG	NG	NG	NG
2.5	NG	NG	NG	NG
3.0	NG	NG	NG	NG
3.5	NG	NG	NG	NG
4.0	3.3	2.3	3.6	2.0
4.5	10.6	4.6	12.3	5.0
5.0	26.3	44.6	15.3	45.3
5.5	59.6	52.3	61.6	52.0
6.0	62.0	62.6	62.6	45.3
6.5	47.0	44.3	47.0	35.3
7.0	49.3	33.0	21.6	17.6

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
The values are mean of three independent enumerations
NG- No growth

In the present study, viability of bacteria was observed only between pH 4.0 and 7.0 and the bacterial count varied with different pH. No viability was observed between pH 1.5 to 3.5 in all the treatments. The maximum viability was observed in pH 6.0 in treatments T_1, T_2 and T_8 (62, 62.6, and 62.6 x 10^8 cfu/g respectively). In treatment T_9, the highest bacterial count was observed in pH 5.5 (52 x 10^8cfu/g).

Table 11. Viability of yeast in fresh tempeh types at different pH levels (x 10^5 cfu/g)

pH	T_1	T_2	T_8	T_9
1.5	NG	NG	NG	NG
2.0	NG	NG	NG	NG
2.5	NG	NG	NG	NG
3.0	0.6	4.3	2.6	4.0
3.5	5.6	32.6	21.3	31.3
4.0	30.0	46.3	42.0	82.0
4.5	32.3	39.0	62.6	75.0
5.0	17.6	27.3	40.3	70.6
5.5	7.0	16.3	24.3	55.3
6.0	1.0	3.3	7.6	16.6
6.5	NG	NG	NG	NG
7.0	NG	NG	NG	NG

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
The values are mean of three independent enumerations
NG- No growth

As revealed in Table 11, viability of yeast was observed between pH 3.0 to 6.0. The yeast count was maximum in pH 4.5 in both T_1 (32.3 x10^5 cfu/g) and T_8 (62.6 x10^5 cfu/g). In treatment T_2 and T_9, the highest yeast count was observed in pH 4.0 (46.3 and 82.0 x10^5 cfu/g respectively). No viability was observed between pH 1.5 to 2.5 and also between 6.5 to 7.0.

Pure culture of *Rhizopus oligosporus* – MTCC 556, used for the preparation of tempeh was tested *in vitro* for their acid tolerance by assessing their biomass at different pH levels and the results are presented in Table 12.

Table 12. Biomass of *Rhizopus oligosporus* – MTCC 556 at different pH levels

pH	Dry weight (g)
1.5	NG
2.0	NG
2.5	NG
3.0	0.04
3.5	0.06
4.0	0.09
4.5	0.16
5.0	0.09
5.5	0.09
6.0	0.08
6.5	0.02
7.0	0.01

As revealed in Table 12, the biomass of *Rhizopus oligosporus* varied with different pH levels. No fungal growth was observed between the pH 1.5 to 2.5. With an increase in pH, the biomass of *Rhizopus oligosporus* reached a maximum of 0.16 g at pH 4.5 and then gradually decreased to a minimum of 0.01g at pH 7.0.

4.4.3.2. Bile acid tolerance of the organisms found in fresh tempeh types

Bile acid tolerance of the organisms in fresh tempeh types (bacteria, yeast and *Rhizopus oligosporus*) were assessed at four different levels of bile salt concentration (1- 4 per cent) and none of the organisms showed tolerance for bile acid in any of the concentration levels.

4.4.3.3. Antimicrobial activities of fresh tempeh types against enteropathogenic bacteria

When the antimicrobial activity of fresh tempeh types against enteropathogenic bacteria was tested *in vitro*, the fresh tempeh as such did not show antibacterial activity (Plates 48 - 51) against any of the enteropathogens studied (*Escherichia coli*, *Salmonella enteritidis*, *Bacillus cereus* and *Staphylococcus aureus*).

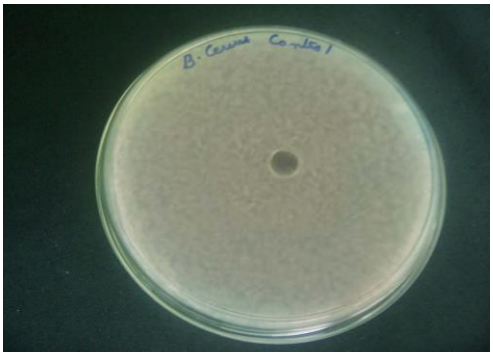
4.4.4. Shelf life studies of selected fresh tempeh types

The selected three fresh tempeh along with the control (T_1) after initial observations were packed in polythene bags of 250 gauge thickness and were kept under refrigerated condition (3 to 7^0C) for 12 days and in a deep freezer (-15 to -18^0C) for one month.

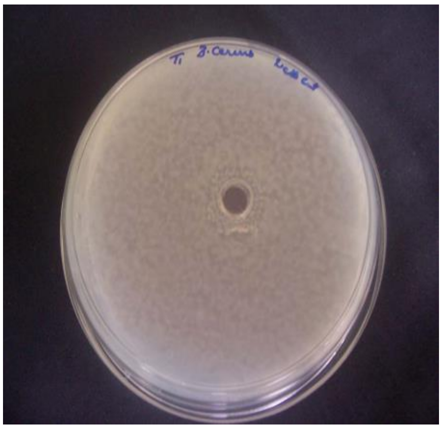
4.4.4.1. Sensory evaluation of tempeh in storage by observations.

Appearance, colour, flavour, and texture of tempeh types stored under refrigerated condition (Plates 52 - 71) and in deep freezer (Plates 72 - 83) were assessed periodically by observations and the observations are presented in Table 13 and 14 respectively.

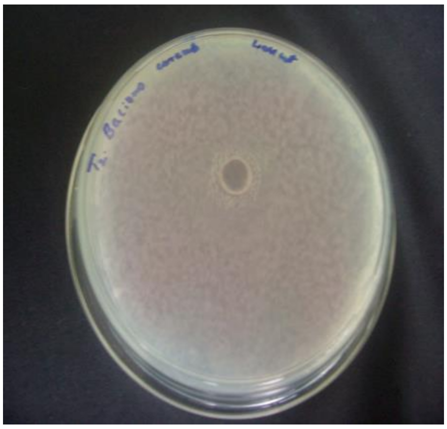
Plate 48. Antagonistic activity test against *Bacillus cereus*



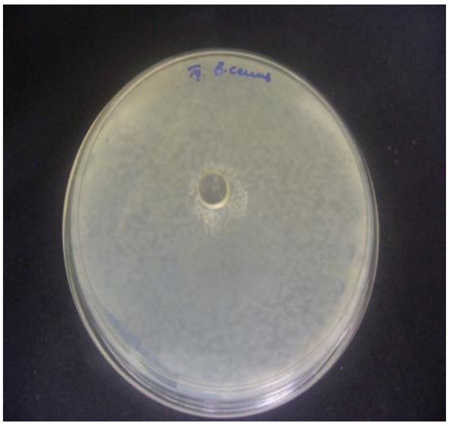
Control



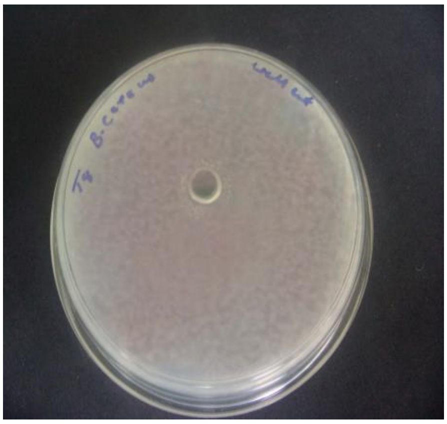
T_1 (100 % soybean)



T_2 (100% green gram)



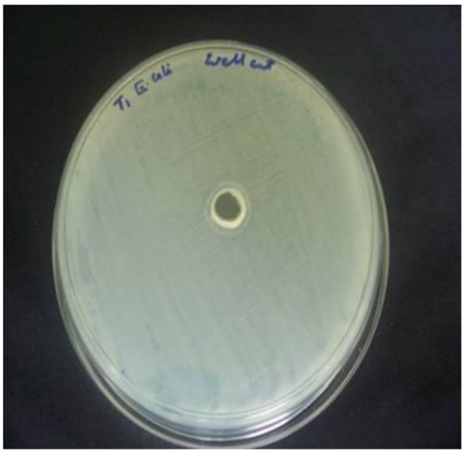
T_8 (green gram 75% + rice 25%)



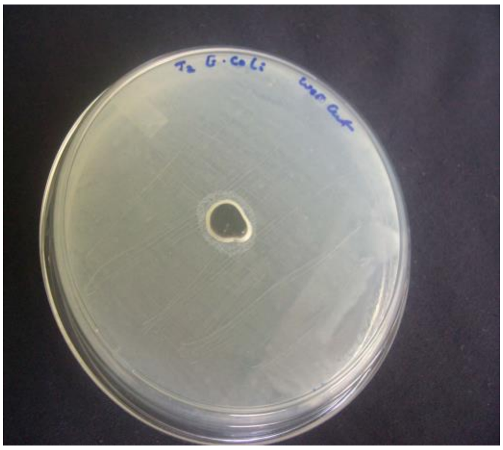
T_9 (green gram 50% + rice 50%)

Plate 49. Antagonistic activity test against *E. coli*

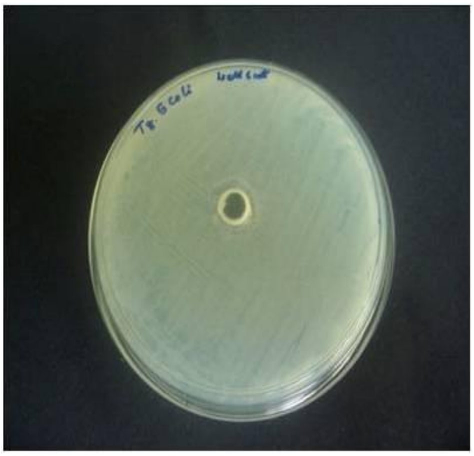
Control



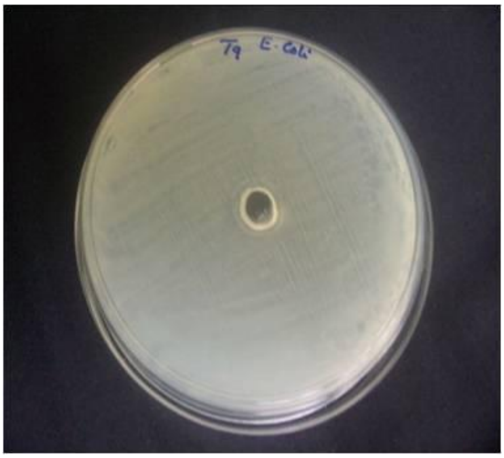
T_1 (100 % soybean)



T_2 (100% green gram)

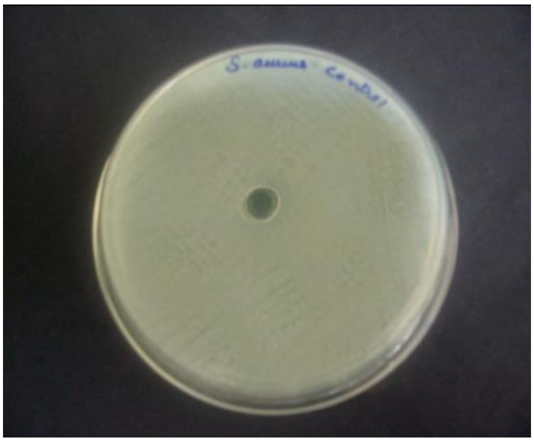


T_8 (green gram 75% + rice 25%)



T_9 (green gram 50% + rice 50%)

Plate 50. Antagonistic activity test against *Staphylococcus aureus*



Control

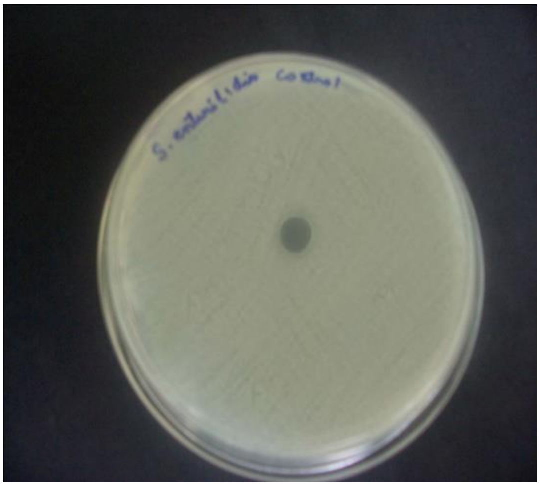
T_1 (100 % soybean)

T_2 (100% green gram)

T_8 (green gram 75% + rice 25%)

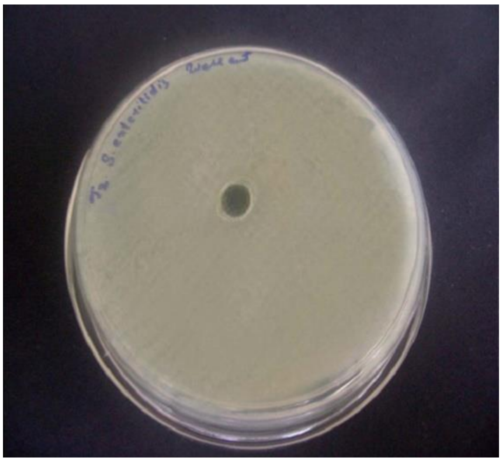
T_9 (green gram 50% + rice 50%)

Plate 51. Antagonistic activity test against *Salmonella enteritidis*



Control

T_1 (100% soybean)



T_2 (100% green gram)

T_8 (green gram 75% + rice 25%)

T_9 (green gram 50% + rice 50%)

Table. 13. Observations on tempeh types in refrigerated storage

Treatment	DAS	Appearance	Colour	Flavour	Texture
T_1	**0**	Cottony white cake covered with mycelium	White colour with small black coloured patches indicating sporulation	Pleasant nutty flavour	Compact and firm texture, without any loose beans
	3	Mycelium less dense - beans were visible	Creamish colour with black coloured patches	No further change	No change
	6	No further change	Cream yellow colour with black coloured patches	Slight change in nutty flavour	No further change
	9	No further change	No change	No further change	No further change
	12	No further change	Colour more darker	No further change	No further change
T_2	**0**	Tempeh covered with mycelium which was less dense than in T_1	Green and creamish colour with gray to black patches	Pleasant nutty smell	Firm texture but less firm than T_1 without any loose beans
	3	Mycelium less dense -beans were more visible	No further change	No further change	No further change
	6	No further change	No further change	No further change	Less firm and slight water retention
	9	No further change	No further change	Decrease in flavour but acceptable	No further change
	12	No further change	No further change	No further change	No further change

T_8	**0**	Appearance similar to T_2, but mycelium less dense than in T_1 and T_2. Green gram and rice were visible	Cream colour with gray to black patches	Pleasant smell	Less firm than T_1 and T_2
	3	Mycelium less dense	No further change	Slight reduction in flavour but acceptable	Less firm with slight water retention
	6	No further change	No further change	No further change	Less firm
	9	Glazed, slimy appearance	Light cream yellow colour	Distinct smell of ammonia and yeast	Slimy and soggy
	12	Glazed, slimy appearance	Light creamish yellow colour	Strong unpleasant smell	Highly slimy and soggy
T_9	**0**	Appearance similar to T_8. Less dense mycelium than in T_1 and T_2. Green gram and rice were visible	Cream colour with grey to black patches	Pleasant smell	Less firm than T_1 and T_2, but comparable to T_8
	3	Mycelium less dense	No further change	Slight reduction in the flavour, but acceptable	Less firm with slight water retention
	6	No further change	Light cream colour	Slight smell of ammonia	Less firm
	9	Slimy appearance	Light cream yellow colour	Unpleasant smell	Slimy and soggy
	12	Highly slimy	No further change	Strong unpleasant smell	Highly slimy and soggy

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage

As revealed in the Table 13, in treatment $T_{1,}$ the initial appearance of the tempeh was a solid cake covered with white cottony mycelium with small black patches and could be lifted out of the cover as a whole. It had a pleasant nutty flavour

Refrigerated storage of 100% soybean tempeh (T_1)

Plate 52. Fresh tempeh

Plate 53. 3rd day of refrigeration

Plate 54. 6th day of refrigeration

Plate 55. 9th day of refrigeration

Plate 56. 12th day of refrigeration

Refrigerated storage of 100% green gram tempeh (T_2)

Plate 57. Fresh tempeh

Plate 58. 3rd day of refrigeration

Plate 59. 6th day of refrigeration

Plate 60. 9th day of refrigeration

Plate 61. 12th day of refrigeration

Refrigerated storage of green gram 75% + rice 25% tempeh (T_8)

Plate 62. Fresh tempeh

Plate 63. 3rd day of refrigeration

Plate. 64. 6th day of refrigeration

Plate 65. 9th day of refrigeration

Plate 66. 12th day of refrigeration

Refrigerated storage of green gram 50% + rice 50% tempeh (T_9)

Plate 67. Fresh tempeh

Plate 68. 3rd day of refrigeration

Plate 69. 6th day of refrigeration

Plate 70. 9th day of refrigeration

Plate 71. 12th day of refrigeration

with firm texture. On the 3rd day of storage, the mycelium was less dense and the beans were visible which gave a creamish colour to the tempeh. There was no noticeable change in the flavour and texture. During the 12th day of storage, there was no change in the appearance, and texture but for the colour, it had become darker. A decrease in the flavour was observed on the 6th day of storage without much change up to the 12th day of storage.

Regarding the treatment T_2, the mycelia coverage was less dense than that of T_1 and the texture was also less firm than T_1. The tempeh had a mixture of green and creamish colour with grey to black patches at certain places. It also had a pleasant nutty flavour comparable to T_1. On the 3rd day, the mycelium appeared less dense and the beans were visible. Until the 12th day no further change in the colour and appearance was noticed. Slight moisture retention was noticed on the 6th day of storage and there was a decrease in flavour on the 9th day, nevertheless the tempeh was acceptable.

The appearance of the tempeh in treatment T_8 was similar to T_2, but mycelium was less dense and less visible than T_1 and T_2. Green gram and rice were visible and it had a pleasant smell and comparatively a less firm texture than T_1 and T_2. The texture was less firm with slight moisture retention from the 3rd day of storage and the mycelium and the flavour were also found to be less. On the 9th day of storage, the tempeh had a glazed slimy appearance and a distinct smell of ammonia and yeast, with a soggy texture. The tempeh was completely spoiled with an unpleasant smell on the 12th day of storage.

The initial appearance of the tempeh in treatment T_9 was similar to T_8 and had a pleasant smell. Its texture was less firm than T_1 and T_2, but comparable to T_8. The storage qualities were also similar to T_8 and was spoiled on the 9th day of storage.

Table. 14. Observations on frozen tempeh types

Treatment	DAS	Appearance	Colour	Flavour	Texture
T_1	**0**	Cottony white cake covered with mycelium	White colour with black coloured patches indicating sporulation	Pleasant nutty smell	Compact and firm, without any loose beans
	15	Slight decrease in mycelium	No further change	Pleasant nutty smell	Intact, without any loose beans
	30	No further change	No further change	Pleasant nutty smell	Intact without any loose beans
T_2	**0**	Tempeh covered with mycelium which is less dense than in T_1	Green and creamish colour with gray to black patches	Pleasant nutty smell	Intact but less firmer than T_1 without any loose beans
	15	Slight decrease in mycelium	No further change	Pleasant nutty smell	Intact
	30	No further change	No further change	Pleasant nutty smell	Intact
T_8	**0**	Appearance similar to T_2, but mycelium less dense. Green gram and rice were visible	Green and creamish colour with gray to black patches	Pleasant smell	Intact, but less firm than T_1 and T_2
	15	Slightly less mycelium	No further change	Pleasant smell	Intact
	30	No further change	No further change	Pleasant smell	Intact

T_9	**0**	Appearance similar to T_8. Less dense mycelium than T_1 and T_2. Green gram and rice were visible	Green and creamish colour with grey to black patches	Pleaseant smell	Less firm than T_1 and T_2, but comparable to T_8
	15	Slightly less mycelium	No further change	Pleasant smell	Intact
	30	No further change	No further change	Pleasant smell	Intact

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage

The storage of selected fresh tempeh types (T_1, T_2, T_8 and T_9) in a deep freezer gave a product comparable to the fresh tempeh even after the 30th day of storage.

4.4.4.2. Total micro flora in tempeh types during storage

The total microbial count in fresh tempeh types and also in stored samples was enumerated and the results are presented below.

4.4.4.2.1. Total bacterial count in tempeh types in refrigerated storage

Total bacterial count in fresh tempeh types and during storage under refrigerated condition is presented in Table 15 and depicted in Figure 17.

Table 15. Total bacterial count in fresh tempeh types and in refrigerated storage (x 10^8 cfu/g)

Treatments	DAS				
	0	**3**	**6**	**9**	**12**
T_1	69.3	52.6	40.6	40.6	42.0
T_2	52.6	46.0	35.0	42.0	39.0
T_8	56.0	47.6	41.6	57.3	65.3
T_9	49.3	42.0	39.6	54.3	70.6

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three independent determinations

Freezer storage of 100% soybean tempeh (T_1)

Plate 72. Fresh tempeh

Plate 73. 15th day of storage

Plate 74. 30th day of storage

Freezer storage of 100% green gram tempeh (T_2)

Plate 75. Fresh tempeh

Plate 76. 15th day of storage

Plate 77. 30th day of storage

Freezer storage of green gram 75% + rice 25% tempeh (T_8)

Plate 78. Fresh

Plate 79. 15th day of storage

Plate 80. 30th day of storage

Freezer storage of green gram 50% + rice 50% tempeh (T_9)

Plate 81. Fresh tempeh

Plate 82. 15th day of storage

Plate 83. 30th day of storage

As revealed in Table 15, in treatment T_1, the total bacterial count was found to be maximum (69.3 x 10^8 cfu/g) initially and the lowest bacterial count (40.6 x 10^8 cfu/g) was observed on the 6^{th} and 9^{th} day of storage. There was a gradual reduction in the bacterial count on storage up to the 9^{th} day and then there was an increase (42 x 10^8 cfu/g) on the 12^{th} day.

In treatment T_2, the initial bacterial count was found to be maximum (52.6 x 10^8 cfu/g) and the lowest bacterial count (35 x 10^8 cfu/g) was observed on the 6^{th} day of storage. There was a decrease in the total bacterial count up to the 6^{th} day of storage and on the 9^{th} day, there was an increase in the bacterial count (42.0 x 10^8 cfu/g). On the 12^{th} day again a reduction in the total bacterial count (39 x 10^8 cfu/g) was observed under refrigerated storage.

The total bacterial count in treatment T_8 decreased gradually with refrigerated storage, and from the 9^{th} day of storage there was an increase in the bacterial count. Maximum bacterial count was observed on the 12^{th} day of storage (65.3 x 10^8 cfu/g) and the minimum was observed on the 6^{th} day (41.6 x 10^8 cfu/g).

Gradual decrease in the total bacterial count was observed in treatment T_9 up to the 6^{th} day of storage with the lowest bacterial count of 39.6 x 10^8 cfu/g. From the 9^{th} day of storage, there was an increase in the bacterial count with a maximum count on the 12^{th} day (70.6 x 10^8 cfu/g).

4.4.4.2.2. Total yeast count in fresh tempeh types and in refrigerated storage

Total yeast count in fresh tempeh types and during storage under refrigerated condition is presented in Table 16 and illustrated in Figure 18.

Table 16. Total yeast count in fresh tempeh types and in refrigerated storage ($x\ 10^5$ cfu/g)

Treatments	DAS				
	0	3	6	9	12
T_1	55.3	31.6	37.0	37.0	39.6
T_2	42.6	33.0	28.6	28.0	36.0
T_8	65.0	59.3	58.0	126.3	192.0
T_9	66.3	57.6	53.0	137.3	168.6

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three independent determinations

In treatment T_1, the maximum yeast count (55.3×10^5 cfu/g) was observed initially and the lowest in the 3rd day of storage (31.6×10^5 cfu/g). During the 6th day, the yeast count showed an increase to 37×10^5 cfu/g. No difference in the yeast count was observed on the 9th day of storage, but during the 12th day again, this was increased to 39×10^5 cfu/g.

A gradual decrease in the total yeast count was observed in treatment T_2 up to the 9th day of storage with the lowest yeast count of 28×10^5 cfu/g Maximum yeast count was observed in fresh tempeh before storage (42.6×10^5 cfu/g).

In treatment T_8, there was a gradual decrease in the total yeast count up to the 6th day of storage with the lowest count of 58×10^5 cfu/g. An increase in the total yeast count was observed on the 9th and 12th day of storage with a maximum count of 192×10^5 cfu/g on the 12th day.

Variations in the total yeast count were observed in treatment T_9 during different storage periods. There was a gradual decrease in the total yeast count up to the 6th day of storage with the lowest count of 53×10^5 cfu/g on the 6th day. Thereafter, an increase in yeast count was observed with a maximum count of 168.6×10^5 cfu/g on the 12th day.

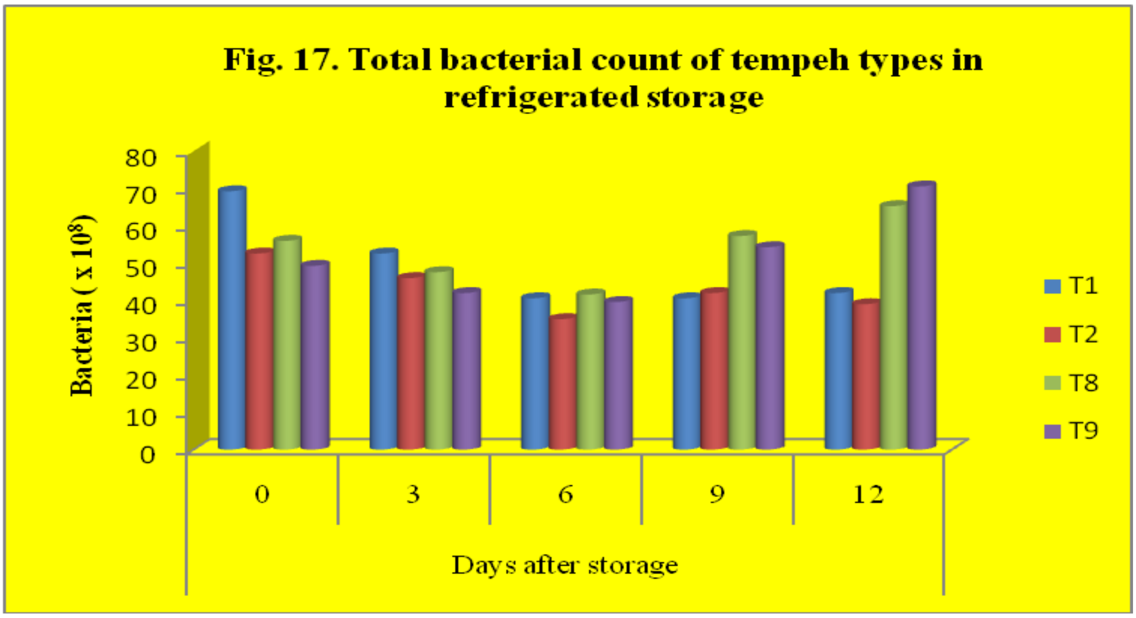


Fig. 17. Total bacterial count of tempeh types in refrigerated storage

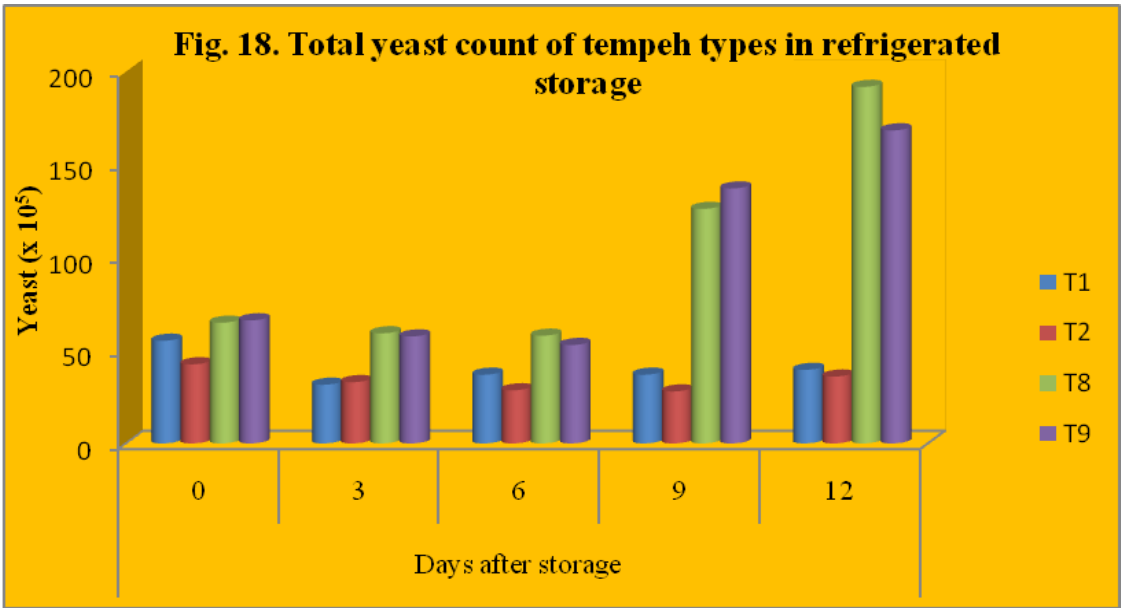


Fig. 18. Total yeast count of tempeh types in refrigerated storage

4.4.4.2.3. Total fungal count in fresh tempeh types and in refrigerated storage

Total fungal count in fresh tempeh types and during storage under refrigerated condition is presented in Table 17 and in Figure 19.

Table 17. Total fungal count in fresh tempeh types and in refrigerated storage ($x\ 10^6$ cfu/g)

Treatments	DAS				
	0	**3**	**6**	**9**	**12**
T_1	5.3	3	3	3	3
T_2	3.6	3	3.3	3	2.3
T_8	3.3	2.6	2.3	1.3	1.0
T_9	2.6	2.3	2.0	1.6	1.3

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three independent determinations

In treatment T_1, total fungal count was maximum (5.3×10^6 cfu/g) initially and during storage on the 3rd day, there was a decrease in fungal count (3.0×10^6 cfu/g) without any change till the 12th day of storage.

In the case of treatment T_2, maximum fungal count (3.6×10^6 cfu/g) was observed initially which decreased to a minimum of 2.3×10^6 cfu/g on the 12th day of storage.

A gradual decline in the total fungal count was observed in treatment T_8 during refrigerated storage. The total fungal count was maximum (3.3×10^6 cfu/g) initially and the lowest count was on the 12th day of storage (1.0×10^6 cfu/g).

Initially, the total fungal count in treatment T_9 was maximum (2.6×10^6 cfu/g) which decreased to the lowest count of 1.3×10^6 cfu/g on the 12th day of storage.

4.4.4.2.4. Total bacterial count in fresh and frozen tempeh

Total bacterial count in fresh tempeh types and during storage in the deep freezer is presented in Table 18 and is given in Figure 20.

Table.18. Total bacterial count in fresh and frozen tempeh (x 10^8 cfu/g)

Treatments	DAS		
	0	15	30
T_1	66.7	0.21	0.17
T_2	53.3	0.60	0.49
T_8	52.6	0.52	0.37
T_9	50.6	0.3	0.22

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three independent determinations

Total bacterial count was found to be high in all the treatments initially. There was a sharp decline in the bacterial count in all the treatments during storage. In treatment T_1, the highest bacterial count of 66.6 x 10^8 cfu/g observed initially before storage, was decreased to 0.17 x10^8 cfu/g on the 30th day of storage. The highest bacterial count in T_2 before storage (53.3 x 10^8 cfu/g) had reached the lowest count of 0.49 x10^8 cfu/g by the 30th day of storage. In treatment T_8, the maximum bacterial count of 52.6 x10^8 cfu/g before storage was found to be reduced to a minimum count of 0.37 x10^8 cfu/g on the 30th day of storage. In treatment T_9 also, the highest bacterial count of 50.6 x10^8 cfu/g initially, was declined to the lowest count of 0.22 x10^8 cfu/g on the 30th day of storage.

4.4.4.2.5. Total yeast count in fresh and frozen tempeh

Total yeast count in fresh tempeh types and during storage in the deep freezer is presented in Table 19 and in Figure 21.

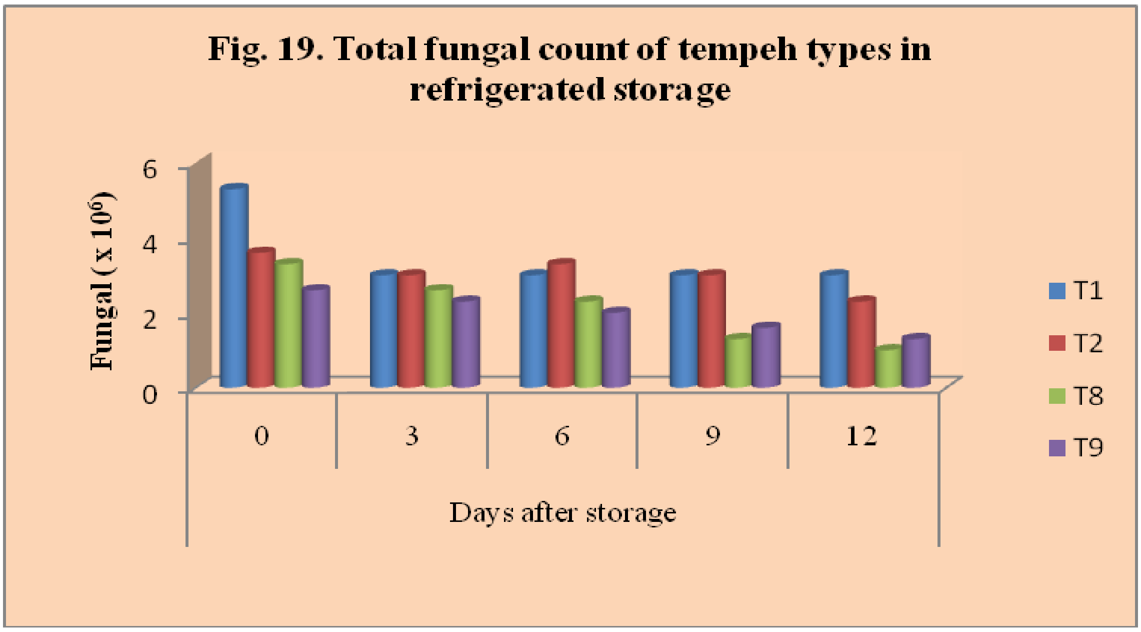


Fig. 19. Total fungal count of tempeh types in refrigerated storage
Fungal (x 10⁶)
6
4
2
0
0
3
6
9
12
Days after storage
T1
T2
T8
T9

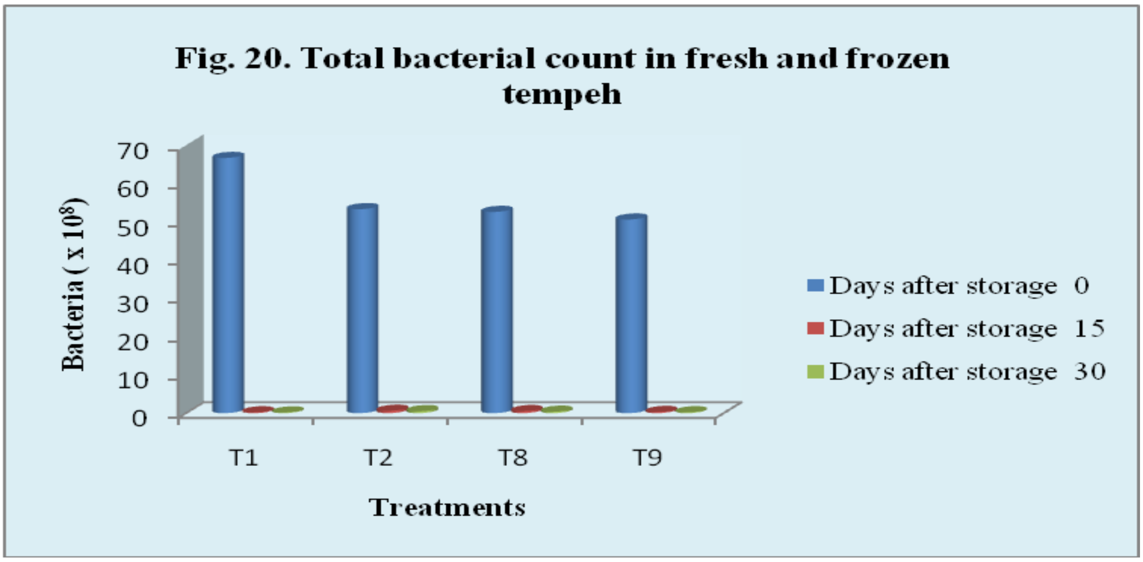


Fig. 20. Total bacterial count in fresh and frozen tempeh
Bacteria (x 10⁸)
70
60
50
40
30
20
10
0
T1
T2
T8
T9
Treatments
Days after storage 0
Days after storage 15
Days after storage 30

Table 19. Total yeast count in fresh and frozen tempeh (x 10^5 cfu/g)

Treatments	DAS		
	0	15	30
T_1	51.6	1.30	0.86
T_2	50.3	0.86	0.63
T_8	60.3	0.70	0.46
T_9	63.6	0.9	0.73

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three independent determinations

As revealed in Table 19, in all the treatments, total yeast count was maximum before storage and the yeast count was reduced to a minimum on the 30th day. In treatment T_1, the maximum yeast count of 51.6 x10^5 cfu/g initially, was decreased to the lowest count of 0.86 x 10^5 cfu/g on the 30th day of storage. In treatment T_2 the highest yeast count of 50.3 x 10^5 cfu/g before storage had reduced to the lowest count of 0.63 x10^5 cfu/g on the 30th day of storage. In the case of treatment T_8 also, the yeast count was found to be maximum (60.3 x10^5 cfu/g) before storage and the minimum count (0.46 x10^5 cfu/g) was on the 30th day of storage. T_9 had the highest yeast count of 63.6 x10^5 cfu/g initially and the lowest count of 0.73 x10^5 cfu/g was observed on the 30th day of storage.

4.4.4.2.6. Total fungal count in fresh and frozen tempeh

Total fungal count in the fresh tempeh types and during storage in the deep freezer is presented in Table 20 and is depicted in Figure 22.

Table 20. Total fungal count in fresh and frozen tempeh (x 10^6 cfu/g)

Treatments	DAS		
	0	15	30
T_1	5.0	0.13	0.1
T_2	3.0	0.1	0.06
T_8	3.3	0.06	0.06
T_9	2.6	0.06	0.03

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three independent determinations

The fungal count in the fresh tempeh types was found to be maximum on the initial day and there was a decrease in the fungal count on storage in all the treatments. The initial fungal count of 5.0 x 10^6 cfu/g in T_1 was reduced to a count of 0.1 x 10^6 cfu/g on the 30th day of storage. In treatment T_2, the highest fungal count of 3.0 x 10^6 cfu/g before storage, had reduced to the lowest count of 0.06 x 10^6 cfu/g on the 30th day of storage. In treatment T_8, the fungal count was reduced from 3.3 to 0.06 x 10^6 cfu/g after 30 days of storage. In T_9, the reduction was from 2.6 to 0.03 x 10^6 cfu/g after 30 days of storage.

4.4.4.3. Acceptability of the products prepared from stored tempeh

Tempeh chips and roast were prepared with the stored tempeh types and sensory evaluation of the tempeh chips and roast were carried out using score cards based on a nine point hedonic scale by the panel of 10 selected judges.

4.4.4.3.1. Acceptability of the chips and roast with tempeh types in refrigerated storage

Organoleptic evaluations of tempeh chips prepared from the selected tempeh types stored under refrigerated condition are presented in Table 21 to Table 26. The acceptability studies with T_8 and T_9 were conducted only up to 6 days of storage beyond which they were found to be spoiled.

Fig. 21. Total yeast count in fresh and frozen tempeh

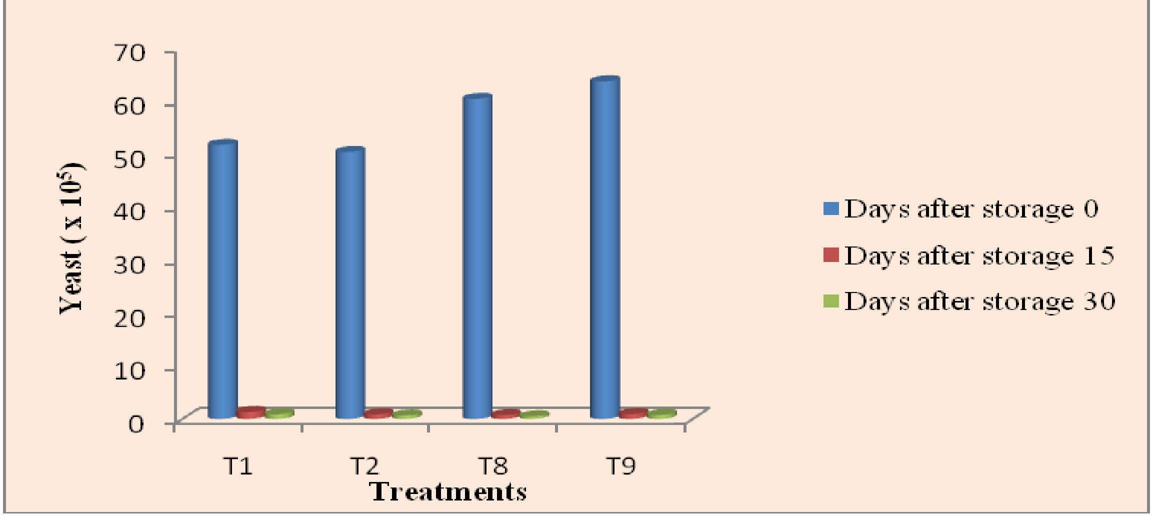


Fig.22. Total fungal count in fresh and frozen tempeh

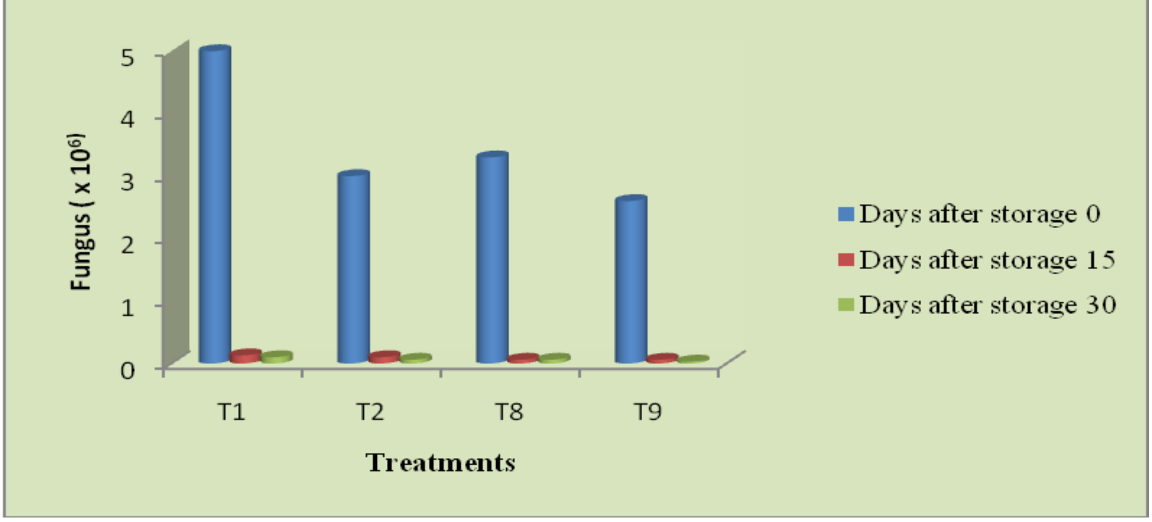


Table 21. Mean score for appearance of chips and roast with tempeh types in refrigerated storage

Treatments	Tempeh chips					Tempeh roast				
	DAS					DAS				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
T_1	8.9	8.5	8.2	8.0	8.0	9.0	9.0	9.0	8.9	8.6
T_2	9.0	8.8	8.6	7.8	7.6	8.8	8.9	9.0	8.7	8.5
T_8	8.0	8.7	7.5	-	-	8.9	8.7	8.6	-	-
T_9	7.5	8.6	7.0	-	-	9.0	8.7	8.7	-	-

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

Table 22. Mean score for colour of chips and roast with tempeh types in refrigerated storage

Treatments	Tempeh chips					Tempeh roast				
	DAS					DAS				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
T_1	8.9	8.8	8.7	8.7	8.7	9.0	8.2	8.6	8.6	8.6
T_2	9.0	8.7	8.5	8.5	8.8	8.8	8.2	8.8	8.5	8.2
T_8	8.0	8.0	8.0	-	-	8.7	8.4	8.0	-	-
T_9	7.8	7.9	7.7	-	-	8.6	8.3	8.1	-	-

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

As revealed in Table 21, there was a gradual reduction in the appearance of the chips prepared with tempeh stored under refrigerated condition. Initially before storage, and during the 3rd and 6th day of storage, the highest mean score for appearance was for T_2 (9.0, 8.8 and 8.6 respectively). T_1 had the highest mean score on the 9th (8.0) and 12th (8.0) day of storage. The lowest mean score for appearance before storage and on the 6th day was for T_9 (7.5 and 7.0 respectively), but during the 3rd day, T_9 had a higher score (8.6) than the control T_1. T_1 had the lowest mean score of 8.5 for appearance on the 3rd day of storage. On the 9th and 12th day of storage, T_1 had a comparatively higher score (8.0) than T_2.

In the case of tempeh roast, (Table 21), roast prepared with T_1 had the highest mean score for appearance before storage and on the 3rd (9.0), 6th (9.0), 9th (8.9) and 12th (8.6) day of storage. T_2 with a mean score of 8.8 initially, showed an increase to 8.9 on the 3rd day and further to 9.0 on the 6th day, comparable to the mean score of control (T_1) on the 6th day. During the 9th and 12th day, the mean score showed a reduction to 8.7 and 8.5 respectively, lower than that of T_1. T_8 with a mean score of 8.9 initially, higher than that of T_2, showed a reduction in the mean score to 8.7 on the 3rd and 8.6 on the 12th day. T_9 with a mean score of 9.0, same as that of T_1 before storage, was reduced to 8.7 during the 6th day, but the score was higher than that of T_8 (8.6) during the 6th day.

There was a gradual reduction in the mean score for colour of chips prepared from stored tempeh T_1 as the storage period advanced (Table 22). Before storage, the mean score of 8.9 for the colour of tempeh chips in T_1, was reduced to 8.8 in the 3rd and 8.7 in the 6th day. However, there was no reduction in the mean score from 8.7 during the 9th and 12th day of storage. Maximum score for colour was for T_1 during the 3rd, 6th and 9th day among all other treatments. Among treatments, maximum score for colour of chips before storage was for T_2 (9.0) which was reduced to 8.7 on the 3rd and to 8.5 on the 6th and 9th day, but, showed an increase in the mean score to 8.8 during the 12th day of storage, a score higher than that of T_1 on the 12th day of storage. T_8 with a mean score of 8.0 before storage retained this score up to the 6th day of storage. T_9 which showed the lowest score before storage (7.8) showed an increase to 7.9 on the 3rd day, but on the 6th day, the mean score was 7.7, the least score among treatments after the 6th day of storage.

With regards to the colour of the tempeh roasts (Table 22), before storage, the highest score was for T_1 (9.0) among the treatments which reduced to a score of 8.2 during the 3rd day, but showed an increase to 8.6 during the 6th, 9th and 12th day. In T_2, a mean score of 8.8 initially, showed a reduction in the mean score to 8.2 on the 3rd day, but on the 6th day, the mean score was again 8.8, the highest score among all the treatments during the 6th day of storage. However, during the 9th and 12th day, the mean score was lower than that of T_1. For T_8, a mean score of 8.7 initially was reduced to a score of 8.0 on the 6th day which was the least score obtained for the colour of roast among all the treatments after 6 days of storage. T_9 with the lowest mean score for colour before storage (8.6) showed a reduction in the mean score during the 3rd (8.3) and the 6th day (8.1) of storage. However, during the 3rd day of storage, mean score for colour of roast was higher in T_8 and T_9 than in T_1 and T_2.

As revealed in Table 23, the mean score of flavour of the chips prepared from the stored tempeh showed a reduction with the advancement of storage period. Before storage and on the 3rd, 6th 9th and 12th day of storage, the highest mean score for flavour was for T_2 (9.0, 8.3, 7.4, 7.4 and 6.6 respectively) among all other treatments. T_1 showed the least score initially and on the 3rd day (7.8 and 7.5 respectively) and also on the 9th and 12th day (6.8 and 6.5 respectively). T_8 with a score of 8.4 initially

Table 23. Mean score for flavour of chips and roast with tempeh types in refrigerated storage

Treatments	Tempeh chips					Tempeh roast				
	DAS					DAS				
	0	3	6	9	12	0	3	6	9	12
T_1	7.8	7.5	7.1	6.8	6.5	8.3	8.3	8.3	8.1	7.8
T_2	9.0	8.3	7.4	7.4	6.6	8.7	8.3	8.2	7.9	7.9
T_8	8.4	8.2	7.0	-	-	8.8	8.2	8.2	-	-
T_9	7.8	7.7	7.1	-	-	8.7	8.2	8.1	-	-

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

Table 24. Mean score for texture of chips and roast with tempeh types in refrigerated storage

Treatments	Tempeh chips					Tempeh roast				
	DAS					DAS				
	0	3	6	9	12	0	3	6	9	12
T_1	8.4	7.6	7.3	7.0	6.8	8.6	7.1	7.0	7.0	7.3
T_2	8.9	8.2	7.5	7.1	6.4	8.6	7.8	7.3	7.2	7.1
T_8	8.0	7.3	7.0	-	-	8.5	8.1	7.7	-	-
T_9	8.4	7.4	7.1	-	-	8.4	7.9	7.2	-	-

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

which was next to that of $T_{2,}$ showed the least score of 7.0 on the 6th day among all the treatments. T_9 also showed the least score of 7.8 like T_1 initially, but showed a score of 7.1 on the 6th day, higher than that of T_8 on the 6th day.

As revealed in Table 23, the mean score for flavour of tempeh roast with T_1 before storage (8.3) showed no difference on the 3rd and 6th day but was reduced to a score of 8.1 on the 9th and 7.8 on the 12th day. In T_2, the mean score of 8.7 before storage, higher than that of T_1 was reduced to 8.3 on the 3rd day, same as that of $T_{1,}$ and further reduced to 8.2 and 7.9 during the 6th, 9th and 12th day of storage. During 12th day the score was higher than that of T_1. T_8 with a maximum score of 8.8 before storage among all the treatments was reduced to a score of 8.2 on the 3rd and 6th day of storage. A mean score of 8.7 in T_9 before storage was reduced to the lowest score of 8.1 on the 6th day.

There was a gradual reduction in the mean score for texture of chips prepared with different tempeh types stored under refrigerated condition (Table 24). Among the four types of tempeh, the highest mean score for texture of chips was for T_2 before storage (8.9) and on the 3rd (8.2), 6th (7.5), and 9th (7.1) day of storage. On the 12th day, the highest mean score for texture was for T_1 (6.8). The lowest mean score was for T_8 before storage, and also on the 3rd and 6th day of storage (8.0, 7.3 and 7.0 respectively). On the 12th day of storage, T_1 had a better score (6.8) than T_2.

The mean score for the texture of the tempeh roasts (Table 24) prepared with tempeh before storage, was found to be high for all the treatments and there was a decrease in the mean score for texture during storage. Initially, the highest mean score (8.6) for the quality attribute texture was for the treatments T_1 and $T_{2.}$ T_1 also showed the maximum score on the 12th day (7.3). The highest mean score for texture on the 3rd (8.1) and 6th (7.7) day was for the treatment $T_{8.}$

As revealed in Table 25, the taste of the tempeh chips varied with the storage period of the tempeh types. Among treatments, T_1 had the least score for taste before storage (7.4) and on the 3rd (7.3), 6th (6.7), 9th (6.6) and 12th (6.1) day of storage. T_2, which had the maximum score of 8.8 on the first day also showed the maximum score in each storage period. T_8 had a mean score of 8.0 before storage, and reduced to the

Table 25. Mean score for taste of chips and roast with tempeh types in refrigerated storage

Treatments	Tempeh chips					Tempeh roast				
	DAS					DAS				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
T_1	7.4	7.3	6.7	6.6	6.1	7.5	7.8	7.2	6.8	6.4
T_2	8.8	8.2	7.6	7.6	7.0	8.3	7.6	7.1	7.2	7.1
T_8	8.0	7.6	7.1	-	-	8.7	8.0	7.4	-	-
T_9	8.4	7.7	7.4	-	-	8.3	7.4	7.0	-	-

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

Table 26. Mean score for overall acceptability of chips and roast with tempeh types in refrigerated storage

Treatments	Tempeh chips					Tempeh roast				
	DAS					DAS				
	0	**3**	**6**	**9**	**12**	**0**	**3**	**6**	**9**	**12**
T_1	8.3	8.2	8.0	7.6	7.0	8.3	7.6	6.9	6.5	6.6
T_2	8.9	8.3	8.1	7.7	7.2	8.7	7.7	7.2	6.9	6.9
T_8	8.3	8.0	7.8	-	-	8.5	8.3	7.4	-	-
T_9	8.1	7.7	7.4	-	-	8.6	8.0	7.6	-	-

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

lowest score of 7.1 after 6 days of storage. The score of T_9 reduced from 8.4 before storage to 7.4 after the 6^{th} day of storage.

As revealed in Table 25, tempeh roast with T_1 had the least score for taste before storage among all the treatments (7.5), which reduced to a score of 6.4 on the 12^{th} day of storage. T_2 with a mean score of 8.3 initially, showed a reduction in the taste score during storage but on 9^{th} and 12^{th} day, had a better score (7.2 and 7.1 respectively) than the control T_1. T_8 had the highest taste score initially and on the 3^{rd} and 6^{th} day of storage (8.7, 8.0 and 7.4 respectively) among all the treatments. For T_9, the initial score of 8.3 was reduced to 7.0 on the 6^{th} day, which was the least score among all the treatments after 6 days of storage.

The overall acceptability score of chips (Table 26) prepared with the stored tempeh was low when compared to the overall acceptability score of chips prepared with fresh tempeh. Among all the treatments, T_2 had the highest mean score for overall acceptability before storage (8.9), and on the 3^{rd} (8.3), 6^{th} (8.1), 9^{th} (7.7) and 12^{th} (7.2) day of storage. T_9 had the lowest mean score for overall acceptability before storage (8.1), and on the 3^{rd} (7.7) and 6^{th} (7.4) day of storage. The overall acceptability of chips prepared with tempeh types stored under refrigerated condition is illustrated in Figure 23.

In tempeh roast also, overall acceptability score was high with fresh tempeh (Table 26) compared to the roast prepared with the stored tempeh. The overall acceptability score was highest for T_2 with fresh tempeh, but on storage on the 3^{rd} day, the highest overall acceptability score was for T_8 (8.3) and on the 6^{th} day, it was for T_9 (7.6). On the 9^{th} and 12^{th} day of storage, T_2 had a comparatively better score (6.9) than the control T_1. The overall acceptability of roast prepared with different tempeh types stored under refrigerated condition is illustrated in Figure 24.

Fig.23. Overall acceptability of tempeh chips with tempeh types in refrigerated storage

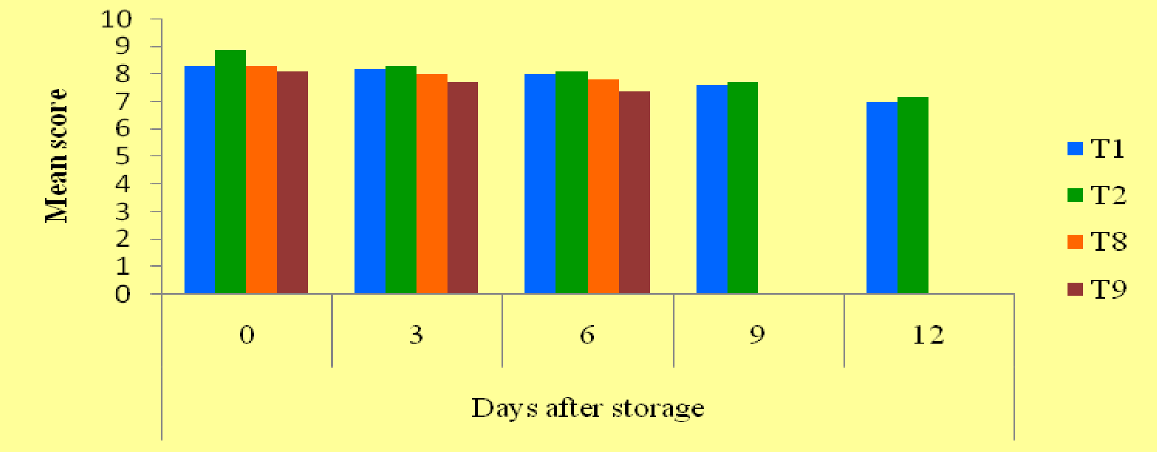
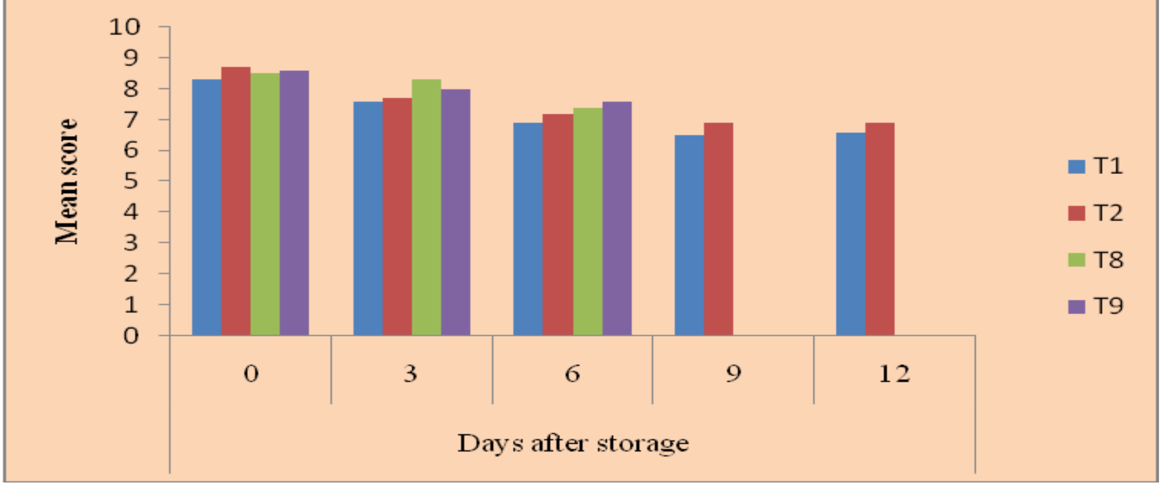


Fig. 24. Overall acceptability of roast with tempeh types in refrigerated storage



4.4.4.3.2. Acceptability of the chips and roast with frozen tempeh

Organoleptic evaluation of tempeh chips and roast prepared from selected tempeh types stored in deep freezer are presented in Table 27 to 32.

Table 27. Mean score for appearance of chips and roast with frozen tempeh

Treatments	Tempeh chips			Tempeh roast		
	DAS			DAS		
	0	15	30	0	15	30
T_1	8.9	8.7	8.8	9.0	9.0	8.9
T_2	9.0	8.9	8.9	8.8	8.7	8.7
T_8	8.0	8.0	8.0	8.9	8.5	8.3
T_9	7.5	7.4	7.4	9.0	8.7	8.1

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations.

As revealed in Table 27, a decrease in the mean score for appearance of the chips prepared with the stored tempeh was observed in all the treatments. Before storage, the maximum mean score for appearance was for the treatment T_2 (9.0) and the minimum score was for T_9 (7.5). On the 15^{th} day of storage, the mean score for appearance ranged from 7.4 to 8.9 with the lowest score in T_9 and the highest in T_2. T_9 had the lowest mean score of 7.4 and T_2 had the maximum score of 8.9 on the 30^{th} day of cold storage.

Regarding the appearance of tempeh roast (Table 27), before cold storage, the highest mean score was for T_1 and T_9 (9.0) and the lowest score was for T_2 (8.8). The mean score for appearance varied from 8.5 to 9.0 on the 15^{th} day of storage with the lowest score for T_8 and highest for $T_{1.}$ On the 30^{th} day of storage, the highest mean score (8.9) was in T_1 and the lowest score (8.1) was in T_9.

Table 28. Mean score for colour of chips and roast with frozen tempeh

Treatments	Tempeh chips			Tempeh roast		
	DAS			DAS		
	0	15	30	0	15	30
T_1	8.9	8.9	8.8	9.0	8.9	8.5
T_2	9.0	9.0	8.7	8.8	8.8	8.4
T_8	8.0	7.9	7.8	8.7	8.5	8.4
T_9	7.8	7.7	7.7	8.6	8.3	8.1

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations.

Before storage, the mean score for the colour of the chips prepared with fresh tempeh types (Table 28) varied from 7.8 to 9.0 with the lowest score for T_9 and the highest for T_2. The tempeh chips prepared on the 15th day of storage got a maximum mean score of 9.0 for T_2 and the lowest score of 7.7 for T_9. On the 30th day of cold storage, maximum score (8.8) was observed in T_1 and the lowest score (7.7) in T_9.

The mean score for the colour of roast with fresh tempeh before storage (Table 28) was highest in T_1 (9.0) and the lowest mean score (8.6) was for T_9. On the 15th day of storage, the mean score for colour ranged from 8.3 to 8.9 with the lowest score in T_9 and the highest score in T_1. The treatment T_9 had the lowest mean score of 8.1 and T_1 had the highest mean score of 8.5 on the 30th day of cold storage.

Table 29. Mean score for flavour of chips and roast with frozen tempeh

Treatments	Tempeh chips			Tempeh roast		
	DAS			DAS		
	0	15	30	0	15	30
T_1	7.8	7.7	7.6	8.3	7.0	7.0
T_2	9.0	8.3	8.0	8.7	8.0	8.0
T_8	8.4	8.0	8.0	8.8	8.0	8.0
T_9	7.8	7.6	7.3	8.7	8.0	8.0

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations

As revealed in Table 29, a decrease in the flavour of the chips was observed on cold storage. Before storage with fresh tempeh, the highest mean score for flavour (9.0) was for T_2 and the lowest score (7.8) for was for T_1 and T_9. On the 15th day of storage, the mean score for flavour ranged from 7.6 to 8.3 with the lowest score in T_9 and the highest score in T_2. The treatment T_9 had the lowest mean score of 7.3 and T_2 and T_8 had the highest mean score of 8.0 on the 30th day of cold storage.

Regarding the flavour of tempeh roast (Table 29), before storage, the highest mean score (8.8) was for T_8 and the lowest score (8.3) was for T_1. The mean score for flavour of the tempeh roast varied from 7.0 to 8.0 with the lowest score in T_1 and the highest (8.0) in $T_{1,}$ T_8 and T_9 respectively on the 15th day of storage. On the 30th day of cold storage, the highest mean score (8.0) was observed in treatments T_1, T_8 and T_9 and the lowest mean score (7.0) was in T_1.

Table 30. Mean score for texture of chips and roast with frozen tempeh

Treatments	Tempeh chips			Tempeh roast		
	DAS			DAS		
	0	15	30	0	15	30
T_1	8.4	8.0	7.8	8.6	7.9	7.7
T_2	8.9	8.1	7.6	8.6	8.0	8.4
T_8	8.0	7.8	7.7	8.5	8.0	8.0
T_9	8.4	7.7	7.5	8.4	8.0	8.0

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations.

The mean score for the texture of the tempeh chips (Table 30) prepared before storage varied between 8.0 to 8.9 with the lowest score for T_8 and the highest for T_2. The tempeh chips prepared on the 15th day of storage got a maximum mean score of 8.1 for the treatment T_2 and the lowest score of 7.7 for T_9. On the 30th day of cold storage, the maximum mean score of 7.8 was in T_1 and the lowest score of 7.5 was observed in T_9.

Before storage with fresh tempeh, the mean score for the texture of the roast (Table 30) was highest in T_1 and T_2 (8.6) and the lowest mean score (8.4) was for T_9. On the 15th day of storage, the mean score for texture ranged from 7.9 to 8.0 with the lowest score in T_1 and highest score in treatments T_2, T_8 and T_9. The treatment T_1 had the lowest mean score of 7.7 and T_2 had the maximum score of 8.4 on the 30th day of storage.

Table 31. Mean score for taste of chips and roast with frozen tempeh

Treatments	Tempeh chips			Tempeh roast		
	DAS			DAS		
	0	15	30	0	15	30
T_1	7.4	7.3	7.4	7.5	7.5	7.4
T_2	8.8	8.5	8.0	8.3	8.0	7.8
T_8	8.0	7.9	7.7	8.7	8.0	8.0
T_9	8.4	8.1	7.9	8.3	8.0	8.0

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations.

As revealed in Table 31, a decrease in the taste of the tempeh chips was observed on storage. Before storage with fresh tempeh, the maximum mean score (8.8) for taste of the tempeh chips was for the treatment T_2 and the lowest mean score (7.4) was for T_1. On the 15th day of storage, the mean score for taste ranged from 7.3 to 8.5 with the lowest score in T_1 and highest score in T_2. The treatment T_1 had the lowest mean score of 7.4 and T_2 had the highest mean score of 8.0 on the 30th day of cold storage.

Regarding the taste of tempeh roast (Table 31), before storage with fresh tempeh, the highest mean score (8.7) was for the treatment T_8 and the lowest score (7.5) was for T_1. The mean score for taste of the tempeh roast varied from 7.5 to 8.0 with the lowest mean score in T_1 and the highest (8.0) in $T_{1,}$ T_8 and T_9 on the 15th day of storage. On the 30th day of cold storage, the highest mean score (8.0) was observed in the treatments T_8 and T_9 and the lowest mean score (7.4) was observed in T_1.

Table 32. Mean score for overall acceptability of chips and roast with frozen tempeh

Treatments	Tempeh chips			Tempeh roast		
	DAS			DAS		
	0	15	30	0	15	30
T_1	8.3	8.1	8.1	8.3	7.9	7.8
T_2	8.9	8.5	8.1	8.7	8.3	8.0
T_8	8.3	8.1	8.1	8.5	8.2	8.0
T_9	8.1	8.0	7.8	8.6	8.3	8.0

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DAS – Days after storage
Values are mean of three evaluations.

A decline in the acceptability of the chips prepared with frozen tempeh (Table 32) was reflected in the decrease of the mean score for overall acceptability. The mean score for the overall acceptability of the fresh tempeh chips prepared before cold storage of tempeh varied between 8.1 to 8.9 with the lowest mean score for the treatment T_9 and the highest for T_2 respectively. The tempeh chips prepared on the 15^{th} day of cold storage got the highest mean score of 8.5 for the treatment T_2 and the lowest score of 8.0 for T_9. On the 30^{th} day of storage, the highest mean score of 8.1 was observed in treatments $T_{1,}$ T_2 and T_8 and the lowest mean score of 7.8 was observed in T_9. The overall acceptability of chips with frozen tempeh types is illustrated in Figure. 25.

The mean score for the overall acceptability of the tempeh roast (Table 32) also showed a decrease in all the treatments on storage. Before storage with fresh tempeh, the mean score for overall acceptability of the tempeh roast was highest in T_2 (8.7) and the lowest mean score (8.3) was for T_1. On the 15^{th} day of cold storage, the mean score for overall acceptability ranged from 7.9 to 8.3 with the lowest mean score in T_1 and the highest score in treatments T_2 and T_9. The treatment T_1 had the lowest mean score of 7.8 and the treatments T_2, T_8 and T_9 had the highest mean score of 8.0 on the 30^{th} day of cold storage. The overall acceptability of roast with frozen tempeh types is illustrated in Figure. 26.

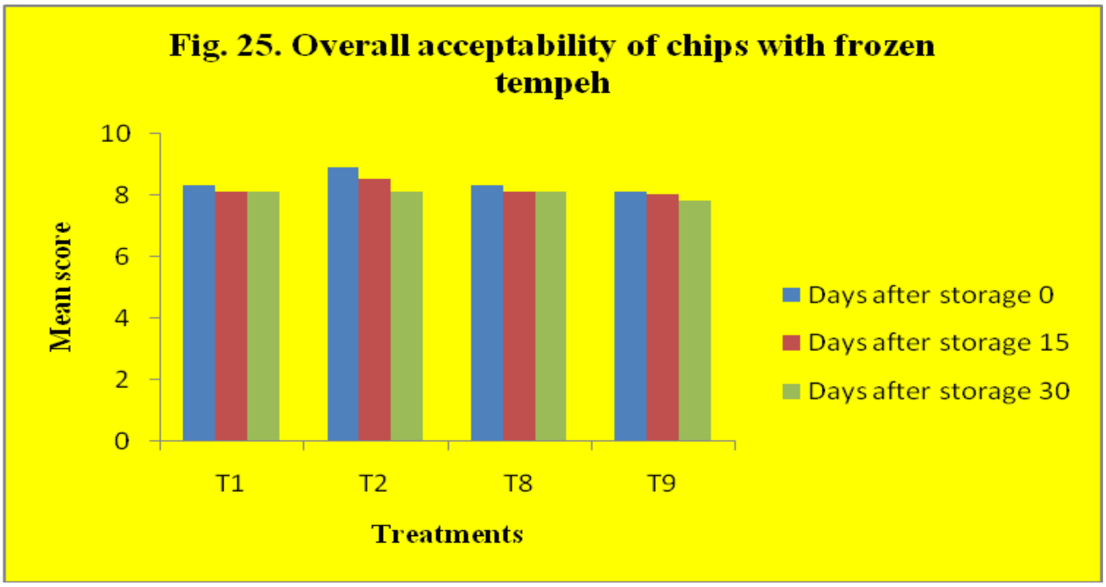


Fig. 25. Overall acceptability of chips with frozen tempeh

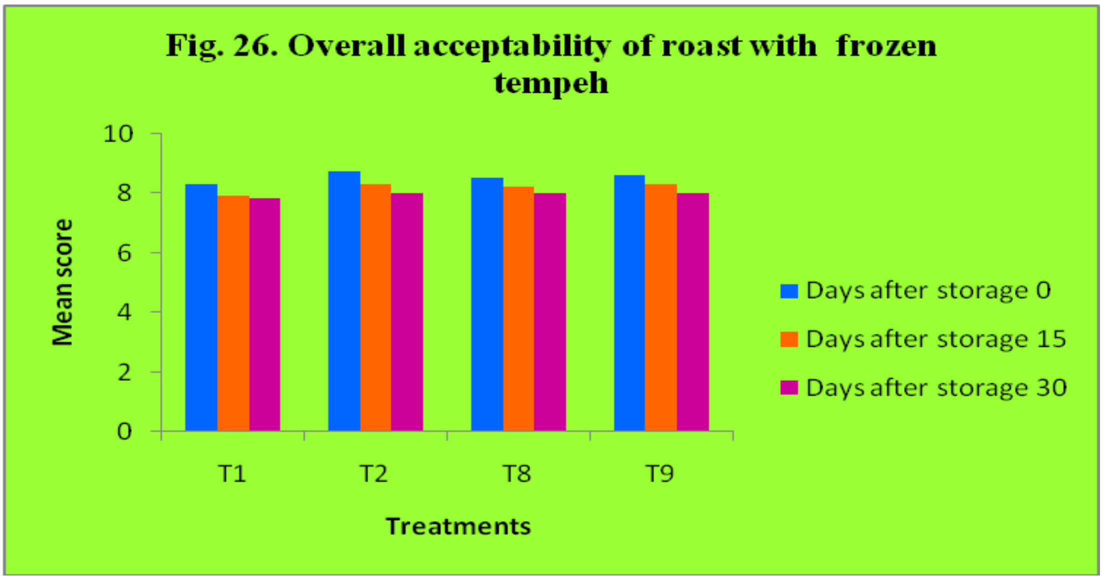


Fig. 26. Overall acceptability of roast with frozen tempeh

4.5. Quality evaluation and shelf life study in tempeh flours

Tempeh flours were prepared (Plates 84 - 87) with the selected tempeh types and the flours were packed in metallised polyester laminate pouches and were stored in room temperature for six months (Plate 88). The flours were analysed for its chemical constituents, *in vitro* protein and starch digestibility, total microbial count and insect infestation initially and also during the 6^{th} month of storage.

4.5.1. Chemical constituents in tempeh flours

The chemical constituents of the tempeh flours were analysed initially and during the 6^{th} month of storage.

Among treatments, variations in chemical constituents were statistically analysed by applying DMRT, and the changes in chemical constituents in each treatment due to storage was analysed by applying paired 't' test and the results are presented in Table 33 to 37.

4.5.1.1. Moisture and Fiber

The moisture and fiber content of tempeh flours are presented in Table 33

Table 33. Moisture and fiber content of tempeh flours on storage

Treatments	Moisture (g/100g)			Fiber (g/100g)		
	Initial	6^{th} month	't' value	Initial	6^{th} month	't' value
T_1	6.53^{ab}	6.93^{ab}	3.46 *	2.87^{b}	3.03^{a}	0.21^{NS}
T_2	6.80^{a}	7.1^{a}	0.14^{NS}	3.30^{a}	3.10^{a}	1.30^{NS}
T_8	6.53^{ab}	6.6^{bc}	1.24^{NS}	2.10^{c}	1.83^{b}	1.24^{NS}
T_9	6.13^{b}	6.23^{c}	2.0^{NS}	1.4^{d}	1.47^{c}	0.25^{NS}

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
*- Significant at 10 % level
NS – Not significant

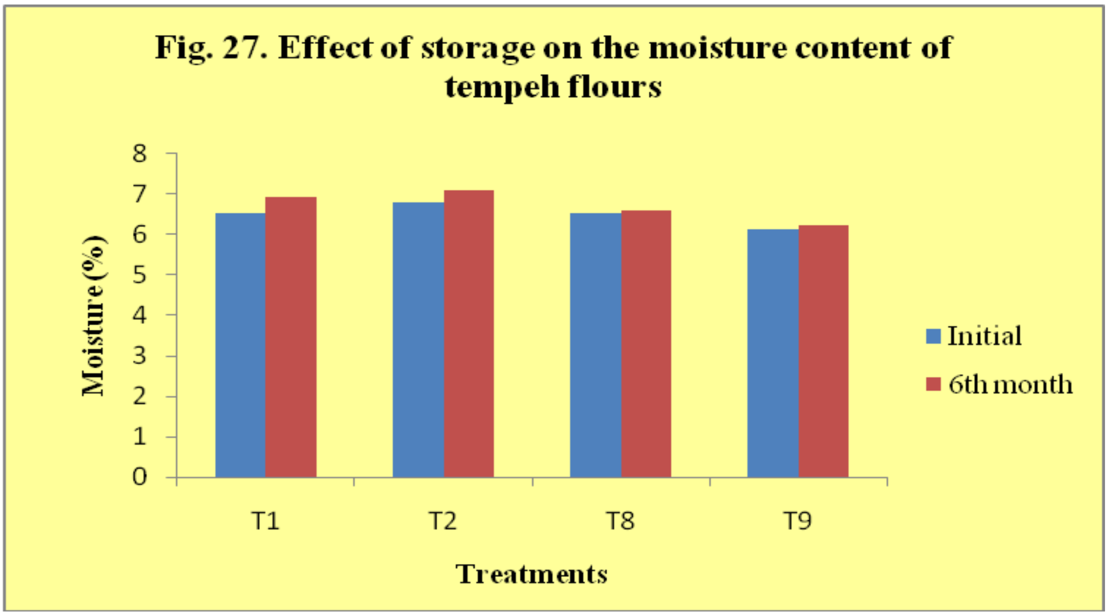


Fig. 27. Effect of storage on the moisture content of tempeh flours
Moisture (%)
8
7
6
5
4
3
2
1
0
T1
T2
T8
T9
Treatments
Initial
6th month

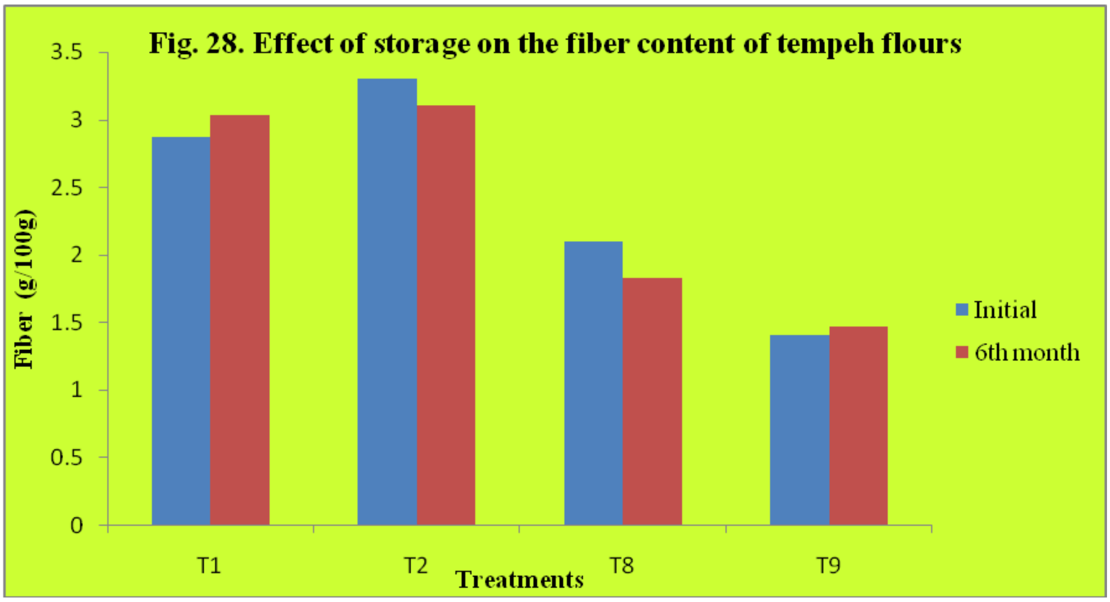


Fig. 28. Effect of storage on the fiber content of tempeh flours
Fiber (g/100g)
3.5
3
2.5
2
1.5
1
0.5
0
T1
T2
T8
T9
Treatments
Initial
6th month

Types of tempeh flour

Plate 84. 100% soybean (T_1)

Plate 85. 100% green gram (T_2)

Plate 86. green gram 75% + rice 25% (T_8)

Plate 87. green gram 50% + rice 50% (T_9)

Plate 88. Tempeh flour stored in metalised polyester laminate pouch

As revealed in Table 33, initially the moisture content in tempeh flours ranged between 6.13 to 6.80 per cent with the lowest moisture content in treatment T_9 and the highest in T_2. Significant difference in the moisture content was observed only between T_2 and T_9. During the 6^{th} month of storage also, the highest moisture content was observed in the treatment T_2 (7.1 %) and lowest in T_9 (6.23 %) and there was significant variation in the moisture content of the treatments $T_{2,}$ T_8 and T_9. Effect of storage on the moisture content of tempeh flour in each treatment showed a significant increase in moisture content only in T_1. In all other tempeh flours, the increase in moisture content after storage was not significant.

Significant difference in the fiber content of tempeh flours was observed initially in all the treatments with the highest fiber content in treatment T_2 (3.30g/100g) and the lowest in T_9 (1.4 g/100g). During the 6^{th} month of storage, maximum fiber content was observed in treatment T_2 (3.10 g/100g) and minimum in T_9 (1.47 g/100g). There was no significant difference in the fiber content of T_1 and T_2, but fiber content in these treatments were significantly high when compared to T_8 and T_9. The variations observed in the fiber content of tempeh flours with each treatment during storage were not significant.

Effect of storage on the moisture and fiber content of tempeh flours are illustrated in Figure 27 and 28 respectively.

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4.5.1.2. Protein and total fats

The protein and total fat content of the tempeh flours are presented in Table 34.

Table 34. Protein and total fat content of tempeh flours on storage

Treatments	Protein (g/100g)			Total fats (g/100g)		
	Initial	6^{th} month	't' value	Initial	6^{th} month	't' value
T_1	43.15^a	42.43^a	2.156^{NS}	20.87^a	20.73^a	0.136^{NS}
T_2	22.45^b	21.85^b	0.754^{NS}	1.13^b	1.13^b	0.00^{NS}
T_8	18.17^c	17.69^c	2.89*	0.87^b	0.93^b	1.00^{NS}
T_9	12.96^d	12.77^d	0.292^{NS}	0.67^b	0.70^b	1.00^{NS}

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
*- Significant at 10 % level
NS – Not significant

Initially among the treatments, the highest protein content was observed in T_1 (43.15 g/100g) and the lowest in T_9 (12.96 g/100g). After storage, the protein content varied between 12.77 g/100g (T_9) and 42.43 g/100g (T_1). Significant difference was observed in the protein content of tempeh flours in all the treatments initially and also during the 6^{th} month of storage, with T_1 having significantly high protein content followed by T_2. There was a reduction in the protein content of tempeh flours during storage, but significant reduction was observed only in T_8.

Initially, the total fat content in the tempeh flours varied from 0.67 to 20.87 g/100g with the lowest fat content in T_9 and highest in T_1. The total fat content during the 6^{th} month was also found to be highest in T_1 (20.73 g/100g) and lowest in T_9 (0.7 g/100g). The treatment T_1 had significantly high fat content when compared to the other treatments initially as well as after storage. There was no significant variation in the fat content of tempeh flours with all the treatments due to storage.

Effect of storage on the protein and total fat content of tempeh flours are illustrated in Figure 29 and 30 respectively.

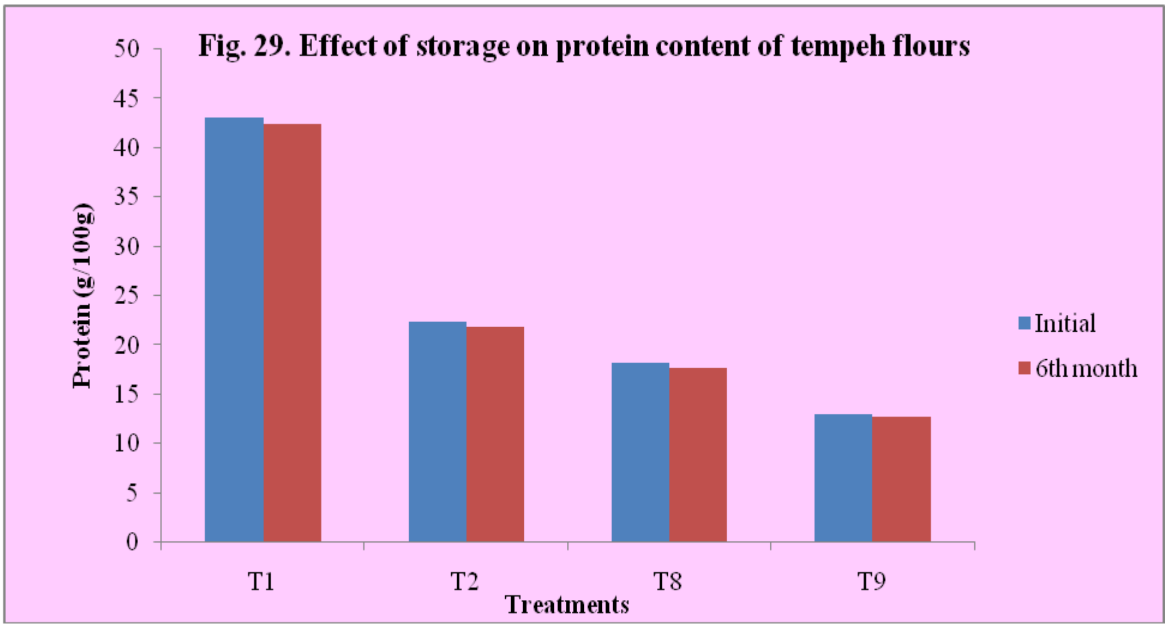


Fig. 29. Effect of storage on protein content of tempeh flours

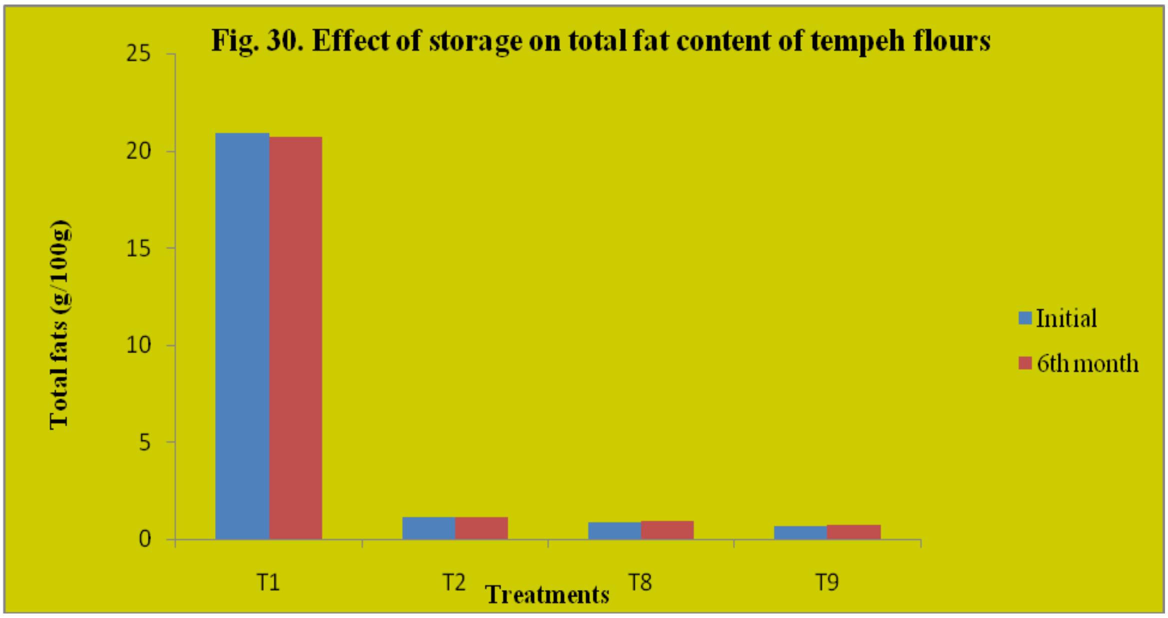


Fig. 30. Effect of storage on total fat content of tempeh flours

4.5.1.3. Starch and riboflavin

The starch and riboflavin content of the tempeh flours are presented in Table 35.

Table 35. Starch and riboflavin content of tempeh flours on storage

Treatments	Starch (g/100g)			Riboflavin (mg/100g)		
	Initial	6th month	't' value	Initial	6th month	't' value
T_1	12.02[d]	11.29[d]	3.0 *	0.021[a]	0.017[a]	0.101[NS]
T_2	34.70[c]	33.48[c]	11.091***	0.03[a]	0.023[a]	1.73[NS]
T_8	43.99[b]	44.47[b]	0.361[NS]	0.0051[b]	0.002[b]	4.34**
T_9	49.38[a]	46.68[a]	16.573***	0.0054[b]	0.0013[b]	15.12***

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
*- Significant at 10 % level, **- Significant at 5% level, ***- Significant at 1% level
NS – Not significant

Among the treatments, starch content (Table 35) was found to be highest in tempeh flour with treatment T_9 (49.38 g/100g) and lowest in T_1 (12.02 g/ 100g) both initially and during the 6th month of storage (46.68 and 11.29 g/100g respectively). DMRT showed a significant variation in the starch content of tempeh flours with different treatments initially as well as after storage. There was a significant reduction in the starch content of tempeh flours with the treatments T_1, T_2 and T_9 after storage (Fig. 31).

Riboflavin content of tempeh flours with different treatments (Table 35 and Fig. 32) initially varied from 0.0051 to 0.03 mg/100g and during the 6th month of storage, this varied from 0.0013 to 0.023 mg/100g. In both the cases the highest riboflavin content was observed in the treatment T_2. The lowest riboflavin was found in treatment T_9 in the initial period and also during the 6th month of storage. On the basis of DMRT, there was no significant difference in the riboflavin content of T_1 and T_2 and among treatments T_8 and T_9 both initially and during storage. But riboflavin

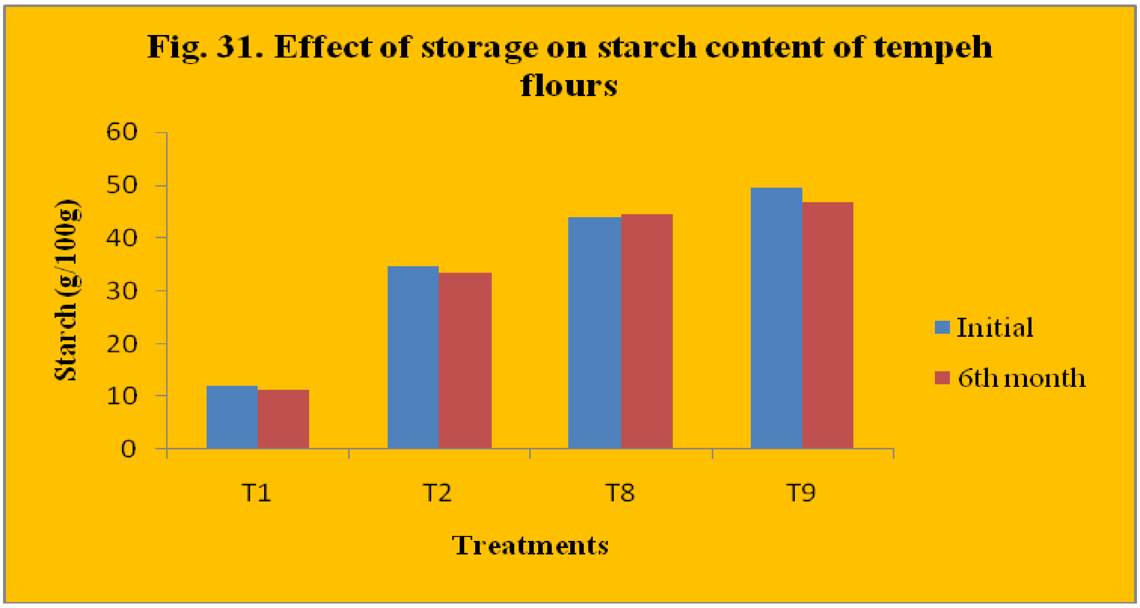


Fig. 31. Effect of storage on starch content of tempeh flours
Starch (g/100g)
60
50
40
30
20
10
0
T1
T2
T8
T9
Treatments
Initial
6th month

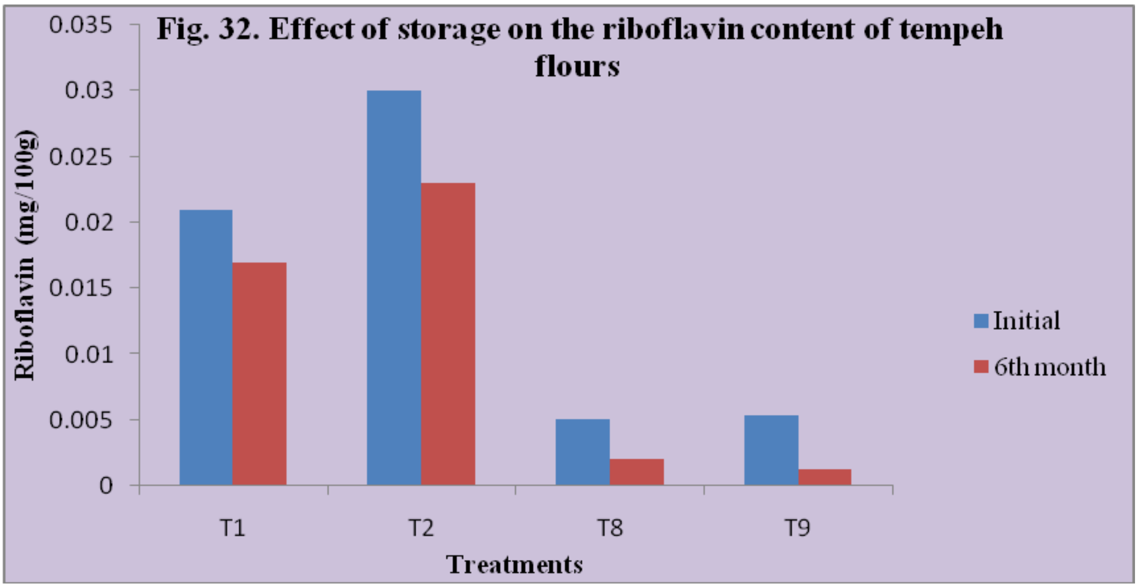


Fig. 32. Effect of storage on the riboflavin content of tempeh flours
Riboflavin (mg/100g)
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
T1
T2
T8
T9
Treatments
Initial
6th month

content of T_1 and T_2 were significantly high when compared to T_8 and T_9 initially and after storage. Effect of storage of tempeh flours in each treatment showed a significant reduction in the riboflavin content in T_8 and T_9.

4.5.1.4. Thiamine

Thiamine was not detected in any of the tempeh flours prepared from the selected tempeh types initially or during the 6^{th} month of storage.

4.5.1.5. Calcium and iron

The calcium and iron content of the tempeh flours are presented in Table 36.

Table 36. Calcium and iron content of tempeh flours on storage

Treatments	Calcium (mg/100g)			Iron (mg/100g)		
	Initial	6^{th} month	't' value	Initial	6^{th} month	't' value
T_1	331.25^a	328.97^a	0.101^{NS}	8.03^a	8.21^a	0.49^{NS}
T_2	113.87^b	112.63^b	0.540^{NS}	3.53^b	3.52^b	0.049^{NS}
T_8	91.34^c	88.03^c	0.816^{NS}	3.13^b	3.14^b	0.064^{NS}
T_9	68.39^d	66.66^d	1.02^{NS}	2.57^c	2.58^c	0.074^{NS}

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
NS – Not significant

As revealed in Table 36 and Figure 33, the calcium content in the tempeh flours initially ranged from 68.39 to 331.25 mg/100g with the lowest calcium content in treatment T_9 and highest in T_1. During the 6^{th} month of storage, there was a reduction in the calcium content with the highest in T_1 (328.97 mg/100g) and lowest in T_9 (66.66 mg/100g). As per DMRT, a significant difference in the calcium content was observed among all the tempeh flours initially and also during the 6^{th} month of storage. The reduction in the calcium content in tempeh flours observed on storage in different treatments was not significant.

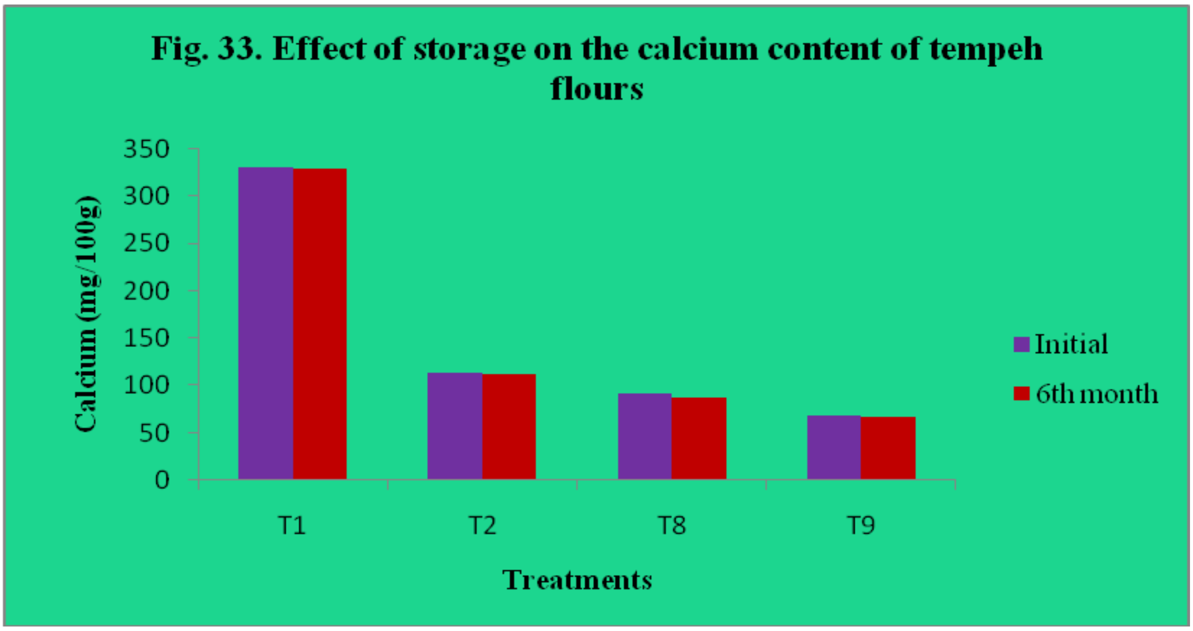


Fig. 33. Effect of storage on the calcium content of tempeh flours
Calcium (mg/100g)
350
300
250
200
150
100
50
0
T1
T2
T8
T9
Treatments
Initial
6th month

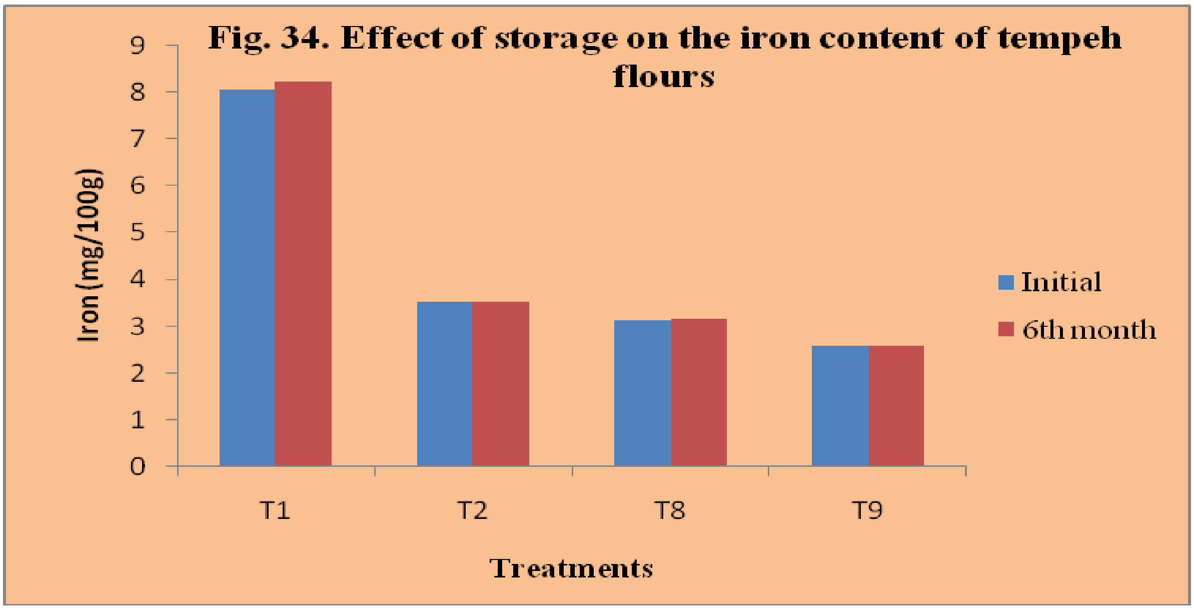


Fig. 34. Effect of storage on the iron content of tempeh flours
Iron (mg/100g)
9
8
7
6
5
4
3
2
1
0
T1
T2
T8
T9
Treatments
Initial
6th month

The iron content (Table 36) was highest in treatment T_1 initially (8.03 mg/100g) and also during the 6th month of storage (8.21 mg/100g). The lowest iron content was observed in treatment T_9 initially (2.57 mg/100g) and also during the 6th month of storage (2.58 mg/100g). As per DMRT among treatments, initially and during the 6th month of storage iron content was found to be significantly high in T_1. There was no significant difference in the iron content of the tempeh flours of T_2 and T_8. Iron content was significantly low in T_9 (Fig 34). There was no significant variation in the iron content of tempeh flours in each treatment on storage.

4.5.1.6. Phosphorus, potassium and zinc

The phosphorus, potassium and zinc content of the tempeh flours are presented in Table 37.

Table 37. Phosphorus, potassium and zinc content of tempeh flours on storage

Treatments	Phosphorus (mg/100g)			Potassium (mg/100g)			Zinc (mg/100g)		
	Initial	6th month	't' value	Initial	6th month	't' value	Initial	6th month	't' value
T_1	601.36^a	595.56^a	0.171^{NS}	659.17^c	653.57^c	0.335^{NS}	4.82^a	4.78^a	1.3^{NS}
T_2	351.69^b	347.99^b	0.132^{NS}	979.68^a	978.93^a	0.556^{NS}	3.33^b	3.41^b	2.8^{NS}
T_8	331.40^b	326.08^{bc}	0.886^{NS}	751.32^b	751.25^b	0.007^{NS}	2.87^c	2.86^c	0.151^{NS}
T_9	268.55^c	267.49^c	0.061^{NS}	559.25^d	563.71^d	0.191^{NS}	2.27^d	2.26^d	0.264^{NS}

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
NS – Not significant

A decrease in the phosphorus (Table 37 and Fig. 35) content of tempeh flours though not significant was observed on storage in all the treatments. Initially among treatments, the highest phosphorus content was in T_1 (601.36 mg/100g) and the lowest in T_9 (268.55 mg/100g). There was no significant difference in the phosphorus content of the treatments T_2 and T_8 but when compared to T_1, phosphorus content was

Fig. 35. Effect of storage on the phosphorus content of tempeh flours

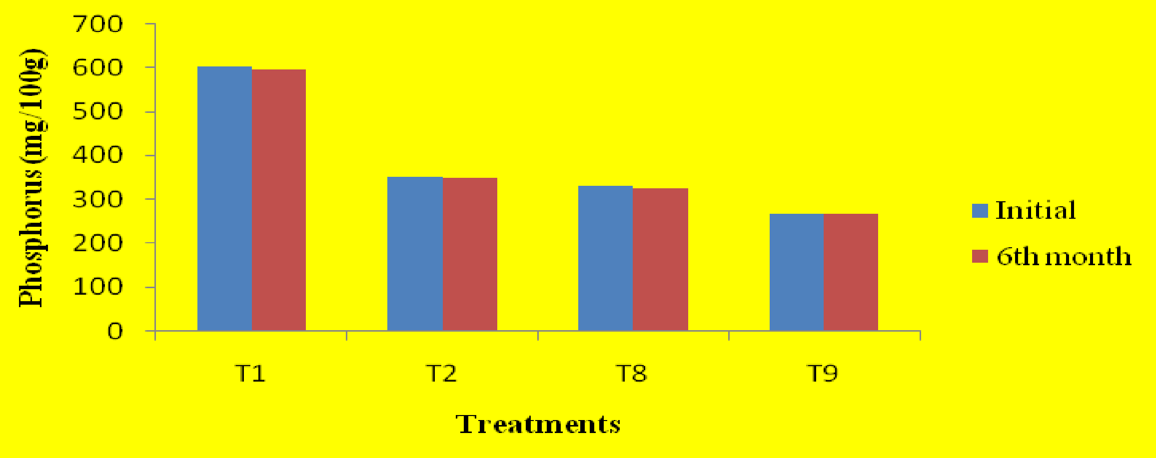
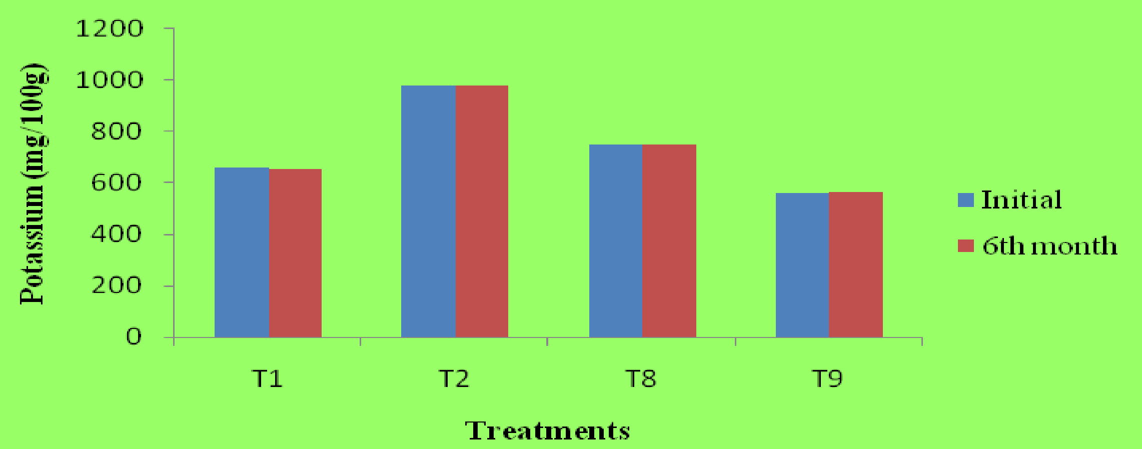


Fig. 36. Effect of storage on the potassium content of tempeh flours



significantly low in T_2 and T_8. During the 6^{th} month of storage, the highest phosphorus content was in T_1 (595.56 mg/100g) and lowest was in T_9 (267.49 mg/100g). The phosphorus content of tempeh flour with T_1 was found to be significantly high initially and even after storage, among all the treatments.

As revealed in Table 37, significant difference in the potassium content of tempeh flours was observed initially among the treatments, with the highest potassium content in treatment T_2 (979.68 mg/100g) and the lowest in T_9 (559.25 mg/100g). During the 6^{th} month of storage also, maximum potassium content was observed in T_2 (978.93 mg/100g) and minimum in T_9 (563.71 mg/100g) and here also significant variation in the potassium content was observed among the treatments. However, after storage (Fig. 36), the variations observed in potassium content in each treatment was not significant in any of the treatments.

Initially, the zinc content (Table 37 and Fig. 37) varied from 2.27 to 4.82 mg/100g with the lowest zinc content in treatment T_9 and highest in T_1. The zinc content during the 6^{th} month was also found to be highest in T_1 (4.78 mg/100g) and lowest in T_9 (2.26 mg/100g). Based on DMRT, the zinc content in tempeh flours initially and also during the 6^{th} month of storage varied significantly among the treatments with significantly high values in T_1. There was no significant variation in the zinc content of tempeh flours with each treatment after storage.

4.5.2 *in vitro* protein digestibility (IVPD) of tempeh flours

IVPD of the tempeh flours were analysed initially and also during the 6^{th} month of storage and is presented in Table 38 and in Figure 38.

Table 38. IVPD of tempeh flours on storage

Treatments	IVPD (%)		't' value
	Initial	6^{th} month	
T_1	75.12^d	75.31^d	16.08***
T_2	90.73^b	91.02^b	5.12**
T_8	90.86^a	91.16^a	5.07**
T_9	88.84^c	89.66^c	56.66***

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
- Significant at 5% level, *- Significant at 1% level

As revealed in Table 38, by applying DMRT a significant difference in the IVPD of different tempeh flours were observed initially and also during the 6^{th} month of storage. The highest IVPD was observed in treatment T_8 initially (90.86%) and also during the 6^{th} month of storage (91.16%). The lowest IVPD was observed in the treatment T_1 initially (75.12%) and after storage (75.31%). As revealed by the't' test, a significant increase in the IVPD was observed in each tempeh flour after storage.

4.5.3. *in vitro* starch digestibility (IVSD) of tempeh flours

The IVSD of the tempeh flours were analysed initially and also during the 6^{th} month of storage and is presented in Table 39 and in Figure 39.

Table 39. IVSD of tempeh flours on storage

Treatments	IVSD (%)		't' value
	Initial	6^{th} month	
T_1	72.18^d	72.89^d	12.51***
T_2	83.08^b	83.24^b	27.7***
T_8	81.23^c	81.80^c	12.341***
T_9	83.86^a	84.78^a	14.34***

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%), T_9 - green gram (50%) + rice (50%)
DMRT column wise comparison
Figures with same super script have no significant difference
***- Significant at 1% level

Fig. 37. Effect of storage on the zinc content of tempeh flours

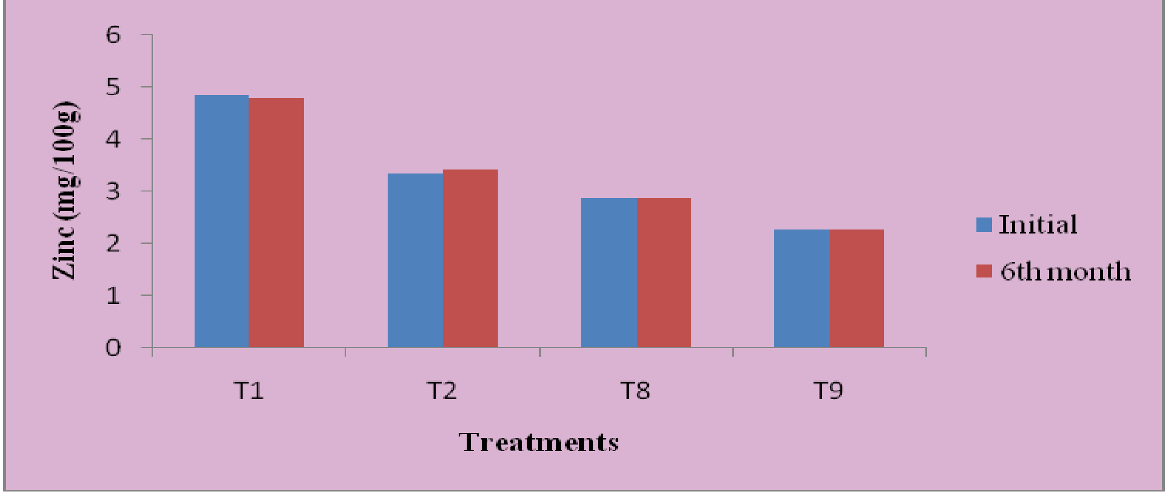
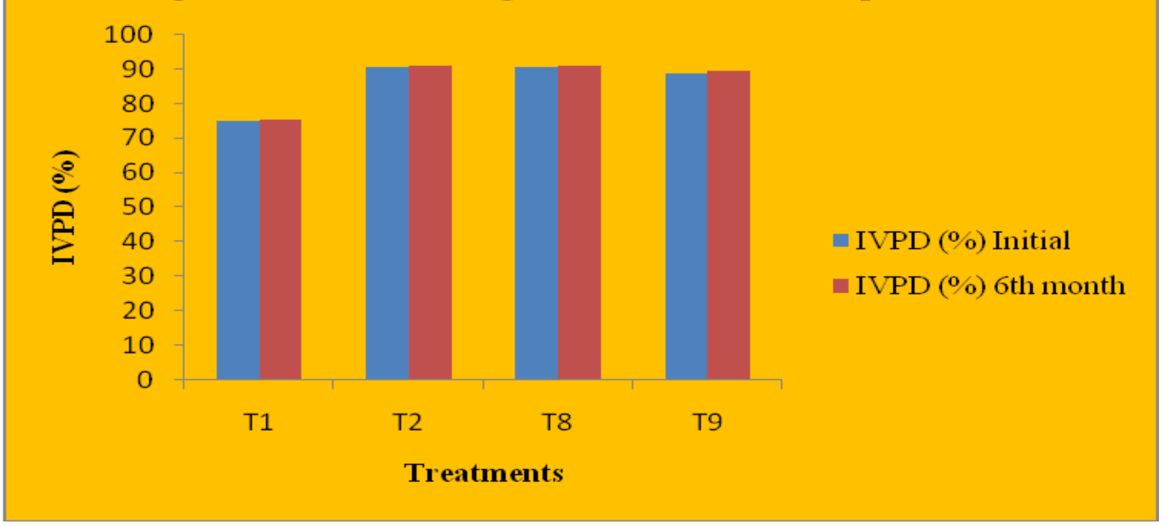


Fig. 38. Effect of storage on the IVPD of tempeh flours



Among treatments, initially the highest IVSD was observed in treatment T_9 (83.86 %) and lowest in T_1 (72.18%). Based on DMRT, a significant variation was observed in the IVSD among the tempeh flours with different treatments initially. After storage also, a significant variation in IVSD was observed among the treatments, with T_9 having significantly high IVSD (84.78%). The increase in the IVSD in each tempeh flour on storage was found to be significant.

4.5.4. Total microbial count in tempeh flours during storage

The total microbial count in tempeh flours were enumerated initially and during the 6^{th} month and the results are presented in the Table 40 and in Figures 40, 41 and 42.

Table 40. Total microbial count of tempeh flours on storage

Treatments	Bacterial count (x 10^5 cfu/g)		Yeast count (x 10^3 cfu/g)		Fungal count (x 10^3 cfu/g)	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
T_1	2.6	5.3	0.66	1.33	0.33	0.66
T_2	3.0	5.0	0.33	1.00	0.33	0.33
T_8	5.0	6.6	2.3	3.3	0.33	0.33
T_9	3.6	5.3	2.0	3.0	0	0

T_1 - 100% soybean, T_2 - 100% green gram, T_8 – green gram (75%) + rice (25%),
T_9 - green gram (50%) + rice (50%)
Values are mean of three independent determinations

As revealed in Table 40, initially, the total bacterial count (Fig.40) of tempeh flours ranged from 2.6 to 3.6 x 10^5 cfu/g with the highest bacterial count in treatment T_9 and the lowest in T_1.There was an increase in the bacterial count on storage. During the sixth month of storage, the maximum bacterial count was observed in treatment T_8 (6.6 x 10^5 cfu/g) and minimum in T_2 (5.0 x 10^5 cfu/g).

Initially the total yeast count in the tempeh flours (Table 40 and Fig. 41) was maximum in treatment T_8 (2.3 x 10^3 cfu/g) and minimum in T_2 (0. 33 x 10^3 cfu/g).

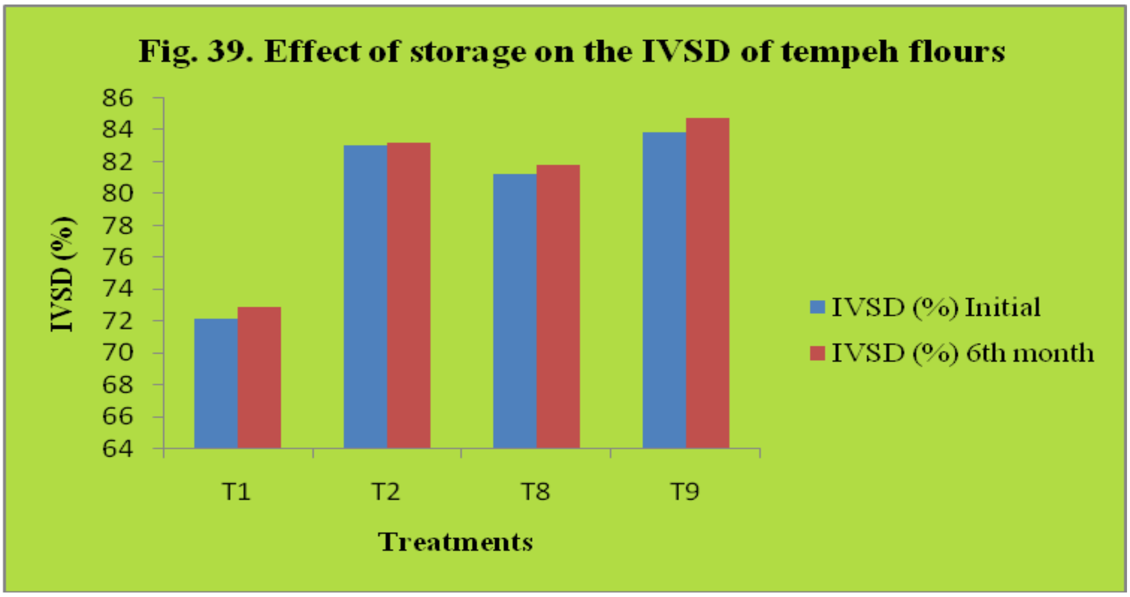


Fig. 39. Effect of storage on the IVSD of tempeh flours

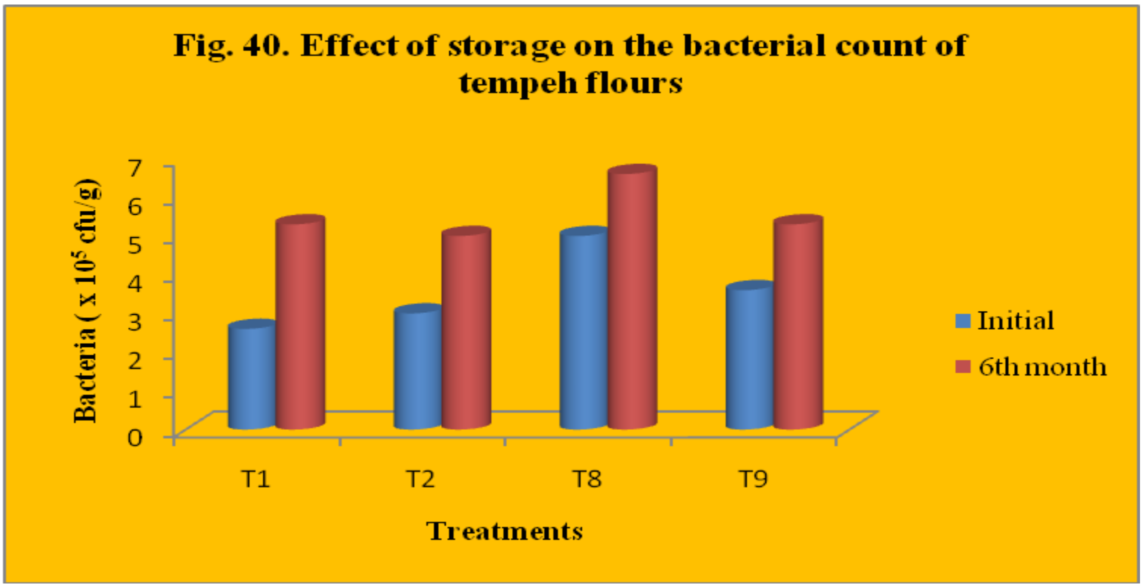


Fig. 40. Effect of storage on the bacterial count of tempeh flours

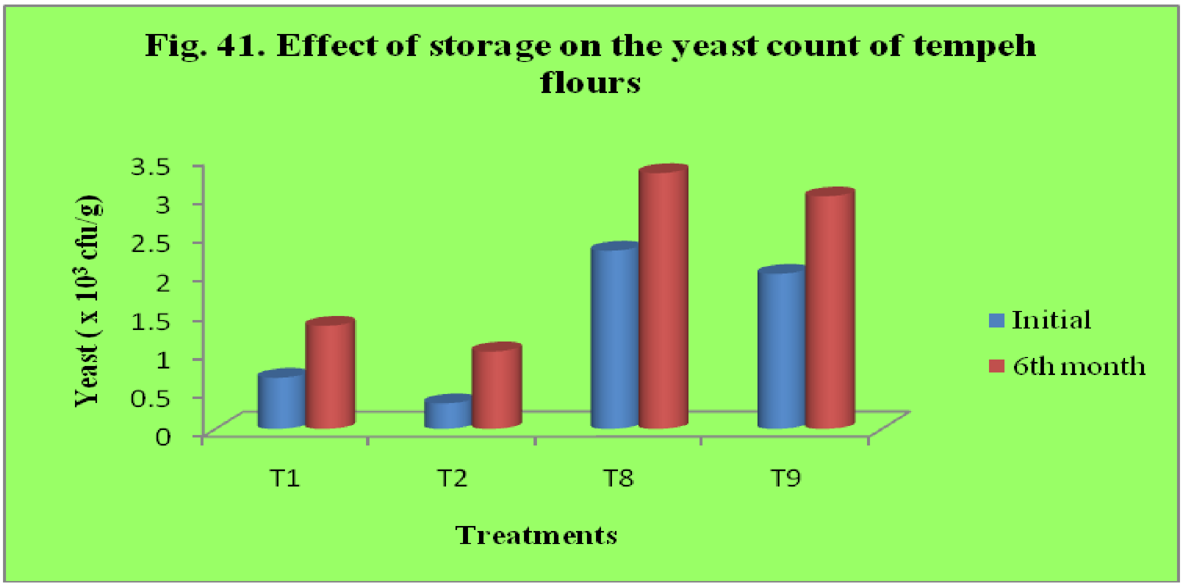


Fig. 41. Effect of storage on the yeast count of tempeh flours
Yeast (x 10³ cfu/g)
3.5
3
2.5
2
1.5
1
0.5
0
T1
T2
T8
T9
Treatments
Initial
6th month

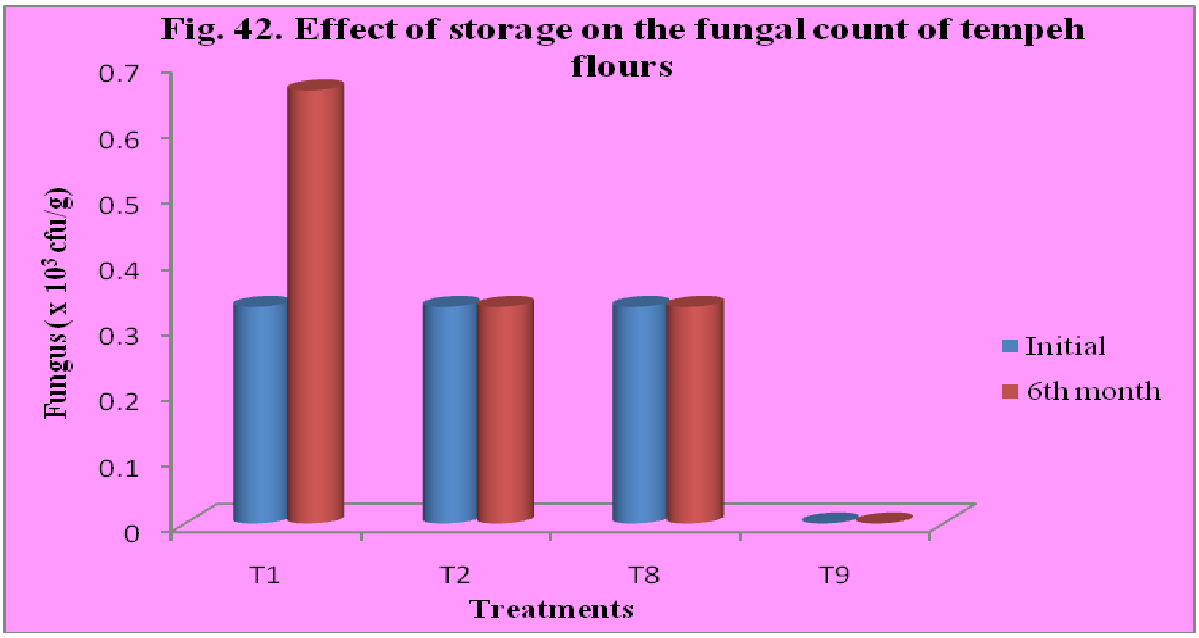


Fig. 42. Effect of storage on the fungal count of tempeh flours
Fungus (x 10³ cfu/g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
T1
T2
T8
T9
Treatments
Initial
6th month

An increase in the total yeast count was observed in all the treatments on storage. During the sixth month of storage, the total yeast count was found to be maximum in T_8 (3.3 x 10^3 cfu/g) and minimum in T_2 (1.00. x 10^3 cfu/g).

Initially, tempeh flours with treatments T_1, T_2 and T_8 had the same total fungal count (Table 40) of 0.33 x 10^3 cfu/g and no fungal growth was observed in the treatment T_9 either initially or during the sixth month of storage. During the 6^{th} month of storage, there was increase in the total fungal count in tempeh flours only with treatment T_1 (0.66 x 10^3 cfu/g) which was found to be the highest among the treatments. In all other tempeh flours, there was no change in the total fugal count on storage. The total fungal count in selected tempeh flours is illustrated in Figure 42.

4.5.5. Insect infestation of tempeh flours

The insect infestation of the stored tempeh flours was assessed initially and also during storage. Insect infestation was not observed in any of the treatments initially or during the 6^{th} month of storage. Tempeh flours after storage were of free flowing without any lump formation or caking of the flour.

4.6. Standardisation of tempeh based instant soup mixes

Tempeh flours prepared from the selected tempeh types were used for standardising soup mixes with suitable blending materials. Four different soup mixes of varying compositions (Plates 89 - 104) were prepared from each of the selected tempeh flour (T_1, T_2, T_8 and T_9). The variations were in the proportion of tempeh flour and corn flour used. For each of the selected tempeh flour (treatments), the composition of the soup mixes prepared were $S_{1(50)}$, $S_{2(55)}$, $S_{3(60)}$ and $S_{4(65)}$, the number in brackets indicating the percentage of tempeh flour used in each treatment. The composition of the soup mixes prepared with treatments T_1, T_2, T_8 and T_9 are given in Table 1 in Chapter 3.

Compositions of soup mixes prepared with T_1 tempeh flour (100% soybean)

Plate 89. $S_{1(50)}$

Plate 90. $S_{2(55)}$

Plate 91. $S_{3(60)}$

Plate 92. $S_{4(65)}$

Compositions of soup mixes prepared with T_2 tempeh flour (100% green gram)

Plate 93. $S_{1(50)}$

Plate 94. $S_{2(55)}$

Plate 95. $S_{3(60)}$

Plate 96. $S_{4(65)}$

Compositions of soup mixes prepared with T_8 tempeh flour (green gram 75% + rice 25%)

Plate 97. $S_{1(50)}$

Plate 98. $S_{2(55)}$

Plate 99. $S_{3(60)}$

Plate 100. $S_{4(65)}$

Compositions of soup mixes prepared with T_8 tempeh flour (green gram 50% + rice 50%)

Plate 101. $S_{1(50)}$

Plate 102. $S_{2(55)}$

Plate 103. $S_{3(60)}$

Plate 104. $S_{4(65)}$

Plate 105. Soup mix stored in metalized polyester laminated pouches

4.6.1. Evaluation of chemical composition of soup mixes

The soup mixes after blending with the prepared vegetable mix, spice mix and other ingredients as indicated in Table 1, were packed in metalised polyester laminate pouches and were stored for six months under ambient conditions (Plate 105). The chemical constituents of the soup mixes were studied initially, and during the 6th month of storage. In each treatment, the variations in the chemical constituents among the different compositions of the soup mixes were statistically analysed by applying DMRT, both initially and during the 6th month of storage. The variations in the chemical constituents of each composition of soup mixes in a treatment initially and after the storage period was compared by applying paired‘t’ test.

4.6.1.1. Moisture

The moisture content of the soup mixes are presented in Table 41 and in Figure 43.

Table 41. Moisture content of soup mixes on storage

Compositions	Moisture (%)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	6.86^a	^{NS}7.00^a	6.60^a	^{NS} 6.70^a	6.86^a	^{NS} 6.90^a	6.67^a	^{NS} 6.96^a
$S_{2(55)}$	7.30^a	^{NS} 7.53^a	6.80^a	^{NS} 6.93^a	7.13^a	^{NS} 7.30^a	6.50^a	**7.10^a
$S_{3(60)}$	6.60^a	^{NS} 6.83^a	6.73^a	^{NS} 7.00^a	6.60^a	^{NS} 7.26^a	6.46^a	***7.13^a
$S_{4(65)}$	6.50^a	^{NS} 6.53^a	6.60^a	^{NS} 6.96^a	6.73^a	**7.30^a	6.53^a	*7.10^a

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
*** - Significant at 1% level, ** - Significant at 5% level, * - Significant at 10% level
NS – Not significant

Initially, the moisture content of different compositions of soup mixes prepared with tempeh flour T_1 varied from 6.50 to 7.30 per cent without significant variation among different compositions. During the 6th month of storage, the moisture content showed an increase in all the compositions which varied from 6.53 to 7.53 per cent without significant variation among themselves. The increase in the moisture content observed in each composition of soup mixes after storage was not significant in treatment T_1.

The initial moisture content of different compositions of soup mixes prepared with tempeh flour T_2 varied from 6.60 to 6.80 per cent without any significant variation among the compositions. During the 6th month of storage, there was an increase in the moisture content in all the compositions which varied from 6.70 to 7.00 per cent and showed no significant variation among them. The increase in moisture content observed in each of the soup mixes after storage was not significant.

In the soup mixes prepared with T_8, the initial moisture content ranged from 6.60 to 7.13 without significant variation among the compositions. During the 6th month of storage, the moisture content showed an increase in all the compositions which varied from 6.90 to 7.30 per cent without significant variation among themselves. However, the increase in moisture content observed in composition $S_{4(65)}$ after storage was found to be significant in treatment T_8.

Initially, the moisture content of different compositions of soup mixes prepared with T_9 varied from 6.46 to 6.67 without significant variation among themselves. During the 6th month of storage, the moisture content showed an increase in all the compositions which varied from 6.96 to 7.13 per cent without significant variation among themselves. The increase in moisture content of soup mixes observed after storage were found to be significant in the compositions $S_{2(55)}$, $S_{3(60)}$ and $S_{4(65)}$ in treatment T_9.

4.6.1.2. Protein

The protein content of the soup mixes are presented in Table 42 and in Figure 44.

Table 42. Protein content of soup mixes on storage

Compositions	Protein (g/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	30.63^c	$^{NS}29.60^b$	13.67^d	$^{NS}13.29^c$	11.46^d	$**10.26^d$	6.73^d	$**5.53^d$
$S_{2(55)}$	31.58^c	$^{NS}30.67^b$	14.93^c	$**13.67^{bc}$	12.61^c	$**11.74^c$	8.56^c	$*7.88^c$
$S_{3(60)}$	34.23^b	$*32.92^a$	15.77^b	$*14.17^b$	13.47^b	$^{NS}13.13^b$	9.40^b	$^{NS}9.09^b$
$S_{4(65)}$	36.08^a	$^{NS}34.65^a$	17.13^a	$^{NS}17.00^a$	15.85^a	$^{NS}14.58^a$	10.54^a	$^{NS}10.13^a$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
** - Significant at 5% level, * - Significant at 10% level
NS – Not significant

Initial protein content of different compositions of soup mixes (Table 42) prepared from T_1 varied from 30.63 to 36.08 g/100g with the lowest protein content in $S_{1(50)}$ and the highest in $S_{4(65)}$. The soup mix $S_{4(65)}$ had significantly high protein content than all the other compositions and the protein content of $S_{1(50)}$ and $S_{2(55)}$ did not vary significantly among themselves but had the least protein content. After storage, the protein content showed a decrease in all the compositions which varied from 29.60 to 34.65 g/100g with the lowest protein content in $S_{1(50)}$ and the highest in $S_{4(65)}$. There was no significant difference between the compositions $S_{1(50)}$ and $S_{2(55)}$ and between $S_{3(60)}$ and $S_{4(65)}$. After the storage period, a reduction in protein content observed in each composition, was significant only in $S_{3(60)}$.

Fig. 43. Effect of storage on the moisture content of soup mixes

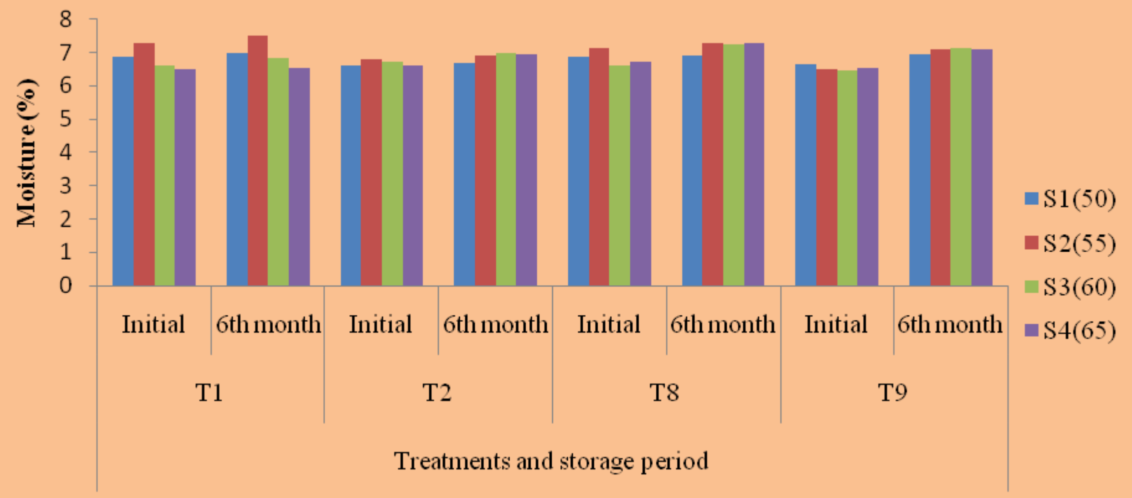
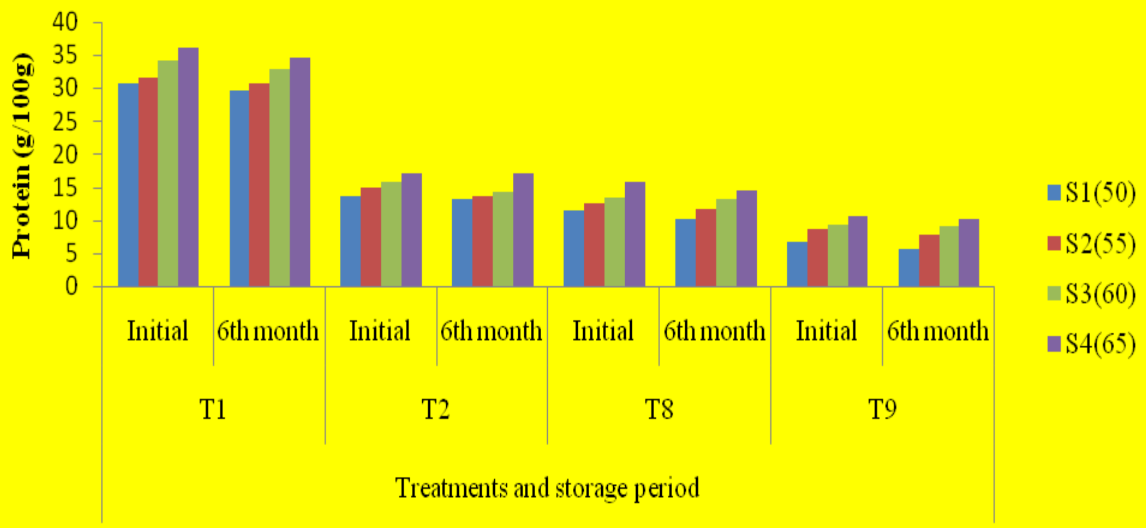


Fig. 44. Effect of storage on the protein content of soup mixes



In the soup mixes prepared with T_2, the initial protein content was highest in the soup mix composition $S_{4(65)}$ (17.13 g/100g) and the lowest was in $S_{1(50)}$ (13.67 g/100g). Significant difference in the protein content was observed among all the soup mixes prepared with T_2. During the 6th month of storage, the protein content showed a decrease in all the compositions and varied from 13.29 to 17.00 g/100g with the lowest protein content in $S_{1(50)}$ and the highest in $S_{4(65)}$ respectively. Protein content was significantly high in $S_{4(65)}$ among all the compositions after storage. The reduction in protein content observed in each composition of soup mixes after storage was found to be significant in $S_{2(55)}$ and $S_{3(60)}$.

There was a significant variation in the initial protein content of all the soup mixes prepared with T_8 which varied from 11.46 to 15.85 g/100g with the lowest and highest protein content in $S_{1(50)}$ and $S_{4(65)}$ respectively. After storage, the protein content showed a reduction in all the compositions which varied between 10.26 to 14.58 g/100g. The highest protein content after storage was in the composition $S_{4(65)}$ and the lowest was in $S_{1(50)}$. After the storage period, the reduction in protein content observed in compositions $S_{1(50)}$ and $S_{2(55)}$ was found to be significant.

Initially, the protein content of soup mixes prepared with T_9 varied between 6.73 to 10.54 g/100g with the lowest and highest protein content in $S_{1(50)}$ and $S_{4(65)}$ respectively and showed significant variation in the protein content among them. After storage, the protein content showed a reduction in all the compositions which varied from 5.53 to 10.13 g/100g and showed significant variation among the compositions. Protein content was significantly high in $S_{4(65)}$ and significantly low in $S_{1(50)}$. The reduction in protein content observed after storage in each composition was found to be significant in $S_{1(50)}$ and $S_{2(55)}$.

4.6.1.3. Starch

The starch content of the soup mixes are presented in Table 43 and in Figure 45.

Table 43. Starch content of soup mixes on storage

Compositions	Starch (g/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
$S_{1(50)}$	23.52^a	$***21.87^a$	42.00^a	^{NS} 41.04^a	51.74^a	$*50.63^a$	53.33^a	$***51.45^a$
$S_{2(55)}$	20.35^b	$*19.07^b$	40.23^b	$*39.19^b$	49.23^b	$*48.23^b$	51.44^b	$**50.25^b$
$S_{3(60)}$	18.33^c	$*17.44^c$	38.01^c	$**36.66^c$	47.68^c	$**46.20^c$	49.78^c	^{NS} 49.63^c
$S_{4(65)}$	16.96^d	$^{NS}16.67^d$	36.58^d	$**35.51^d$	46.46^d	$***45.21^d$	49.08^c	$^{NS}48.71^d$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
*** - Significant at 1% level, ** - Significant at 5% level, * - Significant at 10% level
NS – Not significant

Initially, the starch content of different compositions of soup mixes with tempeh flour T_1 varied from 16.96 to 23.52 g/100g with the lowest starch content in $S_{4(65)}$ and the highest in $S_{1(50)}$ and showed significant difference among different compositions. During the 6^{th} month of storage, the starch content showed a reduction and varied from 16.67 to 21.87 g/ 100g with a significant variation among themselves. The lowest starch content was in $S_{4(65)}$ and the highest was in $S_{1(50)}$. The reduction in starch content observed after storage in each composition was found to be significant except in $S_{4(65)}$.

The soup mixes prepared with T_2 had an initial starch content which ranged from 36.58 to 42.00 g/100g with the highest and lowest in $S_{1(50)}$ and $S_{4(65)}$ respectively with a significant variation in the starch content among the compositions. After storage, there was a reduction in the starch content of all the compositions which ranged from 35.51 to 41.04 g/100g with a significant variation among themselves. Maximum starch content was in $S_{1(50)}$ and the lowest in $S_{4(65)}$. After storage, the reduction in starch content observed in each composition was found to be significant except in $S_{1(50)}$.

Significant variation in the initial starch content of all the soup mix compositions prepared with T_8 was observed with the highest starch content of 50.63 g/100g in $S_{1(50)}$ and the lowest of 45.21 g/100g in $S_{4(65).}$ After storage, the starch content showed a reduction in all the compositions which varied from 45.21 to 50.63 g/100g and also showed significant variation among the compositions. Starch content was significantly high in $S_{1(50)}$ and significantly low in $S_{4(65).}$ The decrease in starch content observed in each composition after storage was found to be significant in all the compositions with T_8.

In the soup mixes prepared with T_9, no significant difference in the initial starch content was observed among the compositions $S_{3(60)}$ and $S_{4(65).}$ Maximum starch content of 53.33 g/100g was observed in $S_{1(50)}$ which was significantly high and the lowest (49.08 g/100g) in $S_{4(65).}$ After the storage period, there was a reduction in the starch content in all the compositions which varied from 48.71 g/100g in $S_{1(50)}$ to 51.45 g/100g in $S_{4(65)}$ with significant variation among all the compositions. After storage, the reduction in starch content observed in compositions $S_{1(50)}$ and $S_{2(55)}$ were found to be significant.

4.6.1.4. Fiber

The fiber content of the soup mixes are presented in Table 44 and in Figure 46.

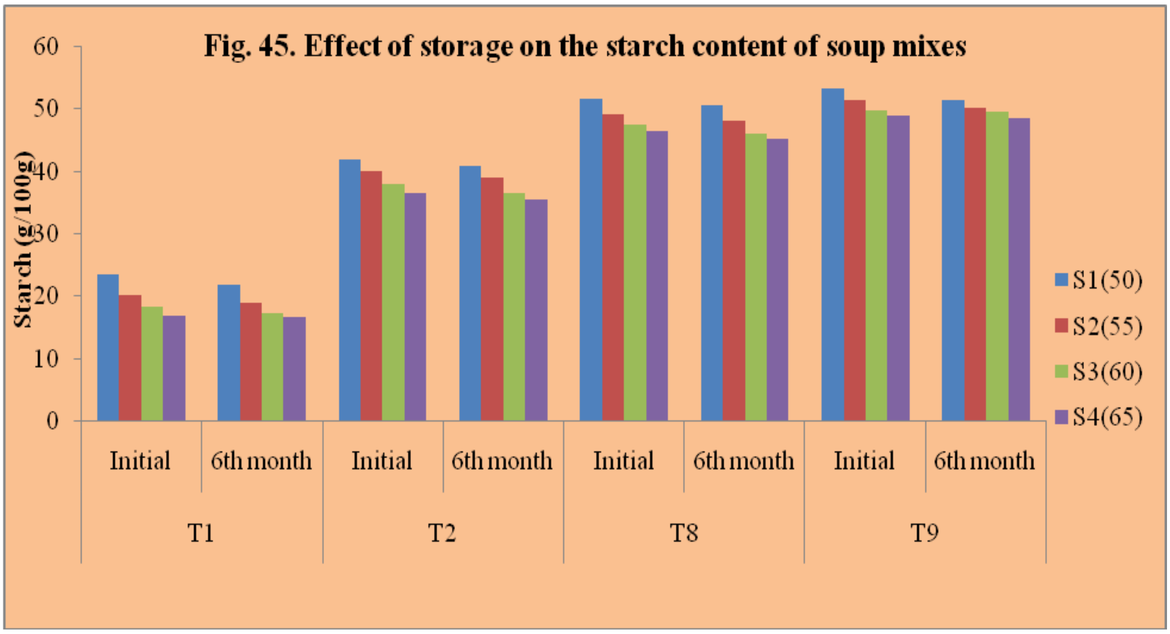


Fig. 45. Effect of storage on the starch content of soup mixes

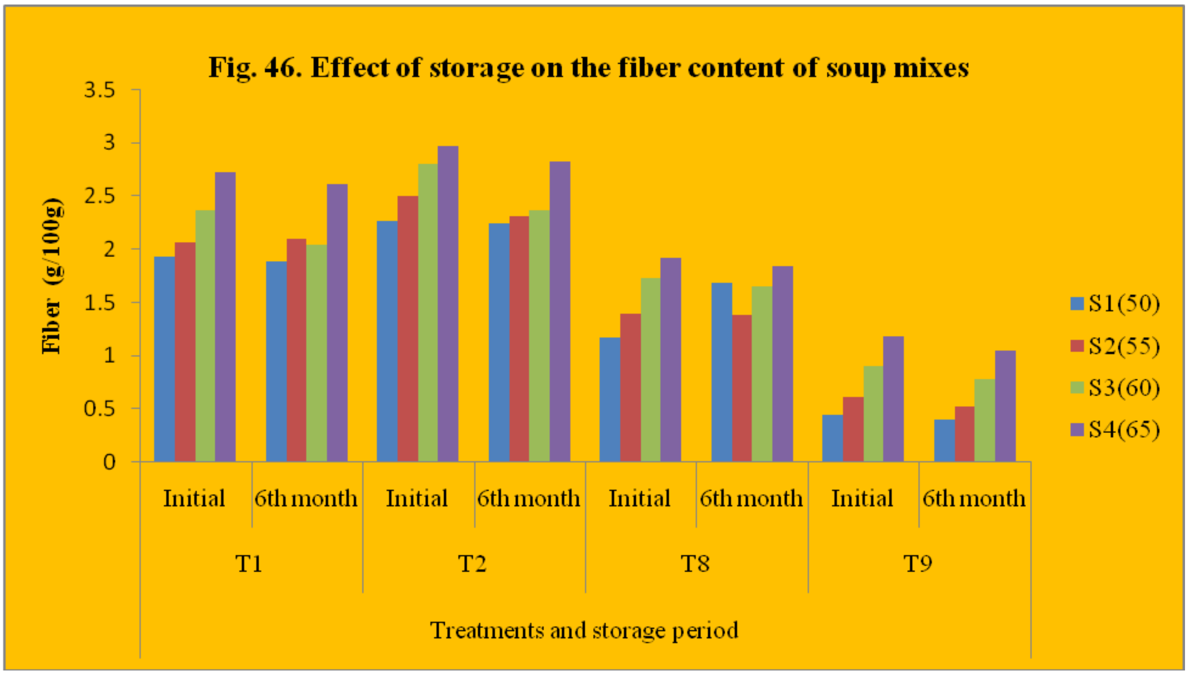


Fig. 46. Effect of storage on the fiber content of soup mixes

Table 44. Fiber content of soup mixes on storage

Compositions	Fiber (g/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	1.93^c	^{NS} 1.89^d	2.27^c	^{NS} 2.25^c	1.17^c	$**1.69^b$	0.45^c	$^{NS}0.40^a$
$S_{2(55)}$	2.07^c	^{NS} 2.10^b	2.50^b	$**2.32^b$	1.40^b	$^{NS}1.39^c$	0.61^c	$^{NS}0.52^a$
$S_{3(60)}$	2.37^b	$*2.05^c$	2.81^a	$***2.37^b$	1.73^a	$^{NS}1.66^b$	0.91^b	$^{NS}0.78^a$
$S_{4(65)}$	2.73^a	^{NS} 2.62^a	2.97^a	$*2.83^a$	1.92^a	$^{NS}1.84^a$	1.18^a	$^{NS}1.05^a$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
*** - Significant at 1% level, ** - Significant at 5% level, * - Significant at 10% level
NS – Not significant

The initial fiber content in different compositions of the soup mixes prepared with T_1 varied from 1.93 to 2.73 g/100g with the lowest fiber content in $S_{1(50)}$ and the highest in $S_{4(65)}$. Fiber content was significantly high in $S_{4(65)}$ followed by $S_{3(60)}$. Significant variation was not observed in the fiber content of $S_{1(50)}$ and $S_{2(55)}$. During the 6th month of storage, the fiber content (Table 44) in the soup mixes prepared with T_1 ranged from 1.89 to 2.62 g/100g with the lowest fiber content in $S_{1(50)}$ and the highest in $S_{4(65)}$ and showed significant variation among the different compositions of soup mixes. After the storage period, the reduction in the fiber content observed in $S_{3(60)}$ was found to be significant.

The initial fiber content was found to be maximum in the soup mixes prepared with the tempeh flour T_2 and this ranged from 2.27 to 2.97 g/100g with the lowest and highest in $S_{1(50)}$ and $S_{4(65)}$ respectively. Statistical analysis showed no significant variation among the compositions $S_{3(60)}$ and $S_{4(65)}$. After storage, there was a reduction in the fiber content in all the compositions. The fiber content ranged from 2.25 to 2.83 g/100g with the lowest fiber content in $S_{1(50)}$ and the highest in $S_{4(65)}$. Significant variation in the fiber content was observed among the compositions except in $S_{2(55)}$ and $S_{3(60)}$ which had no significant variation among them. The reduction in fiber content observed after storage in each composition was found to be significant except in $S_{1(50).}$

In the case of soup mixes prepared with different compositions from T_8, the initial fiber content was highest (1.92 g/100g) in $S_{4(65)}$ and lowest (1.17 g/100g) in $S_{1(50).}$ Among the different compositions, there was a significant variation in the fiber content of the soup mixes but the difference observed in $S_{3(60)}$ and $S_{4(65)}$ were not significant. During the 6th month of storage, the highest fiber content of 1.84 g/100g was in $S_{4(65)}$ and the lowest (1.69 g/100g) in $S_{1(50)}$. After storage, significant difference in the fiber content was observed in the soup mixes prepared with T_8, except among $S_{1(50)}$ and $S_{3(60}$. The variation observed in the fiber content of each composition after storage was significant only in $S_{1(50).}$

Initially, the fiber content of different compositions of soup mixes with tempeh flour T_9 varied from 0.45 g/100g in $S_{1(50)}$ to 1.18 g/100g in $S_{4(65)}$. DMRT showed significant variation among the compositions except among $S_{1(50)}$ and $S_{2(55).}$ After the storage, the fiber content showed a reduction in all the compositions which varied from 0.40 g/100g in $S_{1(50)}$ to 1.05 g/100g in $S_{4(65)}$ without significant variation among the compositions. The reduction in the fiber content observed in each composition after storage was not significant.

4.6.1.5. Total fats

The total fat content of the soup mixes are presented in Table 45 and in Figure 47.

Table 45. Total fat content of soup mixes on storage

Compositions	Total fat (g/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	13.66^{c}	$^{NS}13.73^{c}$	0.76^{b}	^{NS} 0.80^{c}	0.87^{a}	$^{NS}0.80^{a}$	0.73^{a}	$^{NS}0.67^{a}$
$S_{2(55)}$	14.66^{bc}	$^{NS}14.83^{bc}$	0.93^{b}	^{NS} 0.89^{c}	0.90^{a}	$^{NS}0.85^{a}$	0.87^{ab}	$^{NS}0.82^{a}$
$S_{3(60)}$	16.00^{ab}	$^{NS}16.13^{ab}$	1.13^{a}	$^{NS}1.07^{b}$	0.93^{a}	$^{NS}0.88^{a}$	0.93^{a}	$^{NS}0.87^{a}$
$S_{4(65)}$	17.93^{a}	$^{NS}17.93^{a}$	1.20^{a}	$^{NS}1.26^{a}$	1.00^{a}	$^{NS}0.87^{a}$	1.02^{a}	$^{NS}0.87^{a}$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
NS – Not significant

The initial fat content of different compositions of soup mixes prepared with the tempeh flour T_1 ranged from 13.66 to 17.93 g/100g with the lowest and highest fat content in $S_{1(50)}$ and $S_{4(65)}$ respectively. Statistical analysis showed that the composition $S_{4(65)}$ had significantly high fat content and $S_{3(60)}$ was found to be on par with $S_{4(65)}$. The fat content of $S_{1(50)}$ was found to be on par with $S_{2(55)}$. After storage, the total fat content (Table 45) was found to be maximum in the soup mixes prepared with the tempeh flour T_1 and ranged from 13.73 g/100g in $S_{1(50)}$ to 17.93 g/100g in $S_{4(65)}$. The fat content of the composition $S_{3(60)}$ was found to be on par with $S_{4(65)}$ and the composition $S_{1(50)}$ was found to be on par with $S_{2(55)}$. The variation observed in the fat content of each composition after storage was not significant.

Initially, in the soup mixes prepared with T_2 (Table 45), the lowest fat content of 0.76 g/100g was observed in composition $S_{1(50)}$ and the highest of 1.20 g/100g was observed in $S_{4(65)}$. Among compositions, significant variation in the fat content was not observed between $S_{1(50)}$ and $S_{2(55)}$ and also between $S_{3(60)}$ and $S_{4(65)}$. After storage, the lowest fat content of 0.80 g/100g was observed in composition $S_{1(50)}$ and the highest of 1.26 g/100 was observed in $S_{4(65)}$. Among different compositions, significant variations were not observed among $S_{1(50)}$ and $S_{2(55)}$. The variations observed in the total fat content of each composition after storage was not significant.

Regarding the soup mixes prepared with T_8 (Table 45), initially the total fat content ranged from 0.87 to 1.00 g/100g with no significant variation among the treatments. After storage, the fat content showed a reduction in all the compositions which ranged from 0.80 to 0.88 g/100g with no significant variation among them. The reduction in the total fat content after storage in each composition was not significant.

In the case of soup mixes prepared with T_9 (Table 45), the initial total fat content varied from 0.73 to 1.00 g/100g and showed no significant variation among them. A reduction in the fat content was observed after storage which ranged from 0.67 to 0.87 g/100g without significant variation among different compositions. After storage, there was no significant variation in the fat content in all the compositions.

4.6.1.6. Thiamine

Thiamine content was not detected in any of the soup mixes.

4.6.1.7. Riboflavin

The riboflavin content of the soup mixes are presented in Table 46 and in Figure 48.

Table 46. Riboflavin content of soup mixes on storage

Compositions	Riboflavin (mg/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
$S_{1(50)}$	0.025^a	$^{**}0.0084^b$	0.0050^b	$^{NS}0.0036^c$	0.0005^b	ND	0.0002^a	ND
$S_{2(55)}$	0.029^a	$^{***}0.011^{ab}$	0.0070^b	$^{NS}0.0062^c$	0.0008^b	ND	0.0005^a	ND
$S_{3(60)}$	0.051^a	$^{***}0.016^a$	0.016^a	$^{NS}0.012^b$	0.0050^a	ND	0.0007^a	ND
$S_{4(65)}$	0.054^a	$^{*}0.013^{ab}$	0.030^a	$^{NS}0.028^a$	0.0053^a	ND	0.001^a	ND

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
*** - Significant at 1% level, ** - Significant at 5% level, * - Significant at 10% level
NS – Not significant
ND- Not detected

The initial riboflavin content in soup mixes prepared with tempeh flour T_1 (Table 46) ranged from 0.025 to 0.054 mg/100g and showed no significant difference among different compositions. After the 6^{th} month of storage, the riboflavin content showed a reduction in all the compositions which varied from 0.0084 in $S_{1(50)}$ to 0.016 mg/100g in $S_{3(60)}$. DMRT showed that riboflavin was significantly high in $S_{3(60)}$, and compositions $S_{2(55)}$ and $S_{4(65)}$ were on par with $S_{3(60)}$. After storage, the reduction in riboflavin content observed in each composition was found to be significant.

The soup mixes prepared with T_2 (Table 46) had the highest initial riboflavin content of 0.030 mg/100g in $S_{4(65)}$ and the lowest (0.0050 mg/100g) in $S_{1(50)}$. Riboflavin was significantly high in $S_{4(65)}$ and $S_{3(60)}$. A reduction in the riboflavin content was observed in all the compositions after storage. During the 6^{th} month, the highest riboflavin content (0.028 mg/100g) was observed in $S_{4(65)}$ and the lowest (0.0036 mg/100g) in $S_{1(50)}$. $S_{4(65)}$ showed significantly high riboflavin content among the compositions. After storage, the reduction in riboflavin content observed in each composition was not significant.

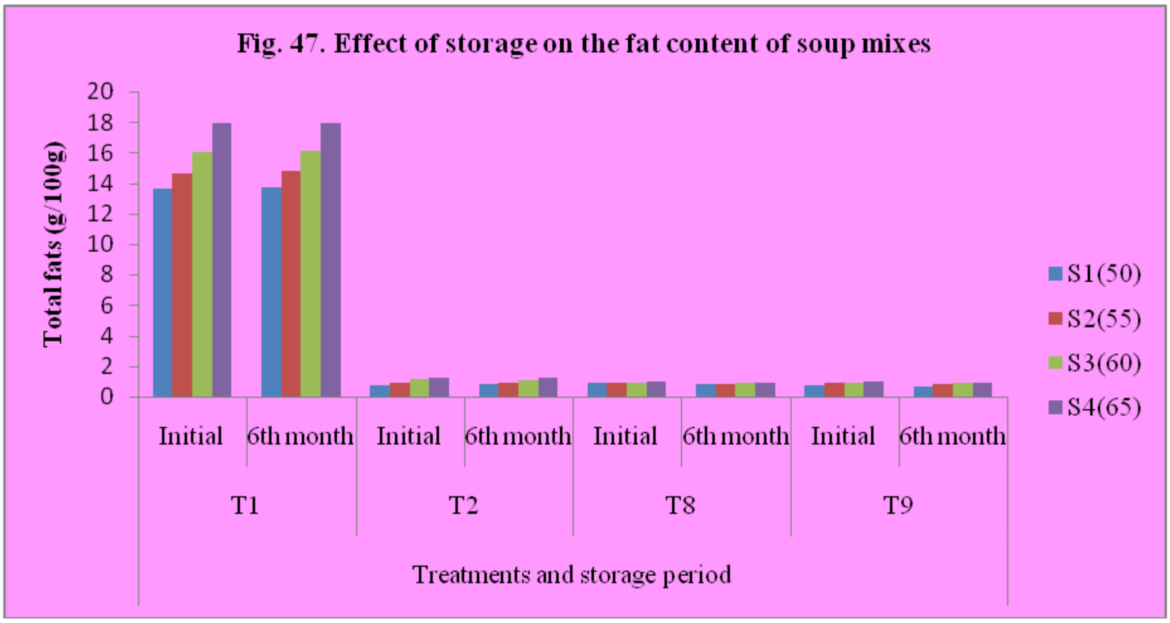


Fig. 47. Effect of storage on the fat content of soup mixes
Total fats (g/100g)
0
2
4
6
8
10
12
14
16
18
20
Initial
6th month
T1
T2
T8
T9
Treatments and storage period
S1(50)
S2(55)
S3(60)
S4(65)

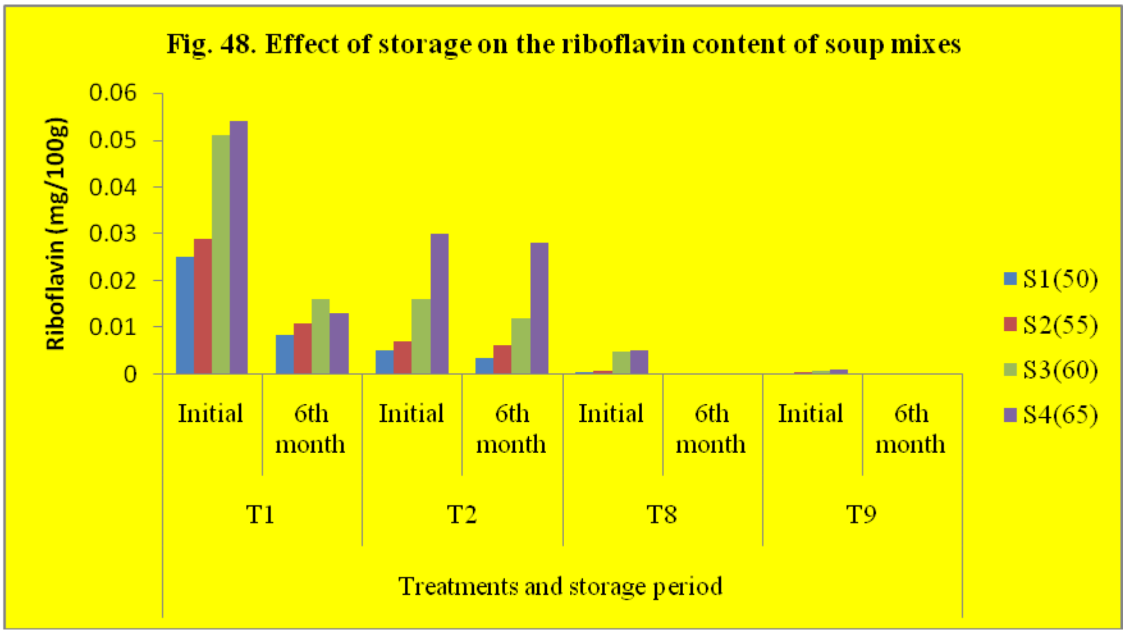


Fig. 48. Effect of storage on the riboflavin content of soup mixes
Riboflavin (mg/100g)
0
0.01
0.02
0.03
0.04
0.05
0.06
Initial
6th month
T1
T2
T8
T9
Treatments and storage period
S1(50)
S2(55)
S3(60)
S4(65)

Initially, the riboflavin content of soup mixes with tempeh flour T_8 (Table 46) varied from 0.0005 to 0.0053 mg/100g; the maximum in $S_{4(65)}$ and the minimum in $S_{1(50)}$. Riboflavin in $S_{4(65)}$ was significantly high and DMRT showed that $S_{3(60)}$ was on par with $S_{4(65)}$. After storage, riboflavin content was not detected in any of the compositions of soup mixes prepared with T_8.

In the case of soup mixes prepared with T_9 (Table 46), the initial riboflavin content varied from 0.0002 to 0.001 mg/100g and showed no significant variation among the compositions. After storage, detectable amount of riboflavin was not observed in any of the compositions of soup mixes prepared with T_9.

4.6.1.8. Calcium

The calcium content of the soup mixes are presented in Table 47 and in Figure 49.

Table 47. Calcium content of soup mixes on storage

Compositions	Calcium (mg/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	261.87^d	$^{NS}259.35^d$	72.77^d	$^{NS}70.69^d$	64.58^d	$^{NS}61.79^d$	41.93^d	$^{NS}39.96^d$
$S_{2(55)}$	285.97^c	$^{**}271.34^c$	78.69^c	$^{NS}75.86^c$	70.03^c	$^{NS}69.70^c$	48.94^c	$^{NS}48.40^c$
$S_{3(60)}$	300.47^b	$^{**}290.64^b$	91.40^b	$^{NS}87.93^b$	77.16^b	$^{NS}74.89^b$	53.58^b	$^{NS}53.04^b$
$S_{4(65)}$	314.00^a	$^{**}298.60^a$	100.20^a	$^{NS}99.20^a$	83.82^a	$^{NS}81.64^a$	62.60^a	$^{NS}61.45^a$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
** - Significant at 5% level, * - Significant at 10% level, NS – Not significant

Initially the calcium content of the soup mixes prepared with T_1 (Table 47) varied from 261.87 mg/100g in $S_{1(50)}$ to 314 mg/100g in $S_{4(65)}$ and showed significant difference among the compositions. After storage, a reduction in the calcium content was observed in all the compositions which varied from 259.35 to 298.60 mg/100g with the highest calcium content in $S_{4(65)}$ and the lowest in $S_{1(50)}$ and showed significant variation among the compositions. The reduction in calcium content observed in each composition during storage was significant except in $S_{1(50)}$.

Significant variation in the calcium content was observed in the different compositions of the soup mixes prepared with T_2 (Table 47) and had an initial calcium content which ranged from 72.77 to 100.20 mg/100g. During the 6th month of storage, the calcium content showed a reduction and maximum calcium content of 99.20 mg/100g was observed in $S_{4(65)}$ and the lowest of 70.69 mg/100g in $S_{1(50)}$ with a significant variation among the compositions. The reduction in the calcium content in each composition after storage was not significant.

In the soup mixes prepared with T_8 (Table 47), the initial calcium content was observed in the range 64.58 to 83.82 mg/100g with the lowest calcium content in $S_{1(50)}$ and the highest in $S_{4(65)}$ and showed significant variation among the compositions. During the 6th month of storage, all the compositions showed a reduction in their calcium content. After storage, the calcium content was observed in the range 61.79 to 81.64 mg/100g with the lowest and highest in $S_{1(50)}$ and $S_{4(65)}$ with significant variation among the different compositions. There was no significant reduction in the calcium content of each composition after storage.

There was a significant variation in the initial calcium content of soup mixes prepared with T_9 (Table 47) and the initial calcium content varied between 41.93 to 62.60 mg/100g. The highest calcium content was in $S_{4(65)}$ and the lowest was in $S_{1(50)}$. After storage, a reduction in the calcium content was observed and varied between 39.96 to 61.45 mg/100g with the highest calcium content in $S_{4(65)}$ and the lowest in $S_{1(50)}$. Calcium content in $S_{4(65)}$ was significantly high when compared to the other compositions after storage. After storage, the reduction in calcium content in each composition was not significant.

4.6.1.9. Iron

The iron content of the soup mixes are presented in Table 48 and in Figure 50.

Table 48. Iron content of soup mixes on storage

Compositions	Iron (mg/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	2.97^d	$^{NS}2.96^d$	1.57^b	$^{NS}1.57^b$	1.37^d	$^{NS}1.38^d$	1.10^d	$^{NS}1.12^c$
$S_{2(55)}$	3.83^c	$^{NS}3.78^c$	1.97^b	$^{NS}1.91^b$	1.77^c	$^{NS}1.72^c$	1.47^c	$^{NS}1.47^b$
$S_{3(60)}$	4.30^b	$^{NS}4.23^b$	2.17^b	$^{NS}2.15^{ab}$	2.00^b	$^{NS}2.07^b$	1.73^b	$^{NS}1.71^a$
$S_{4(65)}$	5.17^a	$^{NS}5.17^a$	2.77^a	$^{NS}2.72^a$	2.40^a	$^{NS}2.37^a$	2.07^a	$^{NS}1.90^a$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
NS – Not significant

The initial iron content of the soup mixes prepared with T_1 (Table 48) varied from 2.97 in $S_{1(50)}$ to 5.17 mg/100g in $S_{4(65)}$ and showed significant difference among the compositions. During the 6^{th} month of storage, the iron content in the soup mixes prepared with T_1 ranged from 2.96 to 5.17 mg/100g with the lowest iron content in $S_{1(50)}$ and the highest in $S_{4(65)}$ respectively and showed significant variation among the compositions. After storage, there was no significant variation in the iron content of any of the soup mixes prepared with T_1.

Initially, the iron content of soup mixes prepared with T_2 (Table 48) ranged from 1.57 to 2.77 mg/100g with the lowest iron content in $S_{1(50)}$ and the highest in $S_{4(65)}$. Iron content in $S_{4(65)}$ was found to be significantly high when compared with the other compositions of soup mixes with T_2. After storage, the highest iron content (2.72 mg/100g) was observed in $S_{4(65)}$ and the lowest (1.57 mg/100g) in $S_{1(50)}$. Iron content in $S_{4(65)}$ was significantly high and $S_{3(60)}$ was on par with $S_{4(65)}$. There was a

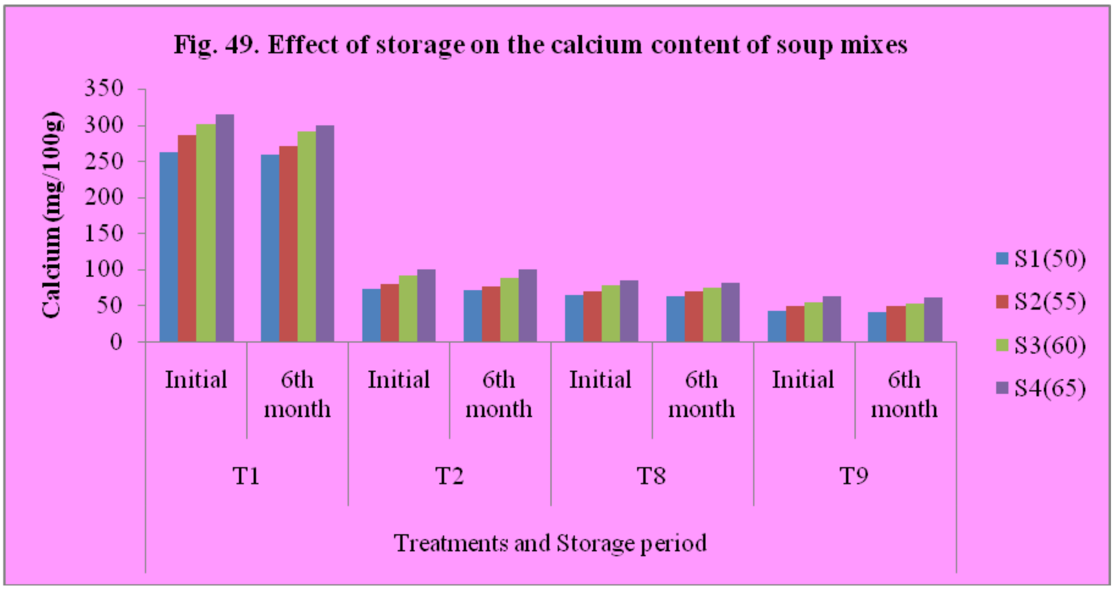


Fig. 49. Effect of storage on the calcium content of soup mixes

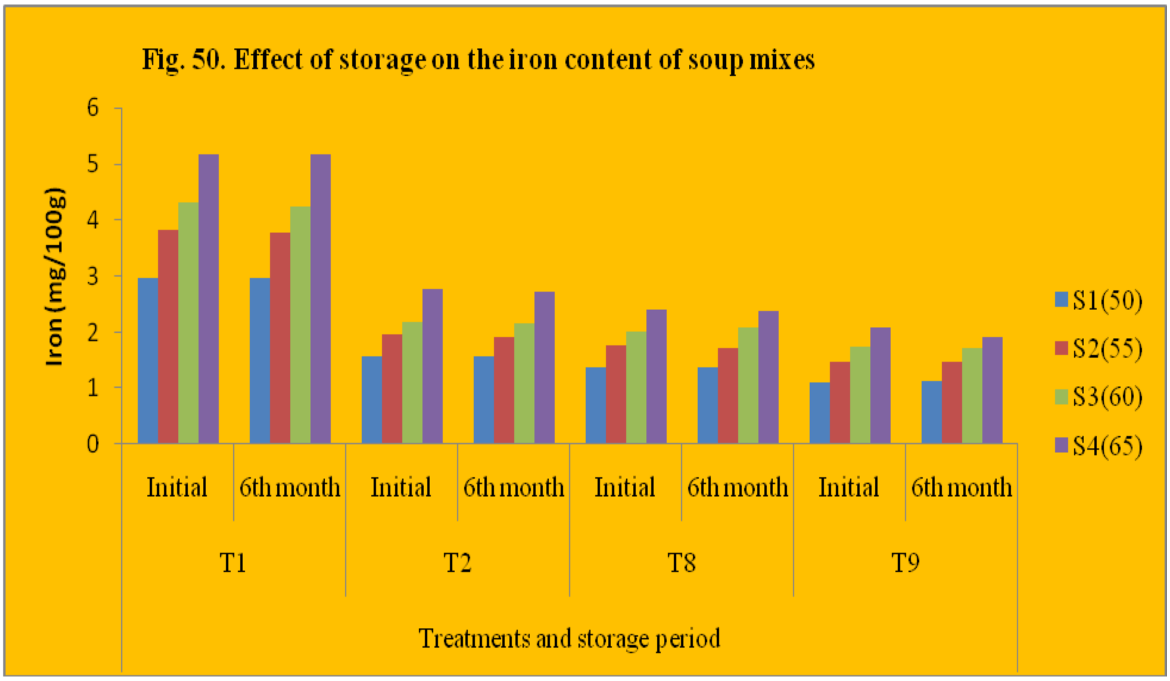


Fig. 50. Effect of storage on the iron content of soup mixes

reduction in the iron content of each composition after storage except in $S_{1(50)}$, but the reduction was not significant.

In the soup mixes prepared from T_8 (Table 48), the initial iron was in the range 1.37 mg/100g in $S_{1(50)}$ to 2.40 mg/100g in $S_{4(65)}$ and showed significant difference among the compositions. After storage, the iron content varied from 1.38 to 2.37 mg/100g with the lowest iron content in $S_{1(50)}$ and the highest in $S_{4(65)}$ with a significant variation among the compositions. The variation observed in the iron content of each composition after storage was not significant.

Significant variation in the initial iron content of the soup mix compositions prepared with T_9 was observed with the maximum iron content (2.07 mg/100g) in $S_{4(65)}$ and the lowest (1.10 mg/100g) in $S_{1(50)}$. After storage the iron content in T_9 varied from 1.12 mg/100g in $S_{1(50)}$ to 1.90 mg/100g in $S_{4(65)}$ and there was no significant variation in the iron content of the compositions $S_{3(60)}$ and $S_{4(65)}$. After storage, the iron content of the different compositions of soup mixes showed no significant difference.

4.6.1.10. Phosphorus

The phosphorus content of the soup mixes are presented in Table 49 and in Figure 51.

Table 49. Phosphorus content of soup mixes on storage

Compositions	Phosphorus (mg/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	481.70^c	$^{NS}482.03^b$	268.77^d	$*259.69^d$	262.86^d	$^{NS}261.75^d$	226.27^d	$^{NS}227.61^d$
$S_{2(55)}$	504.61^b	$**493.71^b$	288.99^c	$*282.17^c$	276.12^c	$^{NS}274.58^c$	241.14^c	$^{NS}238.56^c$
$S_{3(60)}$	521.75^b	$**495.26^b$	307.91^b	$^{NS}302.57^b$	298.45^b	$^{NS}301.88^b$	255.48^b	$^{NS}254.98^b$
$S_{4(65)}$	557.76^a	$^{NS}551.66^a$	335.89^a	$^{NS}324.08^a$	317.11^a	$^{NS}310.19^a$	268.91^a	$^{NS}270.41^a$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
** - Significant at 5% level, * - Significant at 10% level, NS – Not significant

The initial phosphorus content of different compositions of the soup mixes prepared with T_1 (Table 49) varied from 481.70 mg/100g in $S_{1(50)}$ to 557.76 mg/100g in $S_{4(65)}$ and significant difference in the phosphorus content was observed among the compositions except between $S_{2(55)}$ and $S_{3(60)}$. After storage, the phosphorus content varied from 482.03 mg/100g in $S_{1(50)}$ to 551.66 mg/100g in $S_{4(65).}$ Phosphorus content was significantly high in $S_{4(65)}$ among all other compositions. After storage, the reduction in the phosphorus content observed in compositions $S_{2(55)}$ and $S_{3(60)}$ were found to be significant.

Initially, in the soup mixes prepared with T_2 (Table 49), maximum phosphorus content of 335.89 mg/100g was in $S_{4(65)}$ and the lowest (268.77mg/100g) in $S_{1(50)}$ and showed significant difference in the phosphorus content among the compositions. During the 6th month of storage, a reduction in the phosphorus content in all the compositions was observed. The soup mixes showed a significant variation in the phosphorus content among all the compositions and had the highest phosphorus content of 324.08 mg/100g in $S_{4(65)}$ and the lowest (259.69 mg/100g) in $S_{4(65).}$ The reduction in the phosphorus content observed in each composition after storage was found to be significant only in $S_{1(50)}$ and $S_{2(55).}$

Significant variation among the compositions was observed in the soup mixes prepared with T_8 (Table 49), which had a significantly high initial phosphorus content of 317.11 mg/100g in $S_{4(65)}$ and the lowest (262.86 mg/100g) in $S_{1(50)}$. The phosphorus content during the 6^{th} month of storage was in the range of 261.75 to 310.19 mg/100g with the lowest and highest phosphorus content in $S_{1(50)}$ and $S_{4(65)}$ respectively and showed significant variation among the compositions. There was no significant variation in the phosphorus content of each composition after storage.

In the case of soup mixes prepared with T_9 (Table 49), initially significant variation in the phosphorus content was observed ranging between 226.27 mg/100g in $S_{1(50)}$ to 268.91 mg/100g in $S_{4(65)}$. After storage, the phosphorus content varied between 227.61 mg/100g in $S_{1(50)}$ to 270.41 mg/100g in $S_{4(65)}$ and showed significant variation among the compositions. No significant variation in the phosphorus content of each composition was observed after storage.

4.6.1.11. Potassium

The potassium content of the soup mixes are presented in Table 50 and in Figure 52.

Table 50. Potassium content of soup mixes on storage

Compositions	Potassium (mg/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
$S_{1(50)}$	534.02^{c}	$^{NS}531.55^{d}$	914.51^{c}	$^{NS}912.38^{d}$	654.77^{d}	$^{NS}655.35^{d}$	432.93^{d}	$^{NS}432.31^{d}$
$S_{2(55)}$	552.85^{bc}	$^{NS}552.34^{c}$	936.71^{b}	$^{NS}936.35^{c}$	675.04^{c}	$^{NS}673.83^{c}$	455.34^{c}	$^{NS}455.02^{c}$
$S_{3(60)}$	573.57^{b}	$^{NS}578.78^{b}$	955.85^{a}	$^{NS}958.65^{b}$	707.48^{b}	$^{NS}703.21^{b}$	473.24^{b}	$^{NS}473.86^{b}$
$S_{4(65)}$	602.70^{a}	$^{NS}599.92^{a}$	965.57^{a}	$^{NS}961.51^{a}$	733.62^{a}	$^{NS}732.36^{a}$	495.98^{a}	$^{NS}492.95^{a}$

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
NS – Not significant

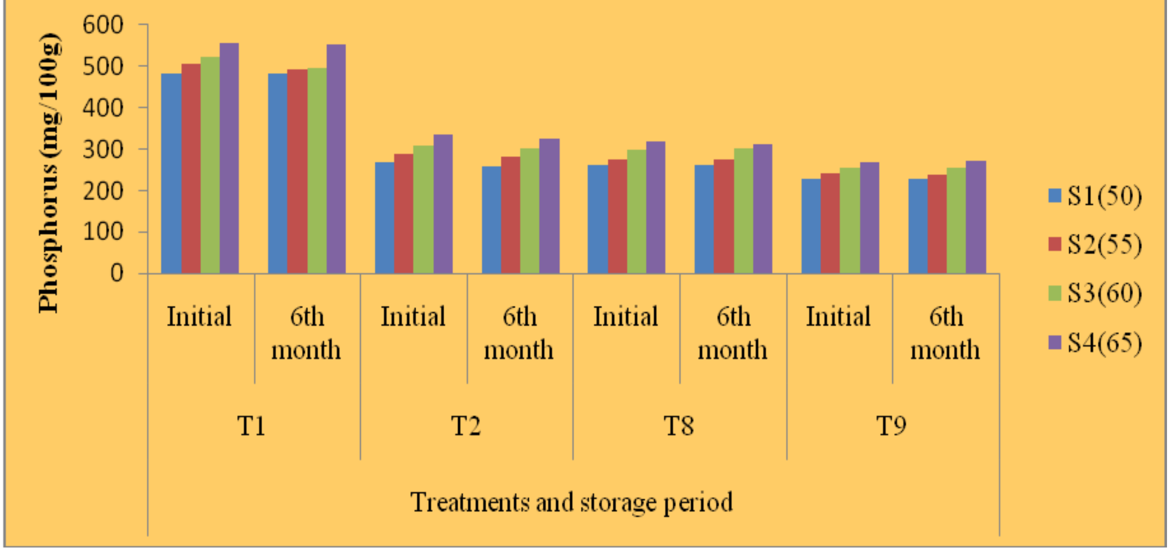


Fig. 51. Effect of storage on the phosphorus content of soup mixes

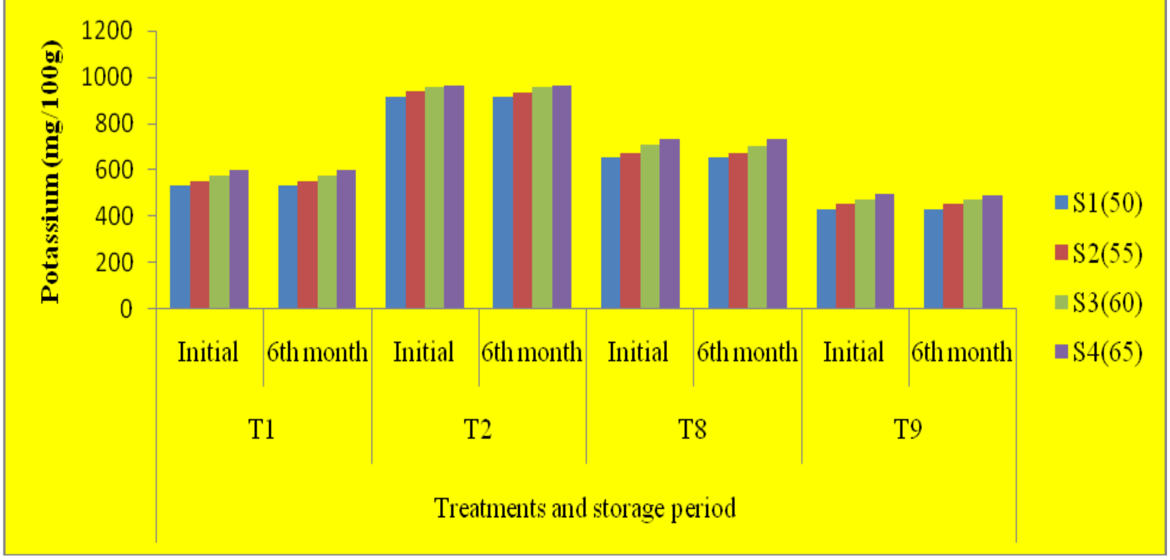


Fig. 52. Effect of storage on the potassium content of soup mixes

The initial potassium content of different compositions of soup mixes with T_1 (Table 50) varied between 534.02 mg/100g in $S_{1(50)}$ to 602.70 mg/100g in $S_{4(65)}$ with a significant variation in the potassium content among the compositions. After storage, the potassium content showed significant variation among the compositions and ranged from 531.55 to 599.92 mg/100g with the lowest and highest potassium content in $S_{1(50)}$ and $S_{4(65)}$ respectively. The changes in the potassium content in each composition after storage were not significant.

The soup mixes prepared with T_2 (Table 50), had an initial potassium content which ranged from 914.51 to 965.57 mg/100g with the lowest and highest potassium content in $S_{1(50)}$ and $S_{4(65)}$ respectively. Significant variation was observed in the potassium content among different compositions of T_2, except between $S_{3(60)}$ and $S_{4(65)}$. During the 6th month of storage, the potassium content ranged from 912.38 mg/100g in $S_{1(50)}$ to 961.51 mg/100g in $S_{4(65)}$ and showed significant variation among the compositions. After storage, the changes in the potassium content of each composition of soup mixes were not significant.

In the soup mixes prepared with T_8 (Table 50), the initial potassium content was in the range of 654.77 mg/100g in $S_{1(50)}$ to 733.62 mg/100g in $S_{4(65)}$ and showed significant variation among different compositions. During the 6th month of storage, $S_{1(50)}$ had the lowest potassium content of 655.35 mg/100g and the highest potassium content of 732.36 mg/100g was observed in $S_{4(65).}$ Significant variation in the potassium content was observed among the different compositions of soup mixes prepared with T_8. After storage, the changes observed in the potassium content of each of the soup mix composition were not significant.

Significant variation in the initial potassium content of soup mixes prepared from T_9 (Table 50) was observed with the highest potassium content of 495.98 mg/100g in $S_{4(65)}$ and the lowest 432.93 mg/100 in $S_{1(50).}$ After storage also, the potassium content showed a significant variation among different compositions and varied between 432.31 mg/100g in $S_{1(50)}$ to 492.95 mg/100g in $S_{4(65)}$. The changes observed in the potassium content of each composition during storage were not significant.

4.6.1.12. Zinc

The zinc content of the soup mixes are presented in Table 51 and in Figure 53.

Table 51. Zinc content of soup mixes on storage

Compositions	Zinc (mg/100g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	3.57^{d}	^{NS} 3.54^{d}	2.92^{b}	$^{NS}3.58^{a}$	2.21^{c}	^{NS} 2.15^{a}	2.43^{b}	^{NS} 2.33^{c}
$S_{2(55)}$	3.88^{c}	^{NS} 3.87^{c}	3.05^{ab}	^{NS} 3.08^{a}	2.33^{c}	^{NS} 2.29^{a}	2.58^{b}	^{NS} 2.50^{b}
$S_{3(60)}$	4.06^{b}	^{NS} 4.06^{b}	3.17^{ab}	^{NS} 3.17^{a}	2.54^{b}	^{NS} 2.54^{a}	2.86^{a}	^{NS} 2.75^{a}
$S_{4(65)}$	4.41^{a}	^{NS} 4.42^{a}	3.29^{a}	^{NS} 3.31^{a}	2.74^{a}	$^{NS}2.70^{a}$	2.15^{c}	^{NS} 2.11^{d}

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
DMRT column wise comparison
Figure with same superscripts form homogenous subsets
NS – Not significant

The initial zinc content in the soup mixes prepared from T_1 (Table 51) ranged from 3.57 to 4.41 mg/100g with the lowest in $S_{1(50)}$ and the highest in $S_{4(65)}$. Significant variation in the zinc content was observed among the soup mixes prepared from T_1. After storage, the zinc content varied from 3.54 to 4.42 mg/100g with the lowest zinc content in $S_{1(50)}$ and the highest in $S_{4(65)}$. Significant variation in the zinc was observed among the compositions. There was no significant variation in the zinc content in any of the soup mix compositions after storage.

In the soup mixes prepared with T_2 (Table 51), the initial zinc content varied between 2.92 to 3.29 mg/100g with the lowest zinc content in $S_{1(50)}$ and the highest in $S_{4(65)}$. Significant variation in the zinc content was observed only between $S_{1(50)}$ and $S_{4(65)}$. After storage, the soup mixes prepared with T_2 had a zinc content ranging from 3.08 mg/100g in $S_{2(55)}$ to 3.58 mg/100g in $S_{1(50)}$ with no significant variation among

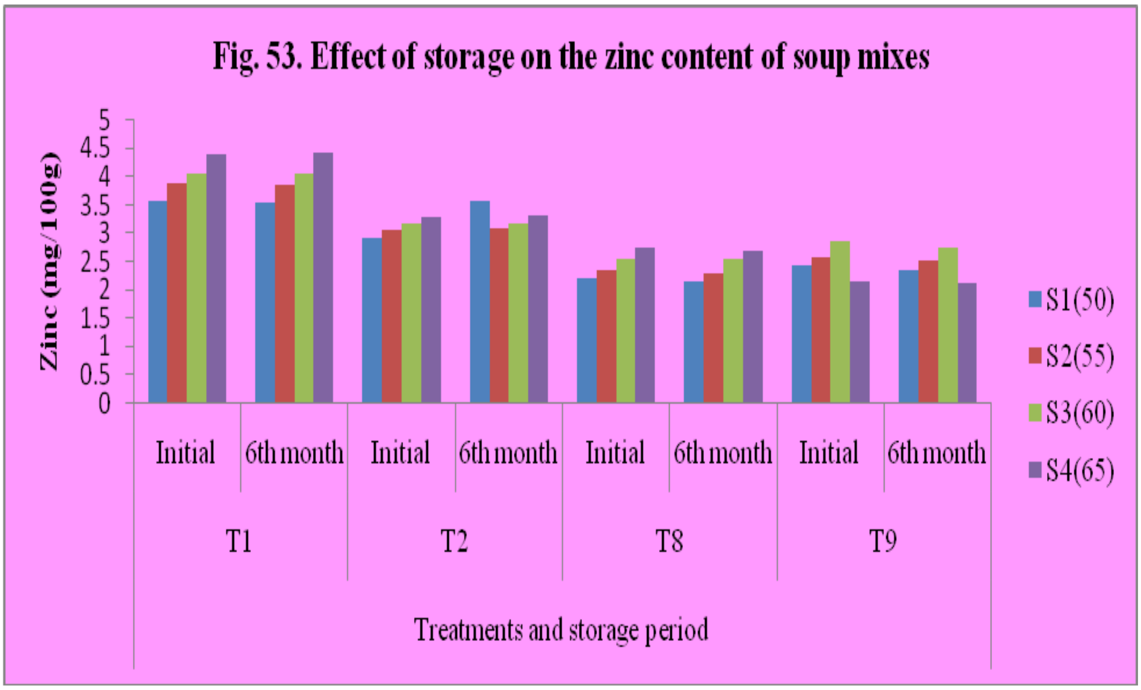


Fig. 53. Effect of storage on the zinc content of soup mixes
Zinc (mg/100g)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Initial
6th month
Initial
6th month
Initial
6th month
Initial
6th month
T1
T2
T8
T9
Treatments and storage period
S1(50)
S2(55)
S3(60)
S4(65)

different compositions. After storage, the zinc content in each composition of soup mixes showed no significant difference.

Initially, the soup mixes prepared with T_8 (Table 51), showed the highest zinc content in $S_{4(65)}$ (2.74 mg/100g) and the lowest in $S_{1(50)}$ (2.21 mg/100g). During the 6th month of storage, the zinc content ranged from 2.15 to 2.70 mg/100g and showed no significant difference among the different compositions. Significant variation in the zinc content was not observed in any of the compositions of soup mixes after storage.

In the case of soup mixes prepared with T_9 (Table 51), the initial zinc content was found to be highest (2.86 mg/100g) in $S_{3(60)}$ and the lowest (2.15 mg/100g) in $S_{4(65)}$. Zinc content in $S_{3(60)}$ was found to be significantly high among all the compositions. The zinc content after storage varied between 2.11 to 2.75 mg/100g with the lowest zinc content in $S_{1(50)}$ and the highest in $S_{4(65)}$. Significant variation in the zinc content of the soup mixes prepared with T_9 was observed among the compositions. After storage, the zinc content of each of the composition of soup mixes showed no significant change.

4.6.2. Acceptability studies

The quantity of water required to make a soup of acceptable consistency with a known quantity of the soup mix was standardised by repeated trials. The soup mix was cooked in water and the time taken was noted. The sensory evaluation of the soups were carried out and the quality attributes namely appearance, colour, flavour, consistency, taste, and overall acceptability were evaluated and acceptability of the products was studied.

4.6.2.1. Water and time required for preparing soups

The water required and the time taken to prepare a soup of acceptable consistency with the soup mixes is presented in Table 52.

Table 52. Water and time required to prepare soup with the soup mixes

Compositions	Treatments							
	T_1		T_2		T_8		T_9	
	Water (ml/100g)	Time (minutes)	Water (ml/100g)	Time (minutes)	Water (ml/100g)	Time (minutes)	Water (ml/100g)	Time (minutes)
$S_{1(50)}$	1100	5	1360	4	1460	4	1530	3
$S_{2(55)}$	1000	5	1290	4	1390	4	1440	3
$S_{3(60)}$	940	5	1220	4	1320	4	1360	3
$S_{4(65)}$	890	6	1140	4	1250	4	1280	3

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

As revealed in Table 52, with treatment T_1, water required to cook the soups with different compositions of soup mixes varied from 890 to 1100 ml/100g, maximum water was required for $S_{1(50)}$ and the minimum water requirement was for $S_{4(65)}$. Maximum time taken to cook the soup (6 minutes) was for $S_{4(65)}$. All other soup mixes were cooked in 5 minutes time.

With treatment T_2, water required varied from 1140 to 1360 ml/100g; maximum for $S_{1(50)}$ and the lowest for $S_{4(65)}$ but all the soup mix compositions took only 4 minutes to cook.

The water required to cook different compositions of soup mixes with T_8 varied from 1250 to 1460 ml/100g; the maximum for $S_{1(50)}$ and the least for $S_{4(65)}$. But all the soup mixes took only 4 minutes to cook.

With T_9 also, water required was maximum for $S_{1(50)}$ (1530 ml/100g) and the least for $S_{4(65)}$ (1280 ml/100g). All the compositions took only 3 minutes as cooking time.

4.6.2.2. Organoleptic evaluation of the soups

The sensory evaluations of the soups prepared (106 - 121) with the soup mixes initially before storage, and also after six months were carried out. The quality attributes namely appearance, colour, flavour, consistency, taste, and overall acceptability were evaluated and the results are presented in Table 53 to 58.

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Table 53. Mean score for appearance of the soups

Compositions	Appearance (Mean score)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
$S_{1(50)}$	8.4	8.3	8.3	8.3	9.0	9.0	8.4	8.4
$S_{2(55)}$	8.8	8.7	8.0	8.0	8.9	8.9	8.2	8.3
$S_{3(60)}$	8.0	8.0	8.0	7.9	8.5	8.2	8.2	8.2
$S_{4(65)}$	8.0	7.9	7.8	7.5	8.3	8.3	8.2	8.2

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

Initially, the mean score for the appearance of the soups prepared with different compositions of tempeh flour T_1 (Table 53) was found to be highest (8.8) in $S_{2(55)}$ and lowest (8.0) in $S_{3(60)}$ and $S_{4(65).}$ During the 6^{th} month of storage, there was a decrease in the mean score for appearance except in $S_{3(60).}$ After storage, the mean score for appearance of soups with T_1 varied from 7.9 to 8.7 with the lowest and highest score in $S_{4(65)}$ and $S_{2(55)}$ respectively.

The mean score for appearance of the soups prepared with the different combinations of T_2 (Table 53), varied from 7.8 to 8.3; the highest in $S_{1(50)}$ and the lowest in $S_{4(65).}$ After storage of soup mixes, the mean score for appearance showed a reduction in $S_{3(60)}$ and $S_{4(65).}$ There was no change in the mean score for appearance in

Soups prepared with soup mixes of T_1 tempeh flour (100% soybean)

Plate 106. $S_{1(50)}$

Plate 107. $S_{2(55)}$

Plate 108. $S_{3(60)}$

Plate 109. $S_{4(65)}$

Soups prepared with soup mixes of T_2 tempeh flour (100% green gram)

Plate 110. $S_{1(50)}$

Plate 111. $S_{2(55)}$

Plate 112. $S_{3(60)}$

Plate 113. $S_{4(65)}$

Soups prepared with soup mixes of T_8 tempeh flour (green gram 75% + rice 25%)

Plate 114. $S_{1(50)}$

Plate 115. $S_{2(55)}$

Plate 116. $S_{3(60)}$

Plate 117. $S_{4(65)}$

Soups prepared with soup mixes of T_9 tempeh flour (green gram 50% + rice 50%)

Plate 118. $S_{1(50)}$

Plate 119. $S_{2(55)}$

Plate 120.$S_{3(60)}$

Plate 121. $S_{4(65)}$

$S_{1(50)}$ and $S_{2(55)}$ after storage. Maximum score for appearance was for $S_{1(50)}$ after storage.

The initial mean score for appearance of the soups prepared with the different compositions of tempeh flour T_8 (Table 53), ranged from 8.3 to 9.0 with the lowest mean score in $S_{4(65)}$ and the highest in $S_{1(50)}$. A decrease in the mean score for appearance after storage of soup mixes was observed only in the soup prepared with the composition $S_{3(60)}$ and the maximum mean score (9.0) for appearance after storage was observed in $S_{1(50)}$ and the lowest (8.2) in $S_{3(60)}$.

In the soups prepared with different compositions of T_9 (Table 53), the maximum score of 8.4 was for $S_{1(50)}$ and a mean score of 8.2 was observed for the other compositions. After storage, the mean score showed an increase in $S_{2(55)}$ (8.3) but there was no change in the mean score of the other compositions. $S_{1(50)}$ had the highest mean score of 8.4 and the lowest score of 8.2 was for $S_{3(60)}$ and $S_{4(65)}$ after storage of the soup mixes.

Table 54. Mean score for colour of the soups

Compositions	Colour (Mean score)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
$S_{1(50)}$	8.4	8.1	8.4	8.2	9.0	9.0	8.7	8.5
$S_{2(55)}$	8.6	8.6	8.2	8.0	8.9	8.8	8.3	8.3
$S_{3(60)}$	8.4	8.1	8.0	8.0	8.8	8.6	8.1	8.2
$S_{4(65)}$	8.0	8.0	7.8	7.8	8.7	8.4	8.4	8.2

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

The initial mean score for the colour of soups with different compositions of T_1 (Table 54) varied from 8.0 to 8.6 with the highest mean score in $S_{2(55)}$ and the lowest score in $S_{4(65)}$. After storage, a decrease in the mean score for colour was observed in the soups with compositions $S_{1(50)}$ and $S_{3(60)}$. The mean score for colour was highest (8.6) in $S_{2(55)}$ and the lowest (8.0) in $S_{4(65)}$ after storage.

Initially, mean score for colour of the soups prepared with the soup mixes from T_2 (Table 54) varied from 7.8 to 8.4, the highest in $S_{1(50)}$ and the lowest in $S_{4(65)}$. A decrease in the mean score was observed in the compositions $S_{1(50)}$ and $S_{2(55)}$ after storage, and the lowest mean score of 7.8 was observed in $S_{4(65)}$ and highest mean score of 8.2 in $S_{1(50)}$.

In the case of soups with different compositions of soup mixes with T_8 (Table 54), the highest initial mean score for colour was observed in $S_{1(50)}$ (9.0) and the lowest (8.7) in $S_{4(65)}$. A decrease in the mean score was observed in the compositions $S_{2(55)}$, $S_{3(60)}$ and $S_{4(65)}$ during the 6th month of storage. After storage, the highest mean score for colour (9.0) was observed in $S_{1(50)}$ and the lowest (8.4) in $S_{4(65)}$.

Initially, the soups prepared from soup mixes with T_9 (Table 54), showed a highest mean score of 8.7 in $S_{1(50)}$ and the lowest (8.1) in $S_{3(60)}$. After storage, $S_{1(50)}$ showed the highest mean score of 8.5 and the lowest score of 8.2 was observed in $S_{3(60)}$ and $S_{4(65)}$.

Table 55. Mean score for flavour of the soups

Compositions	Flavour (Mean score)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	7.8	7.7	7.9	7.7	8.0	8.0	8.3	8.2
$S_{2(55)}$	7.8	7.6	7.9	7.7	7.9	7.7	8.0	7.8
$S_{3(60)}$	7.7	7.5	7.9	7.6	7.9	7.6	7.8	7.8
$S_{4(65)}$	7.5	7.4	7.9	7.4	7.7	7.5	7.6	7.5

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

Initially, the mean score for the flavour of the soups prepared with soup mixes from tempeh flour T_1 (Table 55), was found to be highest (7.8) in $S_{1(50)}$ and $S_{2(55)}$ and lowest (7.5) in $S_{4(65).}$ There was a decrease in the flavour score of all the soups prepared after storage of the soup mixes, which varied from 7.4 to 7.7 with the lowest and highest in $S_{4(65)}$ and $S_{1(50)}$ respectively.

In the soups prepared with different compositions of soup mixes of T_2 (Table 55), the initial mean score for flavour was 7.9 for all the soups. After storage of soup mixes, a decrease in the flavour scores of the soups were observed in all the compositions and the mean score was highest (7.7) in $S_{1(50)}$ and $S_{2(55)}$ and lowest (7.4) in $S_{4(65)}$.

The initial mean score for flavour of the soups prepared with T_8 (Table 55), ranged from 7.7 to 8.0 with the lowest mean score in $S_{4(65)}$ and the highest in $S_{1(50).}$ A decrease in the flavour of the soups were observed except in $S_{1(50)}$ after storage of the soup mixes. The highest score of 8.0 after storage was in $S_{1(50)}$ and the lowest of 7.5 was in $S_{4(65).}$

In the soups prepared with T_9 (Table 55) before storage of the soup mixes, the maximum score of 8.3 was for the composition $S_{1(50)}$ and the lowest score of 7.6 was for $S_{4(65).}$ A decrease in the flavour score of all the soups were observed after storage of soup mixes. The highest mean score of 8.2 was observed in $S_{1(50)}$ and the lowest score of 7.5 was observed in $S_{4(65)}$ after the storage of soup mixes.

Table 56. Mean score for consistency of the soups

Compositions	Consistency (Mean score)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	8.1	8.2	8.0	8.0	8.5	8.3	8.2	8.2
$S_{2(55)}$	8.2	8.1	8.0	8.0	8.3	8.2	8.2	8.5
$S_{3(60)}$	8.0	8.0	8.1	8.0	8.2	8.4	8.1	8.2
$S_{4(65)}$	8.0	8.1	8.0	8.0	8.1	8.1	8.6	8.6

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

In the soups prepared with T_1 (Table 56), initially, the mean score for consistency was found to be highest (8.2) in $S_{2(55)}$ and lowest (8.0) in $S_{3(60)}$ and $S_{4(65)}$. During the 6th month of storage of the soup mixes, the consistency score of the soups with T_1 varied from 8.0 to 8.2 with the lowest and highest score in $S_{3(60)}$ and $S_{1(50)}$respectively. There was an increase in the consistency score of the soups with compositions $S_{1(50)}$ and $S_{4(65)}$ after storage.

Initially, In the soups prepared with T_2 (Table 56), the compositions $S_{1(50)}$ $S_{2(55)}$ and $S_{4(65)}$ had the lowest mean score for consistency (8.0) and the highest mean score (8.1) was in $S_{3(60)}$. The mean score for consistency was found to be the same (8.0) in all the soup compositions after storage.

The initial mean score for consistency in the soups prepared with T_8 (Table 56) ranged from 8.1 to 8.5 with the lowest score in $S_{4(65)}$ and the highest in $S_{1(50)}$. After storage of soup mixes, the maximum score of 8.4 for consistency was observed in $S_{3(60)}$ and the lowest mean score of 8.1 in $S_{4(65)}$.

Regarding the consistency score of different soups prepared with T_9 (Table 56), the initial mean score varied from 8.1 to 8.6 with the lowest and highest score in $S_{3(60)}$ and $S_{4(65)}$ respectively. After storage of the soup mixes, the soup with $S_{4(65)}$ had the highest mean score of 8.6 and the lowest mean score of 8.2 was observed in $S_{1(50)}$and $S_{3(60)}$.

Table 57. Mean score for taste of the soups

Compositions	Taste (Mean score)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	8.0	8.0	8.0	8.0	8.6	8.4	8.3	8.3
$S_{2(55)}$	8.0	8.0	7.7	7.4	8.4	8.3	8.4	8.0
$S_{3(60)}$	6.9	6.6	6.9	6.7	7.8	7.6	8.0	7.8
$S_{4(65)}$	6.8	6.9	6.9	6.6	7.7	7.6	7.7	7.6

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

Initially, the mean score for the taste of the soups prepared from the tempeh flour T_1 (Table 57) was found to be highest (8.0) in $S_{1(50)}$ and $S_{2(55\ )}$ and the lowest (6.8) in $S_{4(65)}$. After storage of the soup mixes, the mean score for taste was found to be highest (8.0) in $S_{1(50)}$ and $S_{2(55)}$ and the lowest (6.6) in $S_{3(60)}$.

In the soups prepared with T_2 (Table 57), the initial mean score for taste varied between 6.9 in $S_{3(60)}$ and $S_{4(65)}$ to 8.0 in $S_{(50)}$. After storage of soup mixes, the lowest mean score of 6.6 was observed in $S_{(65)}$ and the highest mean score of 8.0 in $S_{1(50)}$.

In the case of soups prepared with T_8 (Table 57), the initial mean score for taste ranged from 7.7 to 8.6 with the lowest in $S_{4(65)}$ and the highest in $S_{1(50)}$. After storage of the soup mixes, the highest mean score of 8.4 was observed in $S_{1(50)}$ and the lowest score of 7.6 was in $S_{3(60)}$ and $S_{4(65)}$.

In the soups prepared with T_9 (Table 57), initially, the maximum score of 8.4 was in the composition $S_{2(55)}$ and the lowest score of 7.7 was observed in $S_{4(65)}$. After storage of the soup mixes, the highest mean score of 8.3 was in $S_{1(50)}$ and lowest score of 7.6 was in $S_{4(65)}$.

Table 58. Mean score for overall acceptability of the soups

Compositions	**Overall acceptability (Mean score)**							
	Treatments and storage period							
	T_1		**T_2**		**T_8**		**T_9**	
	Initial	**6^{th} month**	**Initial**	**6^{th} month**	**Initial**	**6^{th} month**	**Initial**	**6^{th} month**
$S_{1(50)}$	8.1	8.0	8.1	8.0	8.4	8.2	8.1	8.0
$S_{2(55)}$	8.2	8.2	8.0	7.8	8.3	8.1	8.1	8.0
$S_{3(60)}$	7.8	7.6	7.7	7.6	8.1	8.0	8.0	7.9
$S_{4(65)}$	7.7	7.5	7.7	7.5	8.0	7.9	8.0	7.8

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
Values are mean of 3 evaluations

The initial mean score for the overall acceptability of soups with T_1 (Table 58) varied from 7.7 to 8.2 with the lowest score in $S_{4(65)}$ and the highest in $S_{2(55)}$. There was a decrease in the overall acceptability score after storage of soup mixes except in $S_{2(55)}$. The mean score varied from 7.5 to 8.2 with the lowest and highest score in $S_{4(65)}$ and $S_{2(55)}$ respectively.

The initial mean score for overall acceptability of the soups with T_2 (Table 58), was observed to be highest in $S_{1(50)}$ (8.1) and the lowest (7.7) in $S_{3(60)}$ and $S_{4(65)}$. After storage, a decrease in the overall acceptability score was observed in all the compositions of soups and the highest mean score of 8.0 was in $S_{1(50)}$ and lowest mean score of 7.5 was in $S_{4(65)}$.

In the case of soups with T_8 (Table 58), the highest initial mean score (8.4) was observed in $S_{1(50)}$ and lowest (8.0) in $S_{4(65)}$. A decrease in the mean score for

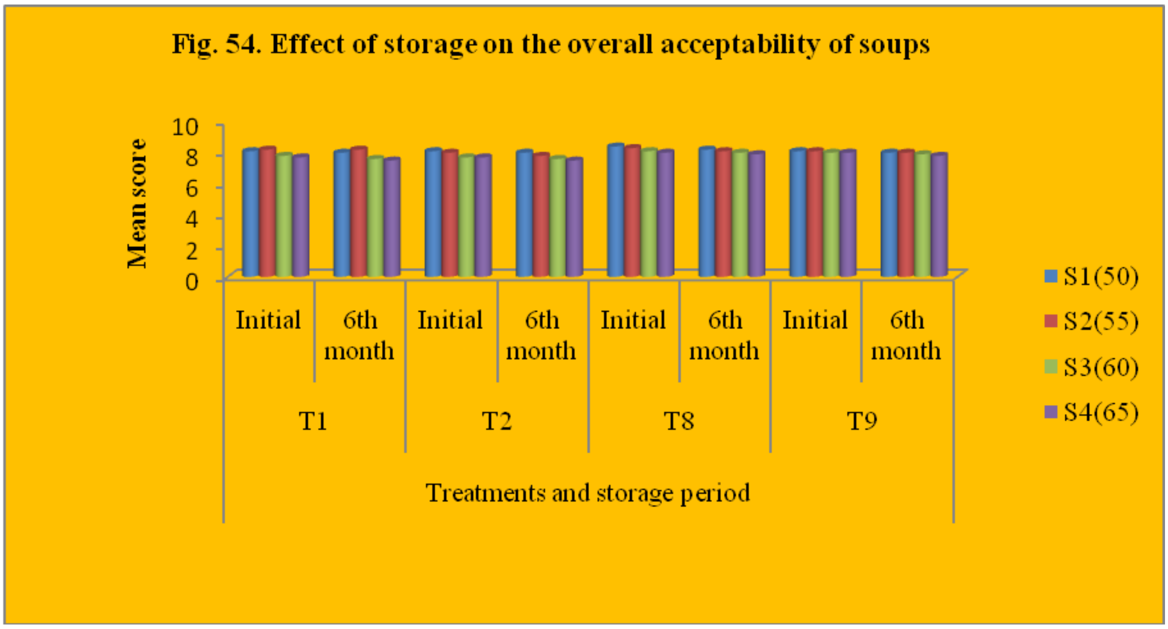
Fig. 54. Effect of storage on the overall acceptability of soups
Mean score
10
8
6
4
2
0
Initial
6th month
T1
T2
T8
T9
Treatments and storage period
S1(50)
S2(55)
S3(60)
S4(65)

overall acceptability was observed in all the compositions of soups after storage of the soup mixes, and the highest overall acceptability mean score of 8.2 was in $S_{1(50)}$ and the lowest score of 7.9 in $S_{4(65)}$.

Initially, in the soups with T_9 (Table 58) the highest mean score of 8.1 was in $S_{1(50)}$ and $S_{2(55)}$ and the lowest mean score of 8.0 was in $S_{3(60)}$ and $S_{4(65)}$. After storage of the soup mixes, there was a decrease in the overall acceptability score of all the compositions of the soups and the highest mean score of 8.0 was in $S_{1(50)}$ and $S_{2(55)}$ and the lowest overall acceptability mean score of 7.8 was in $S_{4(65)}$.

The effect of storage of soup mixes on the overall acceptability of soups is illustrated in Figure 54.

4.6.3. Total micro flora in the soup mixes during storage

The total microbial count in soup mixes was enumerated initially and during the 6th month and the results are presented in the Table 59 to 61 and in Figures 55 to 57.

4.6.3.1. Total bacterial count of soup mixes

The total bacterial count of the soup mixes is presented in Table 59 and in Figure 55.

Table 59. Total bacterial count of soup mixes on storage

Compositions	Total bacterial count (x 10^5 cfu/g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month	Initial	6^{th} month
$S_{1(50)}$	3.3	4.6	4.3	6.6	5.0	7.6	4.6	7.4
$S_{2(55)}$	5.3	8.6	5.0	6.3	4.3	7.3	5.0	8.4
$S_{3(60)}$	3.6	6.0	4.6	6.3	5.3	6.0	4.6	9.3
$S_{4(65)}$	4.6	8.0	5.0	7.0	4.6	6.3	6.0	7.4

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour,
$S_{4(65)}$ - 65% tempeh flour
Values are mean of three independent enumerations

As revealed in Table 59, the initial bacterial count in the soup mixes prepared with T_1 varied from 3.3 to 5.3 x 10^5 cfu/g with the lowest count in $S_{1(50)}$ and the highest in $S_{2(55)}$. After storage, there was an increase in the bacterial count of all the compositions, which varied from 4.6 to 8.6 x 10^5 cfu/g with the highest count in $S_{2(55)}$ and the lowest in $S_{1(50)}$.

In the soup mixes prepared with T_2, the maximum initial bacterial count (5.0 x 10^5 cfu/g) was observed in compositions $S_{2(55)}$ and $S_{4(65)}$ and the lowest (4.3 x 10^5 cfu/g) count in $S_{1(50)}$. During the 6^{th} month of storage, $S_{4(65)}$ had the maximum bacterial count (7.0 x 10^5 cfu/g) and the lowest bacterial count (6.3 x 10^5 cfu/g) was observed in $S_{2(55)}$ and $S_{3(60)}$.

In the soup mixes prepared with T_8, the highest initial bacterial count (5.3 x 10^5 cfu/g) was in $S_{3(60)}$ and the lowest (4.3 x 10^5 cfu/g) in $S_{2(55)}$. An increase in the bacterial count was observed in all the compositions after storage which varied between 6.0 to 7.6 x 10^5 cfu/g with the lowest bacterial count in $S_{3(60)}$ and the highest in $S_{1(50)}$.

The initial total bacterial count in the soup mixes prepared with T_9 ranged from 4.6 to 6.0 x 10^5 cfu/g the lowest in $S_{1(50)}$ and the highest in $S_{4(65)}$. After storage, there was an increase in the bacterial count of all the compositions with the highest bacterial count of 9.3 x 10^5 in $S_{3(60)}$ and the lowest (7.4 x 10^5) in $S_{1(50)}$ and $S_{4(65)}$.

4.6.3.2. Total yeast count of soup mixes

The total yeast count of the soup mixes is presented in Table 60 and in Figure 56.

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Table 60. Total yeast count of soup mixes on storage

Compositions	Total yeast count (x 10^3 cfu/g)							
	Treatments and storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	1.3	3.0	1.6	4.0	2.6	5.6	3.3	4.6
$S_{2(55)}$	2.0	3.5	2.3	4.5	4.0	5.5	4.6	6.5
$S_{3(60)}$	3.3	4.6	3.3	4.0	3.3	5.6	3.3	6.3
$S_{4(65)}$	3.0	4.6	3.0	4.0	4.0	5.6	4.0	6.6

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
Values are mean of three independent enumerations

As revealed in Table 60, the initial yeast count in the soup mixes prepared with T_1 varied from 1.3 to 3.3 x 10^3 cfu/g with the lowest total yeast count in $S_{1(50)}$ and the highest in $S_{3(60)}$. After storage, there was an increase in the yeast count in all the compositions of soup mixes with the highest total count of 4.6 x 10^3 cfu/g in $S_{3(60)}$ and $S_{4(65)}$ and the lowest yeast count of 3.0 x 10^3 cfu/g $S_{1(50)}$.

In the soup mixes prepared with T_2, initially maximum yeast count (3.3 x 10^5 cfu/g) was observed in $S_{3(60)}$ and the lowest (1.6 x 10^3 cfu/g) in $S_{1(50)}$. An increase in the yeast count was observed after storage and the maximum yeast count of 4.5 x 10^3

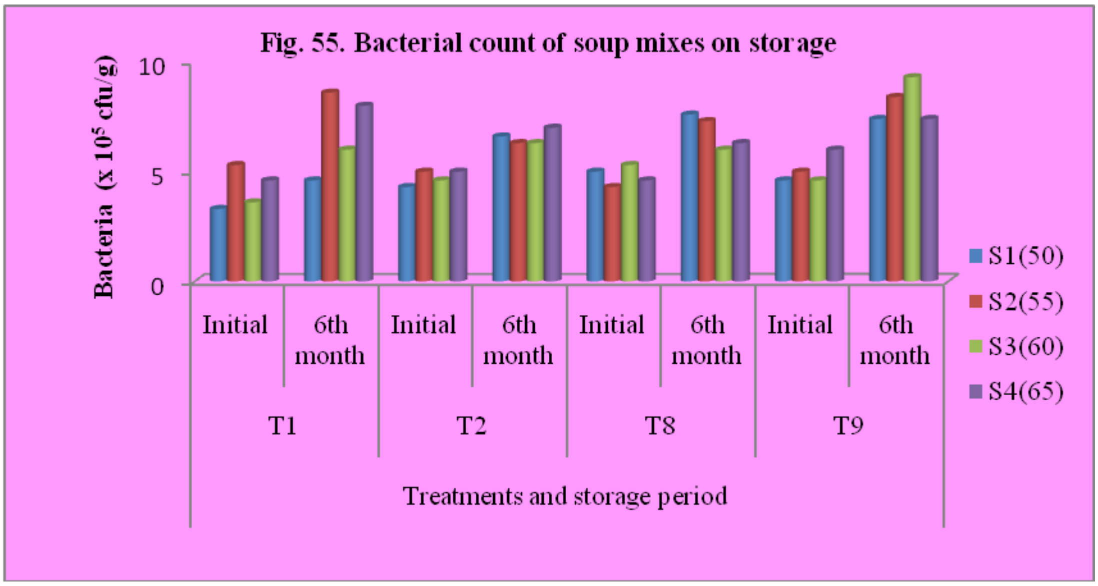

Fig. 55. Bacterial count of soup mixes on storage
Bacteria (x 10⁵ cfu/g)
10
5
0
Initial
6th month
Initial
6th month
Initial
6th month
Initial
6th month
T1
T2
T8
T9
Treatments and storage period
S1(50)
S2(55)
S3(60)
S4(65)

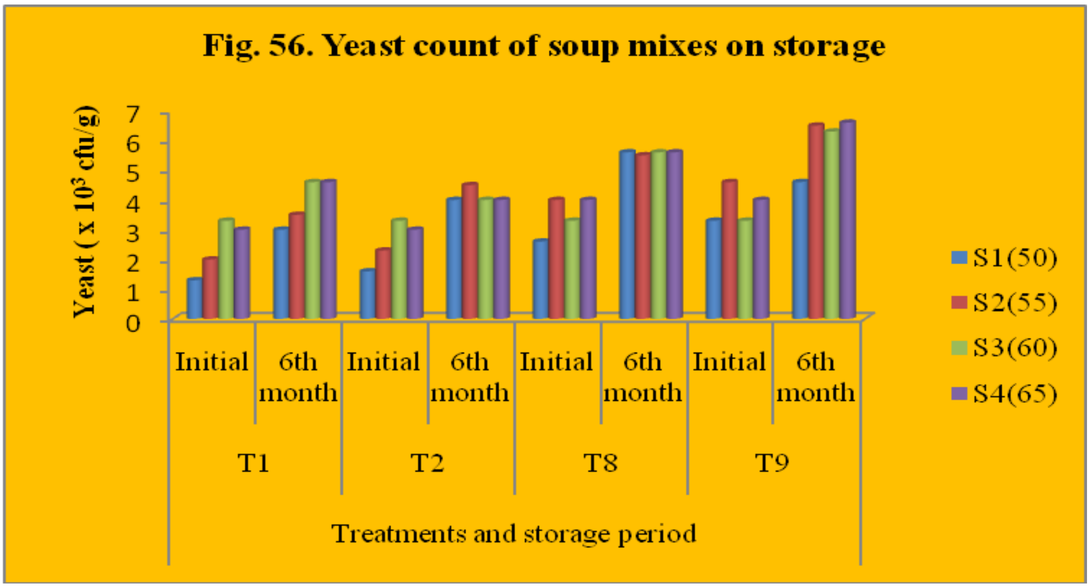

Fig. 56. Yeast count of soup mixes on storage
Yeast (x 10³ cfu/g)
7
6
5
4
3
2
1
0
Initial
6th month
Initial
6th month
Initial
6th month
Initial
6th month
T1
T2
T8
T9
Treatments and storage period
S1(50)
S2(55)
S3(60)
S4(65)

cfu/g was observed in $S_{2(55)}$ and the minimum yeast count of 4.0 x 10^3 cfu/g was observed in $S_{1(50)}$, $S_{3(60)}$ and $S_{4(65)}$.

The soup mixes prepared with T_8 showed the highest initial yeast count (4.0 x 10^3 cfu/g) in $S_{2(55)}$ and $S_{4(65)}$ and the lowest (2.6 x 10^5 cfu/g) in $S_{1(50)}$. During the 6th month of storage, an increase in the yeast count was observed in all the soup mixes which varied from 5.5 to 5.6 x 10^3 cfu/g with the lowest yeast count in $S_{2(55)}$ and highest in $S_{1(50)}$, $S_{3(60)}$ and $S_{4(65)}$.

The initial yeast count in the soup mixes prepared with T_9 ranged from 3.3 x 10^3 cfu/g in $S_{1(50)}$ and $S_{3(60)}$ to 4.6 x 10^3 cfu/g in $S_{2(55)}$. After storage, there was an increase in the yeast count of all the compositions of soup mixes with a maximum yeast count of 6.6 x 10^3 cfu/g in $S_{4(65)}$ and the lowest yeast count of 4.6 x 10^3 cfu/g in $S_{1(50)}$.

4.6.3.3. Total fungal count of soup mixes on storage

The total fungal count of the soup mixes is presented in Table 61 and in Figure 57.

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Table 61. Total fungal count of soup mixes on storage

Compositions	Total fungal count (x 10^3 cfu/g)							
	Treatments and Storage period							
	T_1		T_2		T_8		T_9	
	Initial	6th month	Initial	6th month	Initial	6th month	Initial	6th month
$S_{1(50)}$	1.0	1.3	0.66	0.66	1.0	1.3	0.33	0.66
$S_{2(55)}$	1.3	2.0	1.0	1.3	1.0	1.0	0.33	1.0
$S_{3(60)}$	0.66	1.6	0.33	1.0	1.0	1.0	0.66	1.3
$S_{4(65)}$	2.0	2.6	1.0	1.3	1.3	1.3	0.33	0.66

$S_{1(50)}$ - 50% tempeh flour, $S_{2(55)}$ - 55% tempeh flour, $S_{3(60)}$ - 60% tempeh flour, $S_{4(65)}$ - 65% tempeh flour
Values are mean of three independent enumerations

The initial fungal count in the soup mixes prepared from the tempeh flour T_1 was found to be the highest (2.0 x 10^3 cfu/g) in $S_{4(65)}$ and the lowest (0.66 x 10^3 cfu/g)

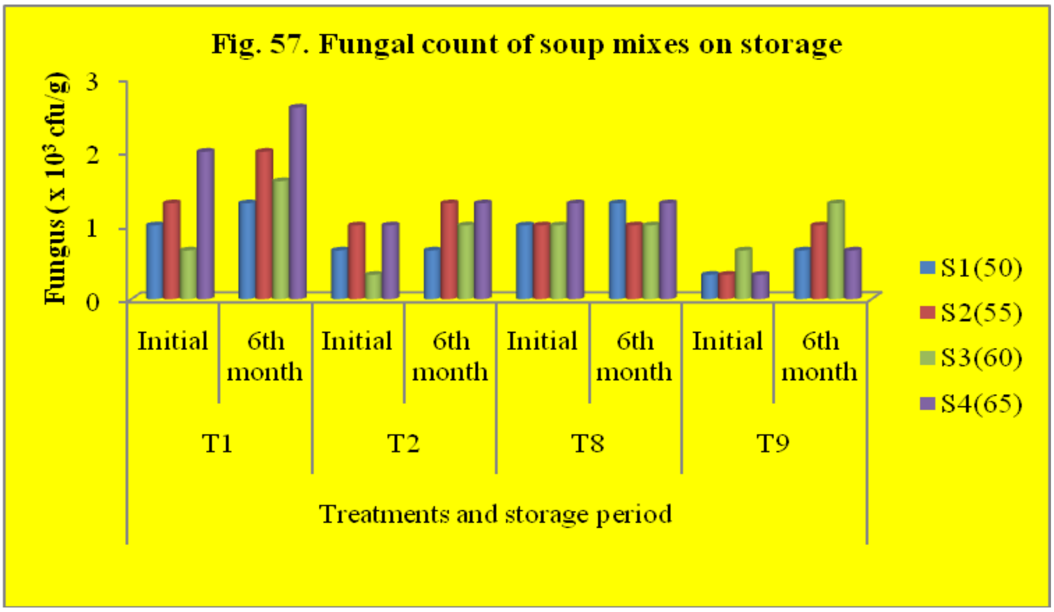


Fig. 57. Fungal count of soup mixes on storage

in $S_{3(60)}$. After storage, there was an increase in the fungal count with S_1 soup mixes which varied from 1.3 to 2.6 x 10^3 cfu/g with the highest fungal count in $S_{4(65)}$ and lowest in $S_{1(50)}$.

The initial fungal count in the soup mixes prepared with T_2 was found to be maximum (1.0 x 10^3 cfu/g) in $S_{2(55)}$ and $S_{4(65)}$ and minimum (0.33 x 10^3 cfu/g) in $S_{3(60)}$. After storage, an increase in the fungal count was observed in the soup mixes $S_{2(55)}$, $S_{3(60)}$ and $S_{4(65)}$. Maximum fungal count after storage (1.3 x 10^5 cfu/g) was observed in $S_{2(55)}$ and $S_{4(65)}$ and minimum (0.66 x 10^3 cfu/g) in $S_{1(50)}$.

Before storage, in the soup mixes prepared from T_8, the fungal count in $S_{1(50)}$, $S_{2(55)}$and $S_{3(60)}$ was found to be the lowest (1.0 x 10^3 cfu/g) and the highest fungal count (1.3 x 10^3 cfu/g) was in $S_{4(65)}$. During the 6^{th} month of storage, an increase in the fungal count was observed in $S_{1(50)}$ and $S_{4(65)}$. After storage, the highest fungal count (3.3 x 10^3 cfu/g) was in $S_{1(50)}$ and $S_{4(65)}$ and the lowest (1.0 x 10^3 cfu/g) in $S_{2(55)}$ and $S_{3(60)}$.

The initial fungal count in the soup mixes prepared with T_9 ranged from 0.33 x 10^3 cfu/g in $S_{1(50)}$, $S_{2(55)}$ and $S_{4(65)}$ to 0.66 x 10^3 cfu/g in $S_{3(60)}$. After storage, there was an increase in the fungal count of all the compositions with T_9, which varied from 0.66 x 10^3 cfu/g in $S_{1(50)}$ and $S_{4(65)}$ to 1.3 x 10^3 cfu/g in $S_{3(60)}$.

4.6.4. Insect infestation in soup mixes during storage

The insect infestation of the soup mixes was assessed initially and also during storage. Insect infestation was not observed in any of the compositions of soup mixes initially or during the 6^{th} month of storage. Soup mixes after storage was of free flowing without any lumps or caking of the flour.

4.6.5. Selection of soup mixes

From the 16 soup mixes developed with 4 different tempeh flours, one soup mix from each tempeh flour (T_1, T_2, T_8 and T_9) was selected based on good acceptability and storage stability with regard to microbial load and maximum retention of nutrients after storage for six months (Fig. 58).

In the soup mixes developed, the total plate count remained within the limit of < 10 x 10^5 cfu/g. Hence all the soup mixes can be considered as microbiologically safe.

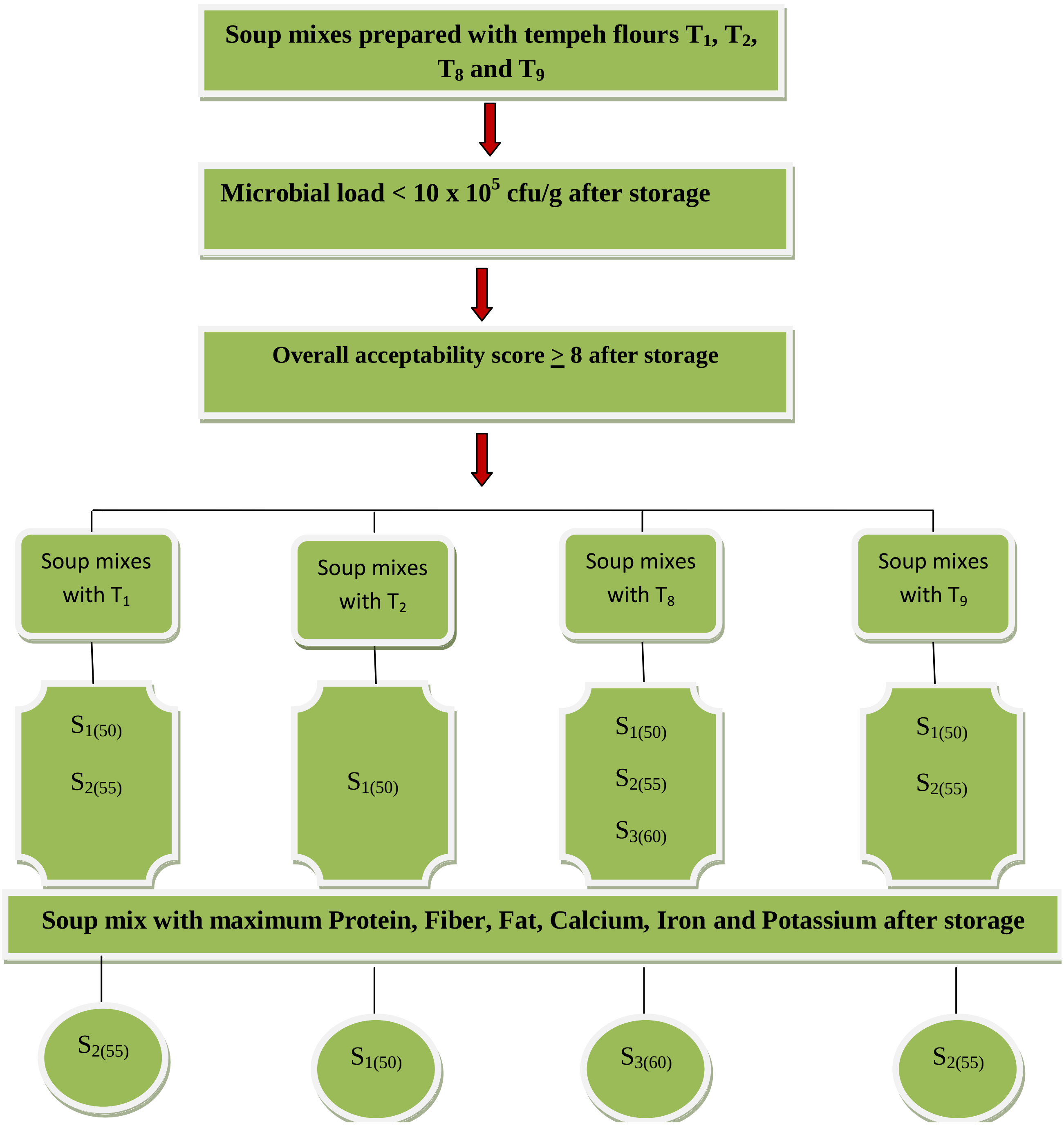
Regarding acceptability, only those soup mixes where the soups prepared after storage had an overall acceptability score $\geq$ 8.0 indicating 'liked very much' in a 9 point hedonic scale was selected. Hence in the soup mixes prepared with T_1 tempeh flour, the compositions $S_{1(50)}$ and $S_{2(55)}$, with T_2 tempeh flour the composition $S_{1(50)}$, with T_8 tempeh flour the compositions $S_{1(50)}$, $S_{2(55)}$ and $S_{3(60)}$ and with T_9 tempeh flour, the compositions $S_{1(50)}$ and $S_{2(55)}$ were selected.

From these soup mixes with each tempeh flour, one soup mix with maximum nutritive value with respect to protein, fiber, fat, calcium, iron and potassium after storage was selected. Thus the soup mix composition $S_{2(55)}$ was selected from T_1 tempeh flour, $S_{1(50)}$ from T_2 tempeh flour, $S_{3(60)}$ from T_8 tempeh flour and $S_{2(55)}$ from T_9 tempeh flour were selected as the most acceptable soup mixes with good storage stability.

4.7. Cost of production

Cost of production of selected fresh tempeh types, tempeh flours (T_1, T_2, T_8 and T_9), and the soup mixes prepared with each of the selected tempeh flour were computed and are presented in Table 62 to 73. For the cost calculation, fixed cost was computed, which included depreciation and interest on the fixed capital of equipments like dryer, vessels and other gadgets used. In the miscellaneous cost, transportation charges and other unforeseen expenditure were included.

Fig. 58. Selection of soup mixes



4.7.1. Cost of production of fresh tempeh

Cost of production of selected fresh tempeh types are presented in Table 62 to 65. The cost per unit pack (500g) of fresh tempeh and benefit cost ratio was also calculated. For the calculation of benefit cost ratio, the selling price was fixed based on the market price of *paneer* (Rs. 200/kg) to which the fresh tempeh was compared.

Table. 62. Cost of production of 20 kg fresh tempeh T_1 (100% soybean)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Soybean	12.35 kg	643.00
	Vinegar	1.25 liters	25.00
	Pure culture		1.00
	Polyethylene bags	40 no’s	16.00
2.	**Other items**		
	L.P.G	30 minutes	3.00
	Labour @ Rs. 250 for 8 hours	4 hours	125.00
	Cleaning materials/ Plastic containers		100.00
	Managerial cost @ Rs. 500 for 8 hours	4 hours	250.00
	Miscellaneous		60.00
	TOTAL		1223.00

Cost of one kilogram fresh tempeh T_1**: Rs. 61.00**

Cost of unit pack of fresh tempeh T_1 (500g)**: Rs. 30.50**

Benefit cost ratio**: 3.28**

Table 63. Cost of production of 20 kg fresh tempeh T_2 (100% green gram)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Green gram	13.10 kg	786.00
	Vinegar	1.3 liters	26.00
	Pure culture	-	1.00
	Polyethylene bags	40 no's	16.00
2.	**Other items**		
	L.P.G	30 minutes	3.00
	Labour @ Rs. 250 for 8 hours	4 hours	125.00
	Cleaning materials/ Plastic containers		100.00
	Managerial cost @ Rs. 500 for 8 hours	4 hours	250.00
	Miscellaneous		60.00
	TOTAL		**1367.00**

Cost of one kilogram fresh tempeh T_2**: Rs.68.00**

Cost of unit pack of fresh tempeh T_2 (500g)**: Rs. 34.00**

Benefit cost ratio**: 2.94**

Table 64. Cost of production of 20 kg fresh tempeh T_8 (75% green gram + 25% rice)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Green gram	10.00 kg	600.00
	Rice	3.30 kg	66.00
	Vinegar	1.3 liters	26.00
	Pure culture		1.00
	Polyethylene bags	40 no's	16.00
2.	**Other items**		
	L.P.G	30 minutes	3.00
	Labour @ Rs. 250 for 8 hours	4 hours	125.00
	Cleaning materials/ Plastic containers		100.00
	Managerial cost @ Rs. 500 for 8 hours	4 hours	250.00
	Miscellaneous		60.00
	TOTAL		**1247.00**

Cost of one kilogram fresh tempeh T_8**: Rs. 62.00**

Cost of unit pack of fresh tempeh T_8 (500g)**: Rs. 31.00**

Benefit cost ratio**: 3.23**

Table 65. Cost of production of 20 kg fresh tempeh T_9 (50% green gram + 50% rice)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Green gram	7.00 kg	420.00
	Rice	7.00 kg	140.00
	Vinegar	1.40 liters	28.00
	Pure culture		1.00
	Polyethylene bags	40 no’s	16.00
2.	**Other items**		
	L.P.G	30 minutes	3.00
	Labour @ Rs. 250 for 8 hours	4 hours	125.00
	Cleaning materials/ Plastic containers		100.00
	Managerial cost @ Rs. 500 for 8 hours	4 hours	250.00
	Miscellaneous		60.00
	TOTAL		**1143.00**

Cost of one kilogram fresh tempeh T_9**: Rs. 57.00**

Cost of unit pack of fresh tempeh T_9 (500g)**: Rs. 28.50**

Benefit cost ratio**: 3.5**

As revealed in Table 62 to 65, the cost of one kilogram of fresh tempeh ranged from Rs. 57.00 to Rs. 68.00 with the highest cost for T_2 (100% green gram tempeh) and the lowest for T_9 (green gram 50% + rice 50% tempeh) respectively. The highest benefit cost ratio of fresh tempeh was for the treatment T_9 (3.5) and the lowest was for T_2 (2.94).

4.7.2. Cost of production of tempeh flours

The cost of production of 20 kg tempeh flour prepared from the selected fresh tempeh types are presented in the Table 66 to 69. Benefit cost ratio of tempeh flour was not calculated since comparable fermented flour is not available in the market.

Table 66. Cost of production of 20 kg T_1 tempeh flour (100% soybean)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Fresh tempeh (T_1)	40 kg	2440.00
	Polyethylene bags	40 no's	16.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	2 hours	62.50
	Electricity charge	9 units	34.00
	Depreciation and interest on fixed inputs		20.00
	Managerial cost @ Rs. 500 for 8 hours	2 hours	125.00
	Miscellaneous		60.00
	TOTAL		**2757.50**

Cost of one kilogram T_1 tempeh flour**: Rs. 138.00.**

Cost of unit pack of T_1 tempeh flour (500g)**: Rs. 69.00.**

Table 67. Cost of production of 20 kg T_2 tempeh flour (100% green gram)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Fresh tempeh (T_2)	37.00 kg	2516.00
	Polyethylene bags	40 no's	16.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	2 hours	62.50
	Electricity charge	9 units	34.00
	Depreciation and interest on fixed inputs		20.00
	Miscellaneous		60.00
	Managerial cost @ Rs. 500 for 8 hours	2 hours	125.00
	TOTAL		**2833.50**

Cost of one kilogram T_2 tempeh flour**: Rs. 142.00**

Cost of unit pack of T_2 tempeh flour (500g)**: Rs. 71.00**

Table 68. Cost of production of 20 kg T_8 tempeh flour (75% green gram + 25% rice)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Fresh tempeh (T_8)	35.7 kg	2213.00
	Polyethylene bags	40 no's	16.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	2 hours	62.50
	Electricity charge	9 units	34.00
	Depreciation and interest on fixed inputs		20.00
	Managerial cost @ Rs. 500 per for 8 hours	2 hours	125.00
	Miscellaneous		60.00
	TOTAL		**2530.50**

Cost of one kilogram T_8 tempeh flour**: Rs. 127.00**

Cost of unit pack of T_8 tempeh flour (500g)**: Rs. 63.50**

Table 69. Cost of production of 20 kg T_9 tempeh flour (50% green gram + 50% rice)

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Fresh tempeh (T_9)	33.26 kg	1896.00
	Polyethylene bags	40 no's	16.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	2 hours	62.50
	Electricity charge	9 units	34.00
	Depreciation and interest on fixed inputs		20.00
	Managerial cost @ Rs. 500 for 8 hours	2 hours	125.00
	Miscellaneous		60.00
	TOTAL		**2213.50**

Cost of one kilogram T_9 tempeh flour**: Rs. 111.00**

Cost of unit pack of T_9 tempeh flour (500g)**: Rs. 55.50**

As revealed in Table 66 to 69, the cost of production of tempeh flours ranged from Rs. 111.00 to 142.00 per kilogram. The production cost was lowest for the tempeh flour prepared with T_9 (green gram 50% + rice 50%) and the highest in T_2 (100% green gram) tempeh flour.

4.7.3. Cost of production of soup mixes

The cost of production of the selected soup mixes prepared with each tempeh flour was computed and is presented in Tables 70 to 73. For the calculation of benefit cost ratio, the selling price of instant soup mixes were fixed based on the selling price of an instant vegetable soup mix available in the market (Rs.32/50g).

Table 70. Cost of production of 10 kg soup mix [$S_{2(55)}$] with T_1 tempeh flour

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Tempeh flour (T_1)	5.5 kg	759.00
	Corn flour	1.5 kg	105.00
	Vegetable mix	1.0 kg	82.00
	Spice mix	0.5 kg	92.00
	Pepper powder	0.5 kg	225.00
	Salt	0.5 kg	5.00
	Sugar	0.3 kg	10.00
	Citric acid	0.2 kg	50.00
	Metalised polyester laminate pouches	200 no's	400.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	8 hours	250.00
	Electricity charge	9 units	34.00
	Managerial cost @ Rs.500 for 8 hours	8 hours	500.00
	Miscellaneous		60.00
	TOTAL		**2572.00**

Cost of one kilogram of soup mix $S_{2(55)}$ with T_1 tempeh flour **: Rs. 257.00**

Cost of unit pack of soup mix (50g) $S_{2(55)}$ with T_1 tempeh flour **: Rs. 13.00**

Benefit cost ratio: **2.46**

Table 71. Cost of production of 10 kg soup mix $S_{1(50)}$ with T_2 tempeh flour

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Tempeh flour (T_2)	5.0 kg	710.00
	Corn flour	2.0 kg	140.00
	Vegetable mix	1.0 kg	82.00
	Spice mix	0.5 kg	92.00
	Pepper powder	0.5 kg	225.00
	Salt	0.5 kg	5.00
	Sugar	0.3 kg	10.00
	Citric acid	0.2 kg	50.00
	Metalised polyester laminate pouches	200 no's	400.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	8 hours	250.00
	Electricity charge	9 units	34.00
	Managerial cost @ Rs. 500 for 8 hours	8 hours	500.00
	Miscellaneous		60.00
	TOTAL		**2558.00**

Cost of one kilogram of soup mix $S_{1(50)}$ with T_2 tempeh flour **: Rs. 256.00**

Cost of unit pack of soup mix (50g) $S_{1(50)}$ with T_2 tempeh flour **: Rs. 13.00**

Benefit cost ratio: **2.46**

Table 72. Cost of production of 10 kg soup mix $S_{3(60)}$ with T_8 tempeh flour

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Tempeh flour (T_8)	6.0 kg	762.00
	Corn flour	1.0 kg	70.00
	Vegetable mix	1.0 kg	82.00
	Spice mix	0.5 kg	92.00
	Pepper powder	0.5 kg	225.00
	Salt	0.5 kg	5.00
	Sugar	0.3 kg	10.00
	Citric acid	0.2 kg	50.00
	Metalised polyester laminate pouches	200 no's	400.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	8 hours	250.00
	Electricity charge	9 units	34.00
	Managerial cost @ Rs. 500 for 8 hours	8 hours	500.00
	Miscellaneous		60.00
	TOTAL		**2540.00**

Cost of one kilogram of soup mix $S_{3(60)}$ with T_8 tempeh flour **: Rs. 254.00**

Cost of unit pack of soup mix (50g) $S_{3(60)}$ with T_8 tempeh flour **: Rs. 13.00**

Benefit cost ratio: **2.46**

Table 73. Cost of production of 10 kg soup mix $S_{2(55)}$ with T_9 tempeh flour

S.No	Items	Quantity	Cost (Rupees)
1.	**Raw materials**		
	Tempeh flour (T_9)	5.5 kg	610.50
	Corn flour	1.5 kg	105.00
	Vegetable mix	1.0 kg	82.00
	Spice mix	0.5 kg	92.00
	Pepper powder	0.5 kg	225.00
	Salt	0.5 kg	5.00
	Sugar	0.3 kg	10.00
	Citric acid	0.2 kg	50.00
	Metalised polyester laminate pouches	200 no's	400.00
2.	**Other items**		
	Labour @ Rs. 250 for 8 hours	8 hours	250.00
	Electricity charge	9 units	34.00
	Managerial cost @ Rs. 500 for 8 hours	8 hours	500.00
	Miscellaneous		60.00
	TOTAL		**2423.50**

Cost of one kilogram of soup mix $S_{2(55)}$ with T_9 tempeh flour **: Rs. 242.00**
Cost of unit pack of soup mix (50g) $S_{2(55)}$ with T_9 tempeh flour **: Rs. 12.00**
Benefit cost ratio**: 2.64**

As revealed in Table 70 to 73, the cost of production was highest (Rs. 257.00 /kg) for the soup mix S_1(55) prepared with T_1 (100% soybean) tempeh flour and the lowest cost of production (Rs. 242.00/ kg.) was for the soup mix $S_{2(55)}$ prepared with T_9 (green gram 50% + rice 50%) tempeh flour. All the soup mixes had a very good benefit cost ratio. The lowest BC ratio (2.46) were for the soup mixes $S_{1(55)}$, $S_{1(50)}$ and $S_{3(60)}$ prepared with T_1 (100% soybean), T_2 (100% green gram) and T_8 (green gram 50% + rice 50%) tempeh flour respectively and the highest BC ratio of 2.64 for the soup mix $S_{2(55)}$ prepared with T_9 (green gram 50% + rice 50%) tempeh flour.

Discussion

5. DISCUSSION

Fermentation is one of the household food technologies reviewed extensively as means by which the nutritive value of plant foods could be improved (Obadina *et al.,* 2008). Fermentation also preserves foods in a wide variety of flavours, aroma and texture. A variety of indigenous fermented foods exist today; however 'Tempeh' has been one of the most widely accepted and researched mould modified fermented product. It is a nutritious oriental fermented food produced by solid state fermentation of soybeans consumed widely in Indonesia.

Tempeh is traditionally made with soybeans only. As tempeh was introduced in Western countries, makers started to experiment with the fermentation of other grains, pulses and cereals all with a unique flavour. As stated by Vaidehi (1993), in Indian situation where varieties of pulses are used, tempeh could be prepared with pulses other than soybeans. Hence, in the present study, tempeh types were standardised using different combinations of cereals, pulses and their quality evaluations as well as acceptability studies were conducted. Secondary products such as tempeh flour and instant soup mixes were also developed and evaluated for their quality attributes. The results of the study are discussed in this chapter.

5.1. Standardisation of tempeh with legumes and cereals.

In the present study, different tempeh types were prepared using different combinations of legumes like green gram, cowpea and soybean and cereals like rice and wheat. The grains were fermented with pure culture of *Rhizopus oligosporus* – MTCC 556. After fermentation, the tempeh prepared with different substrates was covered with fungal mycelium and the entire contents in the polyethylene bags could be lifted out as a whole piece. Thus, the fresh tempeh was a firm white cake with a very good nutty flavour. According to Hachmeister and Fung (1993), in good tempeh, the beans are knitted together by a mat of white mycelia. Babu *et al*. (2009) also reported that fermentation of soybean resulted in a firm textured product with a somewhat nutty flavor and a texture similar to a chewy mushroom. The growth of mycelium was maximum in tempeh prepared with 100 per cent soybean (control) and binding of cotyledons with the fungal hyphae was found to be less in treatments with cowpea. This may be due to the fact that the process of dehulling during the preparation of tempeh was very effective in soybeans whereas in cowpea some hulls still remained after the process. The fungal mycelium was found to adhere firmly to the dehulled cotyledons. Among the cereals used, the combinations with rice had a more firm texture than that of wheat and this may be due to the presence of husk in wheat which might have prevented the easy knitting together by fungal mycelium. The treatments with green gram had a comparable firmness with the control $T_{1.}$ All the treatments had a good flavour.

Similar studies conducted by Srapinkornburee *et al.* (2009) by using red kidney bean as substrate for tempeh production revealed that, tempeh after 48 h fermentation looked much like soybean tempeh and could be easily sliced because of the firm texture. Hachmeister and Fung (1993) developed several tempeh like products with wheat, triticale, yellow sorghum and red sorghum and revealed that the type of grain as well as the strain of *Rhizopus oligosporus* used influenced the product's appearance and flavour. In their study, they found that red sorghum yielded a product with good texture, aroma and appearance, yellow sorghum and triticale were found to be unacceptable substrates for tempeh production, and wheat though produced a product with a desirable aroma and flavour, it crumbled when sliced because of the less firm texture as observed in the present study also in tempeh types with wheat.

Verma and David (1981) developed tempeh with *Bakla* (*Vicia faba Linn*), an indigenous pulse alone and blended with soybean and found that all the tempeh types had a mushroom like odour, which was independent of the strain of *Rhizopus oligosporus* used, and *Bakla* tempeh was more firm than soy tempeh and the firmness decreased with increasing soybean content.

5.2. Acceptability of primary products with fresh tempeh

Tempeh is enjoying a growing popularity across the globe, for its nutty taste and meat like texture. Tempeh also owes its popularity to the fact that it can be consumed in a variety of ways. Today it has become one of the principle choices in the vegetarian cuisine and makes an excellent alternative to the meat. Tempeh is notable for its versatility in how it can be eaten. Because of its firm texture, it can be sliced, cubed, shredded or grated. Similarly, methods of using tempeh are varied; it can be baked, cooked, stir fried, toasted, grilled and stewed. It can be prepared or cooked in any way or can be substituted for meat in stir fries or stews.

Feasibility studies on the acceptability of a new product ought to be conducted to prevent product failure. Hence, in the present study, the twenty different tempeh types prepared with different combinations of cereals and pulses were used to make two preparations like tempeh chips and tempeh roast to conduct organoleptic studies. Among the tempeh chips, the highest mean score for appearance (9.0), colour (9.0), flavour (9.0), texture (8.9) and taste (8.8) was for T_2 (100 % green gram tempeh) which contributed to its high overall acceptability score of 8.9 indicating its acceptability between 'like very much ' to 'like extremely' by the panelists in a nine point hedonic scale. Tempeh chips with an overall acceptability score between 7.0 and 8.0 indicating 'like moderately' to 'like very much' where the chips made with tempeh types in which cowpea or wheat was a constituent substrate (T_6, T_{10}, T_{11}, T_{12}, T_{13}, T_{14}, T_{15}, T_{16} and T_{20}). For all other tempeh chips, the overall acceptability score were above 8.0.

Shurtleff and Aoyagi (2001) also stated that the chips made from tempeh has a very high acceptability and deep frying of tempeh yield chips that are delicate, light and crisp. Srapinkornburee *et al.* (2009) had also found that deep fried red kidney bean tempeh slices were darker in colour compared to the soybean tempeh and had an overall acceptability score of 7.0 in a nine point hedonic scale test.

Regarding tempeh roast, the highest score for appearance and colour was for the control T_1 (9.0). The highest score for flavour was for T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) with a score of 8.8. The maximum score for texture 9.0 was observed in roast prepared with T_5 (green gram 50% + soy 50%). Taste score was highest in T_{11} (cowpea 50% + rice 50%). Overall acceptability score was also highest in T_{11} (8.9). The mean score for the organoleptic qualities like appearance, colour, flavour, texture, taste and overall acceptability of most of the tempeh roasts were between 8.0 and 9.0 indicating 'like very much' to 'like extremely'. But in T_{17} (soy 75% + rice 25%) and T_{18} (soy 50% + rice 50%), even though the score for appearance, colour and texture were above 8.0, the score for flavour and taste was between 6.0 and 7.0 indicating 'like slightly' to 'like moderatly'. However, the overall acceptability score of these tempeh roasts were also between 7.0 and 8.0.

These results revealed that tempeh chips with cowpea as a substrate constituent had a comparatively low score than other chips, but tempeh roast with cowpea as a substrate (T_{11}) had the highest score for taste and overall acceptability. This result is in accordance with the findings of Vaidehi *et al*. (1985) who had also reported that tempeh products like chips and curries showed a high percentage (90%) of acceptability. The high acceptability of tempeh roast as revealed in the present study may be because tempeh fits perfectly to dishes prepared with meat since it absorbs all the flavours and its chewy texture contributing to its taste as meat.

High palatability and acceptability of tempeh products is mainly due to the development of improved flavours and texture due to fermentation (Nout and Ngoddy, 1997). The cultures used in food fermentations are, also contributing secondary reactions to the formation of good flavour and texture (Hansen, 2002). According to Blandino *et al*. (2003), during fermentation, several volatile compounds are formed which contribute to a complex blend of flavours in products. Bejarano *et. al.* (2006) revealed that an important function of the fungus *Rhizopus oligosporus* during tempeh fermentation is the synthesis of enzymes, which hydrolyse some of the substrate constituents and contribute to the development of desirable texture, flavour and aroma of the product.

5.3. *In vitro* starch and protein digestibility (IVSD and IVPD) of fresh tempeh

There are a wide range of processing techniques which could improve the digestibilities of cereals and legumes. Fermentation is a method, by which the nutritive value of plant foods could be improved (Obadina *et al.,* 2008). Fermentation of food significantly lowers the content of antinutrients and thereby improves the nutritive value of foods. In the present study, the IVSD of different tempeh types were found to be significantly high when compared to control T_1 (100% soybean tempeh).

The IVSD of all the fresh tempeh types were found to be above 70 per cent except in T_1 (69.18). IVSD above 80 per cent was observed in tempeh types T_2 (100% green gram), T_4 (green gram 75% + soy 25%), T_5 (green gram 50% +soy 50%), T_8 (green gram 75% + rice 25%) and T_9 (green gram 50%+ rice 50%); the maximum being in T_9 (82.83%). In all these tempeh types with high IVSD, green gram was a substrate constituent.

The high IVSD of tempeh types in the present study is supported by the observations of Urooj and Puttaraj (1994), who also found that the digestibility of starch in bengal gram, cowpea and green gram was increased by fermentation. Elkhalifa *et al*. (2004) also reported an appreciable enhancement (> 96%) in starch digestibility of moth bean due to fermentation. The high starch digestibility of fermented foods may be related to enzymatic properties of microbes, which ferment the substrate. The fermenting micro flora brings about the breakdown of starch to oligosaccharides. The enzymes produced by the fermenting microflora also bring about the cleavage of amylose and amylopectin to maltose and glucose (Sindhu and Khetarpaul, 2002). Bhatia *et al.* (2009) reported that increase in starch digestibility of indigenous fermented foods could be attributed to the significant reduction in phytic acid during fermentation.

IVPD was found to be above 70 per cent in all the tempeh types. Above 80 per cent IVPD was observed in T_2 (88.57%), T_4 (81.23%), T_8 (88.98%), T_9 (86.72%), T_{10} (80.34%), T_{12} (81.32%) and T_{13} (82.08%), the highest being in T_8 (green gram 75% + rice 25%). Here also all the tempeh types with significantly high IVPD were found to be the ones with green gram as a substrate constituent except in T_{10} (cowpea 75% + rice 25%).

The high IVPD of different tempeh types (>70%) in this study is supported by Bozena *et al*. (2008) who reported that tempeh fermentation increased protein bioavailability by about 25 per cent. López and Harry (1990) also reported an increase in the IVPD in common beans as a consequence of tempeh fermentation. Increase in IVPD could be explained by the elimination of antinutritional factors (e.g. hydrolysis of phytic acid during fermentation) and protein denaturation during the cooking step, which results in proteins that are more vulnerable to enzyme action. Vaidehi (1993) reported that increase in the protein efficiency ratio in tempeh is due to the destruction of phytic acid present in soybeans by the fungus *Rhizopus oligosporus* during fermentation. As observed by Sutardi and Buckle (2008), soybeans have the highest levels of phytates, but when they are fermented to make tempeh, the amount of phytic acid decreased by one half. Accordig to Hachmeister and Fung (1993), an important function of *Rhizopus oligosporus* in the fermentation process during the production of tempeh is the synthesis of enzymes, which hydrolyze substrate constituents and which may decrease or eliminate antinutritional constituents; consequently, the nutritional quality of the fermented product may be improved. Yousif (2001) attributed the increase in protein availability in tempeh to enzymatic breakdown during fermentation to partial degradation of complex storage proteins into simpler soluble products. Trypsin inhibitors in legumes are inactivated to a great extent by fermentation (Tripathi and Nath, 2002). Babu *et al*. (2009), revealed that tempeh is easy to digest because the fermentation process breaks down the complex proteins found in soybeans, making it more easily digested than non-fermented soy foods or whole soybeans. Mittal and Garg (1990) also reported tempeh as a highly digestible food and according to Astuti *et al.* (2000), tempeh fermentation increased the bioavailability of proteins, lipids, carbohydrates and minerals.

The high IVSD and IVPD observed in the tempeh types prepared with green gram and rice in the present study can be explained by the fact that both green gram and rice contain low amount of antinutritional factors when compared to soybean, cowpea and wheat and which on fermentation with *Rhizopus oligosporus*, again get reduced and thus increased the digestibility to a maximum.

5.4. Selection of fresh tempeh types with maximum quality attributes

From the twenty different treatments of tempeh, three fresh tempeh types which are most acceptable with high overall acceptability score and with a high protein and starch digestibility, were selected along with the control (100% soybean tempeh) for further nutritional and shelf life studies with fresh tempeh and also for developing tempeh flours and soup mixes. Three treatments were selected based on the acceptability scores of tempeh chips and tempeh roast by applying Kendall's coefficient of concordance (Table 2 and 3) and the observation on IVSD and IVPD were analysed statistically by DMRT (Table 4). From the statistically analysed tables, the three treatments selected considering the acceptability, IVSD and IVPD were T_2 (100% green gram tempeh), T_8 (green gram 75% + rice 25% tempeh) and T_9 (green gram 50% + rice 50% tempeh). Along with the three selected tempeh types, T_1 (100% soybean tempeh) was also selected as control.

5.5. Chemical constituents in selected fresh tempeh types

Tempeh is a low cost nutritious food with high digestibility and it is becoming one of the most preferred and popular indigenous health foods. Hence, in the present study, the three selected tempeh types - T_2 (100% green gram tempeh), T_8 (green gram 75% + rice 25% tempeh) and T_9 (green gram 50% + rice 50% tempeh) along with the control T_1 (100% soybean tempeh) were analysed for their chemical constituents.

The moisture content of different treatments ranged from 40.79 to 55.85 g/100g with significant variation among the treatments. The moisture content was significantly high in T_1 (100% soybean tempeh) and the lowest moisture content was in T_9 (green gram 50% + rice 50% tempeh). The results are in agreement with the findings of Vaidehi (1993), who reported a moisture content of 56 g/100g in 100 per cent soybean tempeh. Hesseltine (1983) reported a higher moisture content in soybean tempeh which ranged from 64.0 to 72.8 per cent.

The protein content of the selected fresh tempeh types varied between 8.36 and 21.09 g/100g with the lowest protein in T_9 (green gram 50% + rice 50% tempeh) and highest in T_1 (100% soybean tempeh). Protein content in T_1 (100% soybean tempeh) was found to be significantly high followed by T_2 (100% green gram tempeh). The high protein content observed in T_1 (100% soybean tempeh) may be due to the high protein content in soybeans than in green gram and rice. The protein content observed for the soybean tempeh in the present study is in accordance with Vaidehi (1993) who reported a protein content of 20.00 g/100g in fresh soybean tempeh. In the present study, the protein content was found to be low when compared to the unfermented soybeans and green gram. The results are in agreement with Reed (1981) who reported that fermentation may not increase the content of protein and amino acids unless ammonia or urea is added as a nitrogen source to the fermentation media. Murata *et al*. (2006) also reported that no increase in protein content existed between tempeh and unfermented soybeans.

Even though there was no increase in the total protein content, the digestibility of protein was found to be very high in the selected tempeh types with green gram (T_2, T_8 and T_9) which ranged from 86.72 to 88.98 per cent when compared to the control T_1 (72.03%). This makes tempeh an easily digestible food with high protein availability.

There was a significant variation in the fiber content among the treatments with the maximum fiber content of 2.52 g/100g in T_2 (100% green gram tempeh) and the least (1.03 g/100g) in T_9 (green gram 50% + rice 50% tempeh). The high fiber content in T_2 (100% green gram tempeh) may be due to the retention of more hulls in green gram when compared to soybeans before tempeh preparation. Significantly low fiber content in the treatments with green gram and rice combinations may be due to the low fiber content in raw rice. In the present study, the total fiber content in tempeh was found to be low when compared to the fiber content in legumes. Cabrejas *et al*. (2004) also revealed that tempeh fermentation significantly decreased the soluble dietary fiber content, and no significant changes were noticed in the insoluble dietary fiber content of beans. Cellulose content of all the samples was also reduced by tempeh fermentation. But Antai and Obong (1992) reported a significant increase in total fiber content in fermented foods. According to Nout and Rombouts (1990), the growth of the fungus during tempeh fermentation reduced the concentration of low molecular weight carbohydrates and increased the dietary fiber content in tempeh.

The total fat content of the selected fresh tempeh types ranged from 0.39 to 10.12 g/100g with highest total fat content in T_1 (100% soybean tempeh). Total fat content in T_1 (10.12 g/100g) was significantly high when compared to all other tempeh types. Babu *et al*. (2009) reported a fat content of 9.2 per cent in soy tempeh, which is in accordance with the fat content observed in T_1 (100% soybean) in the present study. Vaidehi (1993) and Tee *et al*. (1997) reported a lower fat content of 7.5 g/100g in fresh soybean tempeh. Karyadi and Lukito (1996) reported a very low fat content of 4.0 per cent in tempeh. According to Shurtleff and Aoyagi (2001), the decreased fat content in tempeh than that of unfermented soybean may be due to the lipolytic activity of *Rhizopus oligosporus*.

The starch content of the selected fresh tempeh types varied from 5.84 to 29.13 g/100g with the lowest starch content in T_1 (100% soybean tempeh) and the highest in T_9 (green gram 50% + rice 50% tempeh). Hag *et al*. (2002) observed a reduction in the starch content of soybeans from 67 to 59 per cent for standard variety and from 69 to 63 per cent for Ugandi variety during fermentation. The comparatively high values for T_8 (28.49 g/100g) and T_9 (29.13 g/100g) may be due to the combination with rice. Khetarpaul and Chauhan (1990) also reported a reduction in the starch content of grains after fermentation. The reduction in starch content may be due to the hydrolysis of starch to sugars during fermentation.

In the present study, reducing sugar varied from 1.02 to 2.01 g/100g, the maximum in T_2 (100% green gram tempeh) and the minimum in T_9 (green gram 50% + rice 50% tempeh). The total sugar content was found to be highest in T_2 (100% green gram tempeh) and lowest in T_9 (green gram 50% + rice 50% tempeh) (4.21 and 3.21 g/100g respectively). The high reducing and total sugar content in tempeh types in this study is in line with the findings of Khetarpaul and Chauhan (1990) who reported that single as well as mixed culture fermentation of pearl millet flour significantly increased the total amount of soluble sugars, reducing and non-reducing sugar content, with a simultaneous decrease in its starch content.

Thiamine content in different tempeh types was found to be low. The thiamine content of the different treatments ranged between 0.09 to 0.31 mg/100g. The lowest thiamine content was observed in T_9 (green gram 50% + rice 50% tempeh) and highest in T_1 (100% soybean tempeh). The thiamine content in T_1 (100% soybean tempeh) was significantly high than all other treatments. The results are in accordance with the findings of Vaidehi (1993), who reported a thiamin content of 0.28 mg/100g in soybean tempeh. The low thiamine content in tempeh may be due to the soaking and dehulling process during the preparation of tempeh. According to Gopalan *et al*., (1999), removal of outer layer of cereals and pulses results in a drastic decrease in their thiamine content. Chavan and Kadam (1989) stated that changes in the vitamin content of grains with fermentation vary according to the fermentation process, and the raw materials used in the fermentation. According to him, the B group vitamins generally show an increase during fermentation. Many researchers had reported that vitamins, such as thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5) and pyridoxine (B_6) can be produced by the tempeh fungus *Rhizopus oligosporus* (Nout and Rombouts, 1990; Mugula, 1992; Shurtleff and Aoyagi, 2001; Nout and Kiers, 2005). In the present study the production of thiamine during fermentation by *Rhizopus oligosporus* might not have been sufficient enough to cope up with the loss of thiamine due to dehulling and soaking process.

Maximum riboflavin content was found in T_2 (green gram 100%) tempeh (0.30 mg/100g) and the lowest in T_9 (green gram 50% + rice 50%) tempeh (0.19 mg/100g). T_1 (soybean100%) tempeh had a riboflavin content of 0.27 mg/100g. Vaidehi (1993) reported a riboflavin content of 0.65 mg/100g in fresh soybean tempeh. The low riboflavin content of tempeh might be due to the soaking and dehulling of the pulses during the preparation of tempeh. According to Srilakshmi (1999), the B vitamins of the pulses are leached into the water used for soaking pulses. However, the riboflavin content in T_2 (green gram 100%), T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) (0.30, 0.25 and 0.19 mg/100g respectively) were comparatively higher than in the raw ingredients used for tempeh preparations.

β carotene content of the different tempeh types varied from 37.07 to 331.85 μg/100g with the lowest β carotene in T_9 (green gram 50% + rice 50%) tempeh and the highest in T_1 (100% soybean) tempeh. β carotene was found to be significantly high in 100 per cent soybean tempeh when compared to the other three treatments. However, there was a reduction in the β carotene content in all the tempeh types when compared to the unfermented grains. Lin *et al.*, (2007) reported no significant variation in the β carotene content of fermented and unfermented maize porridges. The significantly high β carotene content in 100 per cent soybean tempeh may be due to the high β carotene content in raw soybeans than in green gram.

None of the selected fresh tempeh types in the present study contained detectable amount of vitamin C. Shurtleff and Aoyagi (2001) also reported that fresh tempeh does not contain any vitamin C. According to Gandjar (1978), fermentation of winged bean resulted in the increase of amino nitrogen drastically with complete loss of vitamin C.

It would not be expected that fermentation would increase the mineral content of the product, but the hydrolysis of chelating agents such as phytic acid during fermentation, improves the bioavailability of minerals (Steinkraus, 1996). In the present study, the calcium content of the selected fresh tempeh types ranged from 40.37 to 149.10 mg/100g with the lowest calcium content in T_9 (green gram 50% + rice 50%) tempeh and highest in T_1 (100% soybean) tempeh. Calcium content was significantly high in T_1 when compared to other tempeh types. Vaidehi (1993) also reported a calcium content of 142 mg/100g in fresh soybean tempeh. According to Tee *et al*. (1997) tempeh contains about 129mg calcium / 100 g and in Malaysian diet, tempeh is also one of the calcium rich foods besides milk and dairy products. Karyadi and Lukito (1996) reported a calcium content of 69 mg/100g in tempeh. Studies conducted by Haron *et al*. (2008) showed calcium content of 56.8 + 1.6 mg/100g in tempeh. Babu *et al.* (2009) also stated tempeh as an excellent source of calcium. The significantly high calcium content observed in 100 per cent soybean tempeh may be due to the high calcium content observed in raw soybean when compared to other pulses.

The iron content was significantly high in T_1 (soybean 100%) tempeh (3.6 mg/100g) and there was no significant variation in the iron content of other tempeh types which ranged from 1.53 to 1.9 mg/100g. The iron content in T_1 in the present study was found to be higher than an iron content of 1.5 mg/100g in soybean tempeh as reported by Vaidehi (1993).

The phosphorus content was also significantly high in 100 per cent soybean tempeh (270.61 mg/100g) and significantly low in T_9 (green gram 50% + rice 50%) tempeh (158.52 mg/100g). The results are in accordance with the findings of Shurtleff and Aoyagi (2001) who reported a phosphorus content of 240 mg/100g in fresh soybean tempeh.

The potassium content of the selected fresh tempeh types varied from 296.62 to 525.11 mg/100g with the highest potassium content in T_2 (green gram 100%) and lowest in T_1 (soybean 100%). The lowest zinc content was found in T_9 (green gram 50% + rice 50%) tempeh (1.34mg/100g) and the highest in T_1 (soybean 100%) tempeh (2.17 mg/100g).

According to Kozlowska (1996), fermentation does not usually increase the level of minerals present in foods unless unusual circumstances are present (as in fermenting food in a metal or earthen container), but it decreases the activity of phytic acid content naturally present in grains. In this study also, all the tempeh types, showed a reduction in the total calcium, iron, phosphorus, potassium and zinc when compared to the mineral content in the raw grains used for tempeh preparation. This loss in mineral content can be attributed to soaking and dehulling of legumes in the process of tempeh preparation. Studies conducted by Appukuttan (2010) also revealed that soaking of green gram, bengal gram and horse gram for six hours and dehulling before cooking showed a considerable reduction in calcium, iron, phosphorus, potassium and zinc content.

5.6. ***In vitro*** **availability of minerals from selected fresh tempeh types**

Presence of antinutritional factors is one of the main drawbacks limiting the nutritional and food qualities of legumes. Availability of a nutrient refers to the amount of a nutrient in a food that the body may ultimately use to perform specific physiological functions. Bioavailability of nutrients varies within foods and depends on the concentration of enhancers and inhibitors of absorption. The antinutritional factors in legumes are known to reduce the activity of digestive enzymes, reduce the availability of nutrients for absorption and damage the intestinal tract.

Mineral availability of cereals and pulses can be improved by decreasing the antinutritional factors especially by hydrolysing the phytates. In the present study calcium availability of different tempeh types varied from 49.00 to 61.77 per cent, the highest being in T_9 (green gram 50% + rice 50%) tempeh and the lowest in T_1 (soybean 100%) tempeh. Even though there was a reduction in the total calcium content in tempeh when compared to the raw ingredients, the bioavailability of calcium was found to be very high in different tempeh types. Appukuttan (2010) had reported that in green gram soaked for 12 hours and dehulled, calcium availability was 24.32 per cent whereas in the present study, in T_2 (green gram 100%) tempeh, the calcium availability was found to be 56.63 per cent. The comparatively low calcium availability in T_1 (soybean 100%) tempeh (49 %) may be due to the very high phytate content in raw soybeans.

The availability of iron from different tempeh types was also found to be high which ranged from 64.52 to 66.82 per cent without significant variation among them. Appukuttan (2010) had reported that in green gram soaked for 12 hours and dehulled, iron availability was 30.62 per cent whereas in T_2 (green gram 100%) tempeh, iron availability was 66.07 per cent indicating the effect of tempeh fermentation in increasing the iron availability.

Phosphorus availability from different tempeh types varied from 49.68 to 65.72 per cent with T_1 (soybean 100%) tempeh having significantly low phosphorus availability, compared to other treatments. The study by Appukuttan (2010) revealed that, in green gram soaked for 12 hours and dehulled, phosphorus availability was 48.25 per cent whereas in 100 per cent green gram tempeh (T_2), the phosphorus availability was found to be 61.49 per cent. The comparatively low phosphorus availability in T_1 (soybean 100%) tempeh may be due to the high phytate content in soybean.

Potassium availability was found to be 46.30 to 63.60 per cent in different tempeh types, the highest being in T_9 (green gram 50% + rice 50%) tempeh. Potassium availability in green gram soaked for 12 hours and dehulled was 45.69 per cent as reported by Appukuttan (2010), but after tempeh fermentation , the potassium availability was 54.15 per cent in T_2 (green gram 100%) tempeh.

The availability of zinc was found to be the highest among the minerals studied. Zinc availability varied from 82.23 to 88.99 per cent without significant variation with the tempeh types. Zinc availability reported by Appukuttan (2010) was 62.78 per cent in soaked and dehulled green gram, but in T_2 (100% green gram) tempeh, zinc availability was 86.17 per cent.

The mineral availability studies revealed that the availability of minerals from T_1 (soybean 100%) tempeh was found to be the lowest and the highest availability of minerals was from T_9 (green gram 50% + rice 50%) tempeh. The high availability of minerals as revealed in the present study was supported by Sudermadji and Suparmo (1997) who also found a high bioavailability of elements such as zinc, iron, manganese, calcium and phosphorus in tempeh. Astuti *et al.,* (2000) also revealed that fermentation process of tempeh decreased the phytic acid and enhanced the bioavailability of minerals such as calcium, zinc and iron. Babu *et al.* (2009) reported that fermentation neutralizes the phytate acid present in the soybeans; therefore tempeh does not restrict the body's absorption of minerals. Jood and Khetarpaul (2005) found that reduction in antinutrients due to fermentation may increase the bioavailability of various minerals, but there need not be an increase in the total mineral content in fermented foods. This is in line with the results of the present study which showed a reduction in the total mineral content in all the tempeh types mainly because of soaking and dehulling process in tempeh preparation.

5.7. Probiotic activity of the selected fresh tempeh types

Probiotic foods are those foods which contain a live microbiological culture either as a result of fermentation or as an intentional addition, to beneficially affect the host by improving the intestinal microbial balance (Mark, 2002). These microorganisms interact with the diet and the host contributing to protection against intestinal pathogens through colonisation resistance and providing nutritional and health benefits through their metabolic activities. Probiotics can be bacteria, moulds or yeast. Most probiotic foods are fermented at least partially and among the products which have received the most attention in this regard include fermented milks such as yoghurt and butter milk, frozen desserts such as ice creams, miso kefir, and sauerkraut, certain pickles, tofu and tempeh.

According to Holzapfel (1998), for the use in foods, probiotic microorganisms should not only be capable of surviving passage through the digestive tract but also have the capability to proliferate in the gut. This means, they must be resistant to gastric juices and be able to grow in the presence of bile under conditions in the intestines, or be consumed in a food vehicle that allows them to survive passage through the stomach and exposure to bile. Hence in this study, probiotic characteristics such as acid and bile acid tolerance and antimicrobial activities of the selected fresh tempeh types were studied *in vitro*.

The results of the *in vitro* studies revealed that, there was no bacterial viability in fresh tempeh in different pH levels varying from 1.5 to 3.5, no viable of yeast between pH 1.5 to 2.5 and there was no fungal growth as indicated by the biomass of *Rhizopus oligosporus* between pH 1.5 to 2.5. None of the organisms (bacteria, yeast, *Rhizopus oligosporus*) showed tolerance to a bile acid concentration varying from 1-4 per cent. Fresh tempeh types did not show antibacterial activity against any of the enteropathogens studied (*E.coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Salmonella enteritidis*).

The survival of any microorganisms in the stomach should be pH-HCl dependent (Giannella *et al.*, 1972). In the present study, with tempeh, no organisms were found to survive in pH 1.5 to 2.5. According to Lankahaputhra and Shah (1995), one of the most important criteria for a probiotic organism is their ability to survive in the upper intestine that contains bile. None of the organisms in tempeh showed tolerance to bile acid in any of the concentration levels (1-4) per cent. Further, the developed tempeh types showed no antibacterial activities against enteropathogenic bacteria. Hence, none of the selected tempeh types can be considered as a probiotic food.

Earlier studies conducted by Berghofer *et al*. (2001) had reported that *Rhizopus oligosporus* produced four to five antibacterial compounds during soybean tempeh fermentation. Feng (2006) also reported that tempeh fungus *Rhizopus oligosporus* possesses antimicrobial properties.

According to Clark *et al.* (1993), the acid and bile tolerance and the production of antimicrobial substances by a microorganism is strain depended. Salminen *et al*. (1998) also had reported that the strains of different microorganisms vary in their ability to produce antibacterial substances and the cultural conditions also will influence the amount produced. Hence the pure culture of *Rhizopus* *oligosporus* - MTCC 556 used in the present study for tempeh fermentation may be of a strain without probiotic activity.

5.8. Shelf life of selected fresh tempeh types

Production of foods of consistently acceptable quality with shelf lives adequate for their intended uses, together with correct communication to the consumer of their durability is important to the manufacturer, retailer and the consumer. The consideration which is most important is that, the food must reach the consumer in good condition and retain its quality for the period expected. (Man and Jones, 2000). Consumers are increasingly demanding consistently high food quality, and have corresponding expectations that such quality will be maintained at a high level during the period between purchase and consumption. These expectations are a consequence not only of the primary requirement that the food should remain safe, but also of the need to minimise unwanted changes in sensory qualities and microbial load. The sensory characteristics of most foods deteriorate throughout storage and yet, provided they remain safe, a large degree of change is evidently tolerable to consumers (Kilcast and Subramanian, 2000).

A fermented food is a food that is prepared involving a step where microorganisms (or enzymes) alter the properties of the food. The purpose of fermenting food is often to get a better taste or texture, but one important reason is that it keeps better when fermented. In the present study the selected three fresh tempeh types along with the control (100% soybean tempeh) were packed in polythene bags of 250 gauge thickness and were kept under refrigerated condition (3 to 7^0C) for 12 days and in a deep freezer (-15 to -18^0C) for one month. The shelf life quality of tempeh was assessed by assessing their sensory qualities by observation, by enumerating their microbial load and also by evaluating the organoleptic qualities of tempeh chips and roast prepared from the stored samples. Quality evaluation was done initially and during 3^{rd}, 6^{th}, 9^{th} and 12^{th} day in refrigerated samples and in frozen samples, evaluation was done initially, during the 15^{th} day and 30^{th} day of storage.

5.8.1. Sensory qualities in stored tempeh

The appearance, colour, flavour and texture of the fresh tempeh stored under refrigerated condition up to 12 days showed no changes in the case of T_1 (soybean 100%) and in T_2 (green gram 100%) tempeh. In general, fresh tempeh of good quality is defined by Nout and Rombouts (1990) as a compact and sliceable mass of cooked particles of raw materials covered, penetrated and held together by dense mycelium of *Rhizopus* Spp. In all the selected fresh tempeh types, the grains were under a cottony cover and had a pleasant, nutty flavour. The entire cake can be lifted up as a single cohesive cake, but all the fresh tempeh types showed a small patch of grey mycelium or black sporulation near pin holes in the plastic cover which increased with storage days. Lothony (1992) had reported that the appearance of spores is not harmful, unless there is a change in the flavour and texture, but the gray/black patches will affect the appearance of the tempeh.

In T_8 (green gram 75% + rice 25%) tempeh and T_9 (green gram 50% + rice 50%) tempeh, after the 6th day of refrigerated storage, the tempeh became slimy with a strong smell of ammonia and the texture became soggy indicating the spoilage of tempeh. The changes observed in these tempeh types after 6 days of storage were same as described by Feng (2006). According to the author, in inedible tempeh, the beans are foul or rotten, smelling like ammonia indicating the development of undesirable bacteria due to excess moisture. The tempeh cakes will be wet, slimy, mushy or sticky with a collapsed structure that is limp when bent.

All the tempeh types (T_1, T_2, T_8 and T_9) when stored in a deep freezer gave a product comparable to the fresh tempeh even after 30 days of storage.

The observations on the stored tempeh are in line with Shurtleff and Aoyagi (2001) who reported that the soybean tempeh can be stored in refrigerator for more than a week. In the present study, T_1 (soybean 100%) and T_2 (green gram 100%) tempeh, could be stored up to 12 days in the refrigerator whereas, T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh were stored only up to 6 days in a refrigerator. In contrast, Vaidehi and Rathnamani (1990) in the shelf life studies of soy sunflower tempeh reported that, under refrigerated conditions, soy sunflower tempeh can be stored for three to four days after which they became slimy with slight ammonia smell. In T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh, smell of ammonia developed only after 6 days of storage in the refrigerator and after that the product became slimy. According to Kada *et al*., (2008), the ammonia production in fermented pulses might be due to degradation of proteins by proteolytic bacteria.

The storage of all the selected fresh tempeh types (T_1, T_2, T_8 and T_9) in deep freezer gave intact products comparable to the fresh tempeh after 30 days of storage. This is in line with the findings of Vaidehi and Rathnamani (1990), who reported that the quality of the tempeh was not affected when stored in deep freezer. Shurtleff and Aoyagi (2001) also reported that the best way to store fresh tempeh is by freezing and it can be stored in freezer for more than two months.

5.8.2. Total micro flora in stored tempeh

In the present study the bacterial load in different fresh tempeh types were found to be high which varied from 49.3 to 69.3 x 10^8 cfu/g, the maximum being in T_1 (soybean 100%). This result is in accordance with the results of Ashenafi (1992), who reported an initial bacterial count in the range x 10^8 cfu/g in fresh tempeh. Shurtleff and Aoyagi (2001) also reported a high bacterial count in tempeh. According to Aderibigb and Osegboun (2006), a bacterial count of 8.2 x 10^6 cfu/g was observed in fresh tempeh. A gradual decrease in the bacterial count up to the 6^{th} day of refrigerated storage was observed in all the tempeh types. This may be because at low temperatures, the rate of enzyme reactions in the microorganisms were decreased and thus retarded the growth (Frazier and Westhoff, 1995). According to them, cooler temperatures will prevent the growth of microorganisms, but slow metabolic activity may continue. The slight increase in the bacterial count observed after 6 days of storage in T_1 (soybean 100%) and T_2 (green gram 100%) tempeh may be due to the acclimatization of the bacteria to the new environment. But in T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh, the bacterial count was high when compared to T_1 (soybean 100%) and T_2 (green gram 100%) tempeh after 6^{th} day and also resulted in a slimy tempeh with an unpleasant smell indicating spoilage.

The total yeast count in different fresh tempeh types was in the range of 42.6 to 66.3 x 10^5 cfu/g , the highest in T_9 (green gram 50% + rice 50% tempeh) and lowest in T_2 (green gram 100%) tempeh. These results are also in line with the findings of Ashenafi (1992) who reported a yeast count in the range of x 10^5 cfu/g. Shurtleff and Aoyagi (2001) reported that, yeast also contribute to the fermentation of tempeh. In the present study, a decrease in the yeast count was observed on storage in T_1 (100% soybean) and in T_2 (100% green gram) tempeh, but in T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh, there was an increase in the total yeast count on the 9^{th} and 12^{th} day of storage which resulted in complete spoilage of the tempeh as evidenced by the appearance, flavour and texture. The results indicate that the yeast may also be responsible for the spoilage of tempeh. Shurtleff and Aoyagi (2001) had reported yeast as one of the main spoilage organisms in tempeh.

Rhizopus oligosporus was the only fungus present in tempeh in the present study. This may be due to the use of only pure culture of *Rhizopus oligosporus* – MTCC 556 for tempeh fermentation. Steinkraus (1996) also found only a single predominant mould in Indonesian tempeh which was identified as *Rhizopus oligosporus. Rhizopus oligosporus* is the most preferred species in tempeh fermentation due to its properties such as rapid growth at high temperature (30-42°C), inability to ferment sucrose, high proteolytic and lipolytic activities and production of strong antioxidants (Steinkraus *et al.*, 1983). Hesseltine *et al*. (1963) isolated many fungi from different lots of tempeh made in Indonesia and found that only *Rhizophus* could make tempeh in pure culture fermentation. The total fungal count in the present study was in the range of 10^6 cfu/g (Table 16) which is comparable with the findings of Aderibigb and Osegboun (2006) who reported a total fungal count of 7.17 x 10^6 cfu/g in fresh tempeh. A gradual reduction in the total fungal count was observed on storage. The decrease in the total fungal count may be due to the hazardous effect of low temperature on *Rhizopus oligosporus*. Very low fungal counts were observed in T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh during the 9^{th} and 12^{th} day indicating that the growth of *Rhizopus oligosporus* was inhibited by the overgrowth of yeast in these tempeh types on the 9^{th} and 12^{th} day of storage resulting in spoilage.

The tempeh stored in deep freezer gave a product comparable to that of fresh tempeh (Table 13) and this was reflected in the microbial load also. There was a sharp decline in the microbial count of frozen tempeh in all the treatments (Table 17, 18 and 19). Ashenafi (1992) also reported a sharp decrease in the microbial load of fresh tempeh during cold storage. According to Shurtleff and Aoyagi (2001), freezing is the best way to preserve tempeh and according to him, the microbial load was decreased in freezer storage. Frazier and Westhoff (1995) stated that during the storage of food in frozen condition, the chemical and enzymatic reactions proceed slowly and continuous exposure to freezing temperature kills the vegetative cells of microorganisms. According to them, there is a decrease in the number of viable microorganisms as storage continues, with some species dying more rapidly than others. They also reported that killing rate during freezing is rapid, but it is followed by a gradual reduction of microorganisms and is referred to as 'storage death'.

Traditional tempeh is the result of mixed culture fermentation by a diverse group of microorganisms including moulds, yeast and lactic acid bacteria. *Rhizopus oligosporus* is the dominant tempeh fungus. In the present study, the bacterial count in fresh tempeh was very high, consisting of non pathogenic bacteria such as *Lactococci* and *Klebsiella*, the normal identified microflora found in tempeh and in other naturally fermented foods as reported by Liem *et al.* (1977). According to Suparmo (1989) *Klebsiella* spp. is considered as being the species producing vitamin B_{12} in tempeh. As suggested by Nout, (1989) and Ashenafi and Busse (1991), lactic acid bacteria (LAB) may contribute to the microbial safety and nutritional value of tempeh in many ways.

LAB can improve organoleptic qualities of foods (Stien *et al*., 1999) and can produce folate (Sanna *et al*., 2005; Kariluoto *et al*., 2006). Furthermore LAB can remove raffinose, stachyose and verbascose from legumes (Scalabrini *et al*., 1998; Leroy and Vuyst, 2004), and also proteinase inhibitors from legumes to prevent maldigestion (Holzapfel, 1998). LAB can also degrade phytic acid and tannins from cereals and legumes to increase mineral bioavailability (Sharma and Kapoor, 1996; Holzapfel, 1998). Some LAB are considered as probiotics (Merk *et al*., 2005; Shimosato *et al*., 2006) and both viable and non viable forms showed efficacy in shortening the duration of diarrhea (Ouwehand and Salminen, 1998).

But in the present study, in different tempeh types none of the organisms (bacteria, yeast or fungi) were tolerant to a low pH (1.5 to 2.5) and to different bile acid concentration varying from 1- 4 per cent or showed antibacterial activity against enteropathogens. So the LAB present in the different tempeh types may not be of a probiotic strain.

Pathogenic bacteria do not grow well in acidified substrates and even if they can grow, they are normally controlled by lactic acid bacteria. Koswan and Hesseltine (1979) had also reported that no cases of food poisoning have ever been reported after consuming tempeh. Nout and Rombouts (1990) had suggested that this safety is due to inherent properties of *Rhizopus* Spp., presence of high counts of lactic acid bacteria, incubation under aerobic conditions and the customary heating (cooking) prior to consumption. Therefore, the growth of LAB in fresh tempeh types in this study can contribute to the nutritional value and microbial safety.

During refrigerated storage of fresh tempeh types, the growth of yeast was very high in T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh with a low fungal count of *Rhizopus oligosporus* during the 9th and 12th day of storage which might be due to the inhibition of the growth of tempeh fungus by the high yeast population. This might have resulted in the spoilage of these tempeh types (T_8 and T_9) after 6 days of refrigerated storage. The high starch content of the substrates of these tempeh types (green gram and rice) may be also a factor which helped the yeast to grow well during storage of T_8 and T_9 tempeh. Steinkraus *et al.* (1983) has also detected yeast in commercial and traditional tempeh products. As reported by Samson *et al.* (1987), yeasts are frequently detected in tempeh, but their role is still unknown. But Feng (2006) had observed that yeast at low levels (10^4 cfu/g) did not negatively affect growth of *Rhizopus oligosporus* but did so at higher levels.

5.8.3. Acceptability of products prepared with stored tempeh

Even though the shelf life of the tempeh can be increased by low temperature storage, the acceptability of the products prepared from the stored tempeh is also equally important to the end user. Hence in this study, the acceptability of tempeh chips and roast prepared with tempeh types stored under refrigerated condition and also in deep freezer was studied. Fresh tempeh had a good acceptability score for appearance, colour, flavour, texture, and taste. Since overall acceptability is a reflection of all the quality attributes combined together, the variation in the overall acceptability score will indicate the acceptability of the chips and roast prepared with stored tempeh types.

5.8.3.1. Acceptability of the chips and roast with tempeh types in refrigerated storage

The overall acceptability score of chips (Table 25) with tempeh types in refrigerated storage were low when compared to the overall acceptability score of chips with the respective fresh tempeh types. A gradual reduction in the overall acceptability score was observed on storage. The acceptability score was highest for T_2 (100% green gram) tempeh during all the storage period. Up to the 3rd day of storage, the overall acceptability score was ≥ 8.0 in all the tempeh types except in T_9 (green gram 50% + rice 50%). After 6 days, the score for T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh were below 8.0. By the 12th day, the overall acceptability for T_1 (100% soybean) and T_2 (100% green gram) tempeh were 7.0 and 7.2 respectively indicating the acceptability between 'like slightly' and 'like moderately'. Tempeh types T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) were acceptable only up to 6 days of refrigerated storage beyond which they became spoiled.

In the case of tempeh roast, the overall acceptability was decreased on storage. On the 1st and 3rd day of storage, the acceptability indicated was from 'like moderately' to 'like very much'. Initially there was a high score (≥ 8.0) in all the tempeh types, but after 3 days of storage, overall acceptability score for T_1 (100% soybean) and T_2 (100% green gram) were less than 8.0. On the 6th day, overall acceptability score was higher for T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) compared to T_1 (100% soybean) and T_2 (100% green gram). A lower overall acceptability score of less than 7.0 indicating an acceptability of 'like slightly' to 'like moderately' was observed in T_1 and T_2 for the 9th and 12th day of storage. T_8 and T_9 tempeh types were spoiled after 6 days of refrigerated storage.

The gradual decrease in the overall acceptability of chips and roast with the advancement of storage period might be due to the decrease in the flavour and texture as observed in the sensory evaluation of stored tempeh (Table 12). Vaidehi (1993) reported that the products made from stored tempeh were acceptable unless it is spoiled on storage.

5.8.3.2. Acceptability of the chips and roast with frozen tempeh

A decrease in the overall acceptability score was observed in the tempeh chips and roasts prepared from the tempeh stored in deep freezer. The overall acceptability score indicated an acceptability of 'like very much' to the chips prepared with the tempeh on the 15th day of storage. On the 30th day of storage, the overall acceptability was found to be 'like moderately' in T_9 (green gram 50% + rice 50%) tempeh, but in other tempeh chips, it was 'like very much'. In the case of roast, the acceptability was found to be 'like very much' up to 30th day of storage except in roast prepared with T_1 (100% soybean) tempeh.

Thus the study revealed that frozen tempeh did not reduce the acceptability of the products prepared from it. This is in line with the findings of Vaidehi and Rathnamani (1990), who reported that the quality of the tempeh was not affected when stored in deep freezer. Shurtleff and Aoyagi (2001) also reported that the best way to store fresh tempeh is by freezing and according to them the fresh tempeh can be stored in freezer for more than two months**.**

5.9. Quality evaluation and shelf life of tempeh flours

The flours prepared from different tempeh types were packed in metalised polyester laminate pouches and were stored in room temperature for six months. The stored tempeh flours were evaluated for its chemical constituents, *in vitro* digestibility of proteins and starch and the shelf life attributes such as total micro flora, and insect infestation of the flour were assessed.

5.9.1. Changes in chemical constituents in tempeh flour during storage

Tempeh flours were analysed for moisture, protein, starch, fiber, total fats, thiamine, riboflavin, calcium, iron, phosphorus, potassium and zinc initially and after six months of storage.

Moisture content in different tempeh flours varied from 6.13 to 6.80 per cent initially. Ashenafi (1992) also observed a moisture content of 4.9 to 6.2 per cent in cowpea tempeh flour. Moisture content of all the tempeh flours were found to increase during storage, but significant increase in the moisture content was observed only in T_1 (100% soybean) tempeh flour. Maximum moisture content after storage was in T_2 (100% green gram) tempeh flour (7.1%) after storage. Murugkhar and Jha (2011) reported an increase in the moisture content though lesser than other packaging materials in fermented soy flour packed in laminated pouches. This result is in line with the findings of Liya (2001) in taro flour, Pillai (2001) and Sharon (2003) in breadfruit flour and Lakshmy (2003) in banana flour during storage. The moisture pick up in the flour can be expected to increase with the advancement in storage period, especially when the relative humidity is higher around the storage vicinity.

Significant difference in the fiber content was observed initially among all the tempeh flours which varied from 1.4 to 3.30 g/100g with the highest fiber content in T_2 (100 % green gram tempeh flour) and the lowest in T_9 (green gram 50% + rice 50% tempeh flour). After storage, among different tempeh flours, fiber content was significantly high in T_1 (100% soybean tempeh flour) and T_2 (100 % green gram tempeh flour). Vaidehi (1993) also reported a fiber content of 3.5 g/100g in 100 per cent soy tempeh flour and 2.0 g/100g in 100 per cent green gram tempeh flour. There was no significant change in the fiber content of each flour after storage. The results obtained in this study are in accordance with the findings of Shahzad *et al.* (2005) who reported no significant change in the fiber content of flours prepared with legumes and cereals on storage. Anjum et *al*. (2003) also reported that in commercial (unfortified) and fortified flour samples, storage had no effect on the fiber content.

The protein content in different tempeh flours varied from 12.96 in T_9 (green gram 50% + rice 50% tempeh flour) to 43.15 g/100g in T_1 (100% soybean tempeh flour). The high protein content in 100 per cent soybean tempeh flour may be due to the high protein content of soybeans. Ashenafi (1992) observed a protein content of about 28 per cent in cowpea tempeh flour. Vaidehi and Rathnamani (1990) observed a protein content of about 28.9 per cent in dried soy-sunflower tempeh. But Cuevas-Rodriguez *et al.* (2004) reported a protein content of 14.2 per cent in maize tempeh flour. The variations in the protein content observed in this study may be due to the protein content of the substrates used for tempeh preparation. There was a reduction in the protein content of the tempeh flours during storage, but significant reduction was observed only in T_8 (green gram75% + rice 25% tempeh flour). Shahzad *et al.* (2005) reported a decrease in the protein content in composite flours on storage. According to them, the decrease in protein content on storage was due to the absorption of moisture from the atmosphere that further accelerated the proteolytic activity. Goldin (1998) suggested that decrease in protein content may be due to the browning reaction which is accelerated by the increase in moisture content during storage. Sharon (2010) also reported a decrease in the protein content of unfermented and fermented flours on storage.

Initially the total fat content in the tempeh flours varied from 0.67 to 20.87 g/100g with lowest fat content in T_9 (green gram 50%+ rice 50% tempeh flour) and the highest in T_1 (100% soybean tempeh flour). The results are in accordance with Vaidehi (1993) who reported a fat content of 24.3g/100g in 100 per cent soybean tempeh flour. A low fat content of 1.8 to 2.0 g/100g was observed by Ashenafi (1992) in cowpea tempeh flour. There was no significant variation in the fat content of all the tempeh flours due to storage. Afoakwa (2004) also reported that fat content of the legume and cereal based weaning flour showed no significant variation on storage but Shahzad *et al.* (2005) reported a decrease in the fat content of the composite flour on storage, which was attributed to the development of oxidative rancidity.

The starch content of tempeh flours varied from 12.02 to 49.38 g/100g the highest in T_9 (green gram 50% + rice 50% tempeh flour) and the lowest in T_1 (100% soybean tempeh flour) before storage. This high starch content in T_9 may be due to 50 per cent rice as a substrate constituent in T_9. Cuevas-Rodriguez *et al.* (2004) reported a starch content of 56.9 g/100g in maize tempeh flour. On storage, a significant reduction in the starch content was observed in the tempeh flours except in T_8 (green gram 75% + rice 25% tempeh flour). Bejarano *et al*. (2006) observed that total starch values were slightly higher in untreated chickpea flour than in tempeh flour (49.2 *vs.* 48.4 g/100 g of dry flour). The gradual decrease in the starch content with advancement in storage period may be due to conversion of starch to sugars. The results are in accordance with the findings of Sharon (2010), who reported a gradual decrease in the starch content of fermented and unfermented food mixtures on storage. The result was also in line with the findings of Esuoso and Bamiro (1995) and Pillai (2001) who reported a decrease in the starch content in breadfruit flour on storage. Lakshmy (2003) also reported a decrease in the starch content in banana flour on storage.

A significant decrease in the riboflavin content was observed during the storage of T_8 (green gram75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh flours. There was no thiamin content in any of the tempeh flours after storage. The results are in accordance with Sharon (2010) who reported a decrease in the thiamin and riboflavin content in fermented and unfermented food mixtures on storage. According to Beizadea (2009), thiamine is one of the most unstable B vitamins and heat treatment drastically reduces the thiamine content in foods. In the present study, loss of thiamine in tempeh flours may be due to the drying process in tempeh flour preparation. Rosado *et. al*. (2005) also reported that storage time affected the stability of riboflavin and thiamin in fortified and non fortified corn masa flour, while the cooking process produced considerable losses of both vitamins.

There was no thiamine content in any of the tempeh flours and riboflavin content was found to be very low which ranged from 0.0051 to 0.021 mg/100g. But earlier studies conducted by Wang and Hesseltine (1981) had reported that tempeh is rich in thiamine, riboflavin and pyridoxine and is an especially rich vegetarian source of vitamin B_{12}. Vaidehi (1993) reported a thiamine content of 0.13 mg/100g and a riboflavin content of 0.49 mg/100g in soybean tempeh flour. In a blend of soy-sunflower tempeh flour, the thiamine and riboflavin value as reported by Vaidehi and Rathnamani (1990) were 0.15 and 3,1 mg/100g respectively. As pointed out by Nout and Rombouts (1990), Mugula (1992), Shurtleff and Aoyagi (2001) and Nout and Kiers (2005), many vitamins such as niacin (B_3), riboflavin (B_2), pyridoxine (B_6), panthothenic acid and thiamine (B_1) can be produced by the tempeh fungus *Rhizopus* *oligosporus*, but this effect is strain dependent. In the present study the *Rhizopus* strain used for tempeh fermentation (*Rhizopus oligosporus* MTCC – 556) may not have such property, which can be attributed to the low thiamine and riboflavin content in the fresh tempeh types as well as in tempeh flours.

Babu *et al.* (2009) observed tempeh as an excellent source of calcium. In the present study, the calcium content in the tempeh flours initially ranged from 68.39 to 331.25 mg/100g with the lowest calcium content in T_9 (green gram 50% + rice 50%) tempeh flour and highest in T_1 (100% soybean) tempeh flour. Reddy (2006) also reported a calcium content in the range 71.14 to 326.23 mg/100g in different types of tempeh flours prepared with millets and soybean. The results obtained are in line with the findings of Vaidehi (1993), who reported a calcium content of 358 mg/100g in 100 per cent soybean tempeh flour. A reduction in the calcium content was observed on storage, which was not significant. Similar findings were reported by Misra and Kulshrestha (2003) who reported that storage of potato flour for six months did not result in any significant changes in the calcium or any other mineral content. A gradual reduction in the calcium, content of banana flour was observed during storage by Lakshmy (2003). Sharon (2010) also reported a decrease in the calcium content on of fermented and unfermented food mixtures on storage.

The iron content was highest (8.03 mg/100g) in 100 per cent soybean tempeh flour (T_1) and the lowest iron content (2.57 mg/100g) was detected in the treatment T_9 (green gram 50% + rice 50%) tempeh flour. Vaidehi (1993) reported an iron content ranging from 4.9 to 13.5 mg/ 100g in different combinations of tempeh flour and according to her 100 per cent soybean tempeh flour contains 11.6 mg/100g of iron. In the present study, significant variation was not observed in the iron content on storage. Similar finding were reported by Rosado *et. al*. (2005) who reported that on storage, iron content showed no significant variation in fortified corn masa flour. Misra and Kulshrestha (2003) also reported that storage of potato flour for six months did not result in any significant changes in the mineral content. Lakshmy (2003) and Sharon (2010) reported a reduction in iron content in banana flour and fermented food mixtures respectively on storage.

Among different tempeh flours, the highest phosphorus content (601.36 mg/100g) was in 100 per cent soybean tempeh flour (T_1) and the lowest (268.55

mg/100g) in T_9 (green gram 50% + rice 50%) tempeh flour. Reddy (2006) reported a phosphorus content of 691.30 mg/100g in 100 per cent soybean tempeh flour which is in line with the findings of the present study. A reduction in the phosphorus content though not significant was observed on storage in all the tempeh flours. Misra and Kulshrestha (2003) also reported that storage of potato flour for six months did not result in any significant changes in the phosphorus content. However, Lakshmy (2003) reported a gradual reduction in the phosphorus content of banana flour on storage.

Significant difference in the potassium content was observed among different tempeh flours with the highest potassium content of 979.68 mg/100g in T_2 (100% green gram tempeh flour) and lowest potassium content of 559.25 mg/100g in T_9 (green gram 50% + rice 50%) tempeh flour. Shurtleff and Aoyagi (2001) had also reported tempeh, as a food having high amount of potassium. After storage, there was no significant variation in the potassium content of each tempeh flour. This finding is supported by Lakshmy (2003) who reported a decrease in the potassium content which was not significant in banana flours during storage.

The zinc content varied from 2.27 to 4.82 mg/100g with the lowest zinc content in T_9 (green gram 50% + rice 50%) tempeh flour and the highest in T_1 (100% soybean) tempeh flour and there was no significant variation in the zinc content of tempeh flour after storage. According to Shurtleff and Aoyagi (2001), tempeh is a good source of zinc as soybean contain good amount of zinc and according to Murata *et al.* (2006), fermentation of tempeh does not bring about any change on the mineral content. Misra and Kulshrestha (2003) also reported that storage of potato flour for six months did not result in any significant change in the mineral content.

5.9.2. *in vitro* protein and starch digestibility (IVPD and IVSD) of selected tempeh flours

Fermentation of food is an important method which significantly lowers the antinutrient contents and thereby improves the nutritional value of foods. Mittal and Garg (1990) reported tempeh as a highly digestible food. The IVPD of tempeh flours in the present study ranged between 75.12 to 90.86 per cent, the highest in T_8 (green gram 75% + 25%) and the lowest in T_1 (100% soybean) tempeh flour. Bejarano *et al*. (2006) reported an *in vitro* protein digestibility of 83.20 per cent in chickpea tempeh flour. Cuevas-Rodríguez *et al.* (2004) also reported a protein digestibility which varied from 80.4 to 83.6 per cent in maize tempeh flours. Bożena *et al*. (2008) reported that tempeh fermentation increased protein bioavailability by about 25 per cent. A significant increase in the IVPD was observed in each tempeh flour after storage which ranged from 72.89 to 84.78 per cent. A similar increase in IVPD was observed in fermented food mixtures on storage by Sharon (2010).

The IVSD of different tempeh flours in the present study ranged from 72.18 to 83.86 per cent, the highest in T_9 (green gram 50% + rice 50%) and lowest in T_1 (100% soybean) tempeh flour. A significant increase in the IVSD was observed in each tempeh flour on storage. This result is in line with the findings of Sharon (2010) who observed a significant increase in IVSD of fermented food mixtures on storage.

In the present study, an increase in the *in vitro* protein and starch digestibility was observed in tempeh flours when compared to the fresh tempeh. Awada *et al.* (2005) reported an increase in the protein and starch digestibilities of maize and lentil flours on application of heat and they attributed the increase in protein digestibilitiy to the reduction in antinutritional factors on heating. According to them, the increase in starch digestibility of flours could be attributed to the rupturing of starch granules on heat treatment. Shimelis and Rakshit (2007) also reported an increase in the protein and starch digestibilities in kidney bean and they attributed this increase in the protein and starch digestibilities to the heat-sensitive nature of saponins, trypsin inhibitors and phytohaemagglutinins, which diminished drastically to undetectable levels when heating processes were employed. Rehman and Shah (2005) also reported that thermal processing increased protein and starch digestibilities of food legumes. In the present study also, the higher IVPD and IVSD in tempeh flours than in fresh tempeh can be attributed to the heat processing applied in the preparation of tempeh flours.

5.9.3. Total microbial count in tempeh flours during storage

There was a decrease in the total microbial count when fresh tempeh was processed to tempeh flour. The decrease in the total microbial count might be due to the heat treatments and also due to the decrease in the moisture content on drying. Frazier and Westhoff (1995), also reported that microorganisms have an absolute demand for water, for without water no growth can occur and hence a decrease in the moisture levels will bring down the total microbial count in foods. In the different tempeh flours, the bacterial count varied from 5.0 to 6.6 x 10^5 cfu/g after storage. Yeast count was found to vary from 1.00 to 3.0 x 10^3 cfu/g and a fungal count of 0 to 0.33 x 10^3 cfu/g. A gradual increase in the total microbial count was observed in all the tempeh flours after storage. Lakshmy (2003) and Herken *et al.* (2006) also reported an increase in the microbial count in banana flour and cow pea flour respectively after storage.   According to Bera *et al.* (2001), the growth of fungi and bacteria in the food samples are influenced by moisture content, high or low relative humidity, temperature of storage and type of samples. The increase in the microbial count in tempeh flours after six months of storage can thus be correlated with the increase in moisture content observed on storage. After storage, the lowest bacterial (5.0 x 10^5 cfu/g), yeast (1.00 x 10^3 cfu/g) and fungal (0.33 x 10^3 cfu/g) count was observed in T_2 (100% green gram) tempeh flour and the highest values in T_8 (green gram 75% + 25%) tempeh flour after storage. However when converted to total plate count, the count varied from 5.01 to 6.63 x 10^5 cfu/g the lowest in T_2 (100% green gram) tempeh flour and highest in T_8 (green gram 75% + 25%) tempeh flour. Thus in all the selected tempeh flours after storage in laminated pouches for six months, the total microbial count remained within the prescribed limit of < 10 x 10^5 cfu/g for edible flours (ISI, 1988). Hence all the tempeh flours can be considered as microbiologically safe even after six months of storage.

5.9.4. Insect infestation of tempeh flours

Insect infestation was not observed in any of the tempeh flours initially or after six months of storage. Tempeh flours after storage was observed to be of smooth flowing without any lump formation or caking of the flour. Muhammad *et al*. (2003) reported moisture as an important factor which affects the insect infestation of flours during storage. According to the author, flours having moisture less than 9 per cent showed no insect infestation. In the present study also in the tempeh flours after storage the moisture content was low which varied from 6.23 to 7.1 per cent. This may be the reason for the absence of insect infestation in tempeh flours during storage.

5.10. Standardisation of tempeh based instant soup mixes

With more and more couples going out for work, rise in income levels, changing food habits and life styles, the demand for convenience foods is growing fast. The consumer is also happy about the convenience that these foods offer along with competitive pricing, improved quality, trendier and better packaging.

Convenience food is not only the food which can be consumed with convenience; it is also the food which can be prepared with ease. The most important aspect on convenience food is that it saves time and labour. The extended shelf life makes them easy to consume even after a couple of months after the products hit the market shelves. Instant foods or mixes are considered as an important part of convenience foods. The very term 'instant food' means simple, fast and convenient food, which is easy and fast to prepare besides being hygienic, free from microbial contamination and also convenient to eat.

Because of its high nutritional value and acceptability, tempeh products will have an impact on the consumers and can also solve the problem of under nutrition. Hence in the present study, an attempt has been made to develop a second generation tempeh product viz. instant soup mixes with high acceptability. Tempeh flours prepared from the selected tempeh types were used for standardising soup mixes. Corn flour was used as one of the blending materials so as to make an acceptable consistency to the soups. Vegetable mix increased the nutritive value and appearance of the soups. The spice mix helped to increase the flavour and acceptability of the instant soup mixes developed. For each tempeh flour (T_1, T_2, T_8 and T_9) four different compositions of soup mixes $S_{1(50)}$, $S_{2(55)}$, $S_{3(60)}$ and $S_{4(65)}$ were prepared and evaluated. The number in brackets in each composition indicates the percentage of tempeh flour used as a constituent in soup mix.

5.10.1. Chemical composition of soup mixes

The chemical constituents of the soup mixes before and after storage in metalised polyester laminate pouches for six months were studied under ambient conditions.

The initial moisture content of the different compositions of soup mixes with four treatments of tempeh flour varied between 6.50 to 7.53 per cent. The results are in accordance with the findings of Srivastava *et al.* (2001), who reported a moisture content of 6.66 per cent in millet based convenience mix but Semwal *et al.* (2001) reported a moisture content of 5.1 per cent in instant vegetable pulav mix. The low moisture content observed in the soup mixes will be helpful for an extended shelf life. On storage, there was an increase in the moisture content of all the soup mixes with a significant increase in the soup mixes prepared with T_9 (green gram 50% + rice 50% tempeh flour). Rao *et al.* (2011) also reported an increase in the moisture content of the instant tomato pickle mix stored in metalized polyester laminate pouches. According to them, increase in the moisture content was irrespective of the packaging material during the storage period of six months. Maximum moisture content after storage (7.53%) was observed in soup mix prepared with T_1 (100% soybean) tempeh flour with a composition of 55 per cent tempeh flour ($S_{2(55)}$).

The protein content of the different compositions of soup mixes with the four treatments of tempeh flours varied from 6.73 to 36.08 g/100g. The protein content of the different soup mixes depended mainly on the type and the proportion of tempeh flour used. The high protein content observed in all the compositions of soup mixes prepared with T_1 (100% soybean) tempeh flour can be attributed to the high protein content of the soybean tempeh flour. A protein content that ranged from 26.8 to 29.2 g/100g was reported by Ereifej (1995) in soup mixes prepared with different varieties of lentil flours. Abeysinghe and Illepurema (2006) reported a protein content of 16.10 per cent in instant soup mixes developed by them. After storage, there was a reduction in the protein content in all the compositions of the soup mixes prepared with the four types of tempeh flour. According to Shahzad *et al.* (2005), the decrease in protein content on storage was due to the absorption of moisture from the atmosphere that further accelerates the proteolytic activity. After storage, maximum protein content was observed in composition $S_{4(65)}$ with 65 per cent tempeh flour in all the soup mixes prepared with T_1, T_2, T_8 and T_9 tempeh flours.

All the compositions of soup mixes had higher starch content when compared to the starch content of the tempeh flours. The starch content of different compositions of soup mixes ranged from 16.96 to 53.33 g/100g. The increased starch content observed in soup mixes is due to the addition of corn flour as an ingredient in all the soup mixes. On storage, a reduction in the starch content was observed in all the soup mixes. The gradual decrease in the starch content with advancement in storage period may be due to the hydrolysis of starch to sugars. The results are in accordance with the findings of Sharon (2010), who reported a gradual decrease in the starch content of fermented and unfermented food mixtures on storage. In the present study, after storage , maximum starch content was observed in composition $S_{1(50)}$ with the maximum amount of corn flour (50%) in all the soup mixes prepared with T_1, T_2, T_8 and T_9 tempeh flours.

The fiber content in different compositions of soup mixes ranged from 0.45 to 2.97 g/100g. The highest fiber content was observed in the soup mixes prepared with T_2 (100% green gram tempeh flour) and the lowest fiber content was observed in the soup mixes prepared with T_9 (green gram 50% + rice 50%) tempeh flour. This can be explained by the comparatively high fiber content in T_2 tempeh flour and lower fiber content in T_9 tempeh flour. Ereifej (1995) reported a fiber content that ranged from 1.5 to 2.7 g/100g in the soup mixes prepared with different varieties of lentil flours. A significant reduction in the fiber content on storage was observed mainly in soup mixes with T_2 (100% green gram) tempeh flour. Anjum *et al*. (2003) reported that for commercial (unfortified) and fortified flour samples, storage had no effect on fiber content. According to Ahmad (1996), a decreasing trend in fiber content on storage may be due to moisture absorption. After storage, maximum fiber content was observed in compositions $S_{4(65)}$ with 65 per cent tempeh flour in all the soup mixes prepared with T_1, T_2, T_8 and T_9 tempeh flours.

There was a variation in the fat content of the soup mixes prepared with different types of tempeh flours. The fat content in different combinations of soup mixes varied from 0.67 to 17.93 g/100g before storage. The highest fat content was observed in the soup mixes prepared with T_1 (100% soybean) tempeh flour and the lowest fat content was in the soup mixes prepared with T_9 (green gram 50% + rice 50%) tempeh flour. In the case of tempeh flour also, the fat content was highest in T_1 (100% soybean) tempeh flour and lowest in T_9 (green gram 50% + rice 50%) tempeh flour. Ereifej (1995) reported a fat content that ranged from 9.3 to 11.5 g/100g in the soup mixes prepared with different varieties of lentil flour. Abeysinghe and Illepurema (2006) reported a fat content of 4.10 g/100g in the instant soup mixes prepared by them. The comparatively high fat content in their soup mixes was due to the addition of vegetable oil as a constituent. In this study, the low fat content observed in the soup mixes prepared with tempeh flours other than T_1 (100% soybean tempeh flour) can be attributed to the non inclusion of any direct sources of oils as a constituent ingredient. There was no significant variation in the fat content of different combinations of soup mixes after storage. Afoakwa (2004) also reported that fat content of the legume and cereal based weaning flour showed no significant variation on storage. The fat content of the soup mixes was found to be maximum in composition $S_{4(65)}$ with 65 per cent tempeh flour in T_1, T_2, T_8 and T_9 tempeh flours after storage.

Thiamine content was not detected in any of the compositions of soup mixes. None of the tempeh flours had detectable thiamine content which may be the reason for this. According to Beizadea (2009), thiamine is one of the most unstable B vitamins and heat treatment drastically reduces the thiamine content in foods. Hence in the present study, lack of thiamine in the soup mixes can be attributed to the drying processes in the preparation of tempeh flour and also of the other ingredients in the soup mixes with no thiamine.

The riboflavin content in the different compositions of soup mixes was low which ranged from 0.0002 to 0.054 mg/100g. The selected tempeh flours also had very low riboflavin content. After storage, a further reduction in the riboflavin content was observed and with T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh flour, there was no riboflavin content in any of the soup mixes. According to Rosado *et.al*. (2005), storage period negatively affected the stability of riboflavin content in foods.

Calcium content in different compositions of soup mixes varied from 39.96 to 314.00 mg/100g. Calcium content was highest in the soup mixes prepared T_1 (100% soybean) tempeh flour. Ereifej (1995) reported a calcium content that ranged from 71.2 to 125.0 mg/100g in the soup mixes prepared with different varieties of lentil flours. This is in line with the calcium content of soup mixes prepared with T_2 (100% green gram tempeh flour) which varied from 72.77 to 100.20 mg/100g. Calcium content was found to be comparatively low in soup mixes prepared with T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh flour where rice is also an ingredient. A reduction in the calcium content of the soup mixes was observed on storage which was not significant except in the soup mixes with T_1 (100% soybean) tempeh flour. Sharon (2010) also reported a reduction in the calcium content on storage of fermented and unfermented food mixtures.

The iron content in different compositions of soup mixes ranged from 1.10 to 5.17 mg/100g. Ereifej (1995) reported an iron content that ranged from 4.9 to 6.0 mg/100g in the soup mixes prepared with different varieties of lentils. Abeysinghe and Illepurema (2006) reported an iron content of 4.61 mg/100g in instant vegetable soup mix. Srivastava *et al.* (2001) reported an iron content of 4.2 mg/100g in proso millet based convenience mix. In the present study, there was no significant variation in the iron content of the soup mixes after storage. Iron content was found to be maximum in composition $S_{4(65)}$ with 65 per cent tempeh flour in all the soup mixes prepared with T_1, T_2, T_8 and T_9 tempeh flours.

The phosphorus content ranged from 226.27 to 557.76 mg/100g in different compositions of soup mixes before storage. The soup mixes prepared with T_1 (100% soybean) tempeh flour had the highest phosphorus content as in the case observed with tempeh flour also. Ereifej (1995) reported a phosphorus content which varied from 432.9 to 470.3 mg/100g in the soup mixes prepared with different lentil flours. After storage, there was not much variation in the phosphorus content of most of the soup mixes. Aba (2003) also reported that no significant change was observed in the phosphorus content of the dried tomato powder mix on storage.

Potassium content of the soup mixes varied from 432.93 to 965.57 mg/100g. The potassium content in T_2 (100% green gram tempeh flour) soup mixes were found to be high (914.51 to 965.57 mg/100g), since T_2 tempeh flour had comparatively high potassium content. However, Ereifej (1995) had reported a higher potassium content that varied from 1813.0 to 2496.0 mg/100g in the soup mixes prepared with different lentil flours. There was no significant variation in the potassium content of different compositions of soup mixes after storage.

All the different compositions of soup mixes had a good amount of zinc which ranged from 2.15 to 4.41 mg/100g. A zinc content that varied from 3.5 to 3.9 mg/100g was reported by Ereifej (1995) in soup mixes prepared with different lentil flours. According to Shurtleff and Aoyagi (2001), tempeh is a good source of zinc as soybean contain good amount of zinc. There was no significant variation in the zinc content of different compositions of soup mixes after six months of storage.

5.10.2. Water and time required for preparing soups

In instant soup mixes, reconstitution of the soup powders in water to make soup of acceptable consistency and also the time taken to prepare the soups are very important. According to Abeysinghe and Illepurema (2006), any dehydrated soup mix should be rehydratable and cookable within minimum time period and should be nutritious and palatable.

The cooking time of the instant soup mixes varied from 3-6 minutes. Soup mixes with T_1 (100% soybean) tempeh flour except the composition $S_{4(65)}$ took 5 minutes for cooking whereas, the soup mix with $S_{4(65)}$ with 65 per cent tempeh flour (T_1) took the maximum time of 6 minutes. All the compositions of soup mixes with T_2 (100% green gram) and T_8 (green gram 75% + rice 25%) tempeh flour took 4 minutes and the soup mixes with T_9 (green gram 50% + rice 50%) tempeh flour took the least time of 3 minutes to cook.

The amount of water required to cook the instant soup mixes was found to be maximum in soup mixes with T_9 (green gram 50% + rice 50%) tempeh flour which varied from 1280 to 1530 ml/100g of soup mix. T_9 tempeh flour contained 50 per cent rice and hence the starch content in T_9 tempeh flour was also comparatively high. Amount of water required to cook soup mixes with T_8 (green gram 75% + rice 25%)

tempeh flour was also comparatively high which contained 25 per cent rice. So also in the soup mixes with T_1 (100% soybean) and T_2 (100% green gram) tempeh flour, the composition $S_{1(50)}$ which contained 20 per cent corn flour, took more quantity of water to cook the soup. Starch granules absorb more water to swell and gelatinize while cooking. This may be the reason for the higher amount of water required to cook these soup mixes with more starch content.

5.10.3. Organoleptic qualities of the soups

In comparison to freshly prepared soup, instant dry soup should possess desired quality representing the dominant flavour and aroma of the ingredients used. It is desirable that the product be free from off flavours, off taste, unacceptable aroma and texture even after storage (Abeysinghe and Illepurema, 2006). Hence in the present study organoleptic evaluation of the soups were conducted before storage and after six months of storage of soup mixes in metalized polyester laminate pouches. Appearance, colour, flavour, consistency and taste were assessed by the selected panel of ten judges and overall acceptability which is a reflection of the above said quality attributes was also assessed.

The overall acceptability score of different compositions of soups with T_1 (100% soybean), T_2 (100% green gram), T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh flour varied from 7.7 to 8.4 before storage and varied from 7.5 to 8.2 after storage indicating an acceptability of 'like moderately' to 'like very much' in a nine point hedonic scale. In general, the overall acceptability score of the soups were the lowest in composition $S_{4(65)}$ with all the tempeh flours. This may be due to the low mean score for flavour and taste of the soups with a higher percentage (65%) of each tempeh flour. The reduction in the overall acceptability score of soups with a higher proportion of tempeh flour may be due to the unfamiliar strong fermented flavour of tempeh flours which might have masked the flavour of spices in these soups. There was a slight reduction in the flavour and taste score of soups prepared with the stored soup mixes which might have contributed to a reduction in the overall acceptability score of soups after storage. However, the soup mixes with $S_{1(50)}$ and $S_{2(55)}$ composition with all the tempeh flours were found to be highly acceptable with an overall acceptability score of ≥ 8.0 (like very much) even after storage. Ereifej (1995) and Abeysinghe and Illepurema (2006) also reported that the dehydrated soup mixes prepared by them were highly acceptable and comparable to the commercially available soup mixes.

5.10.4. Total micro flora in the soup mixes during storage

The microbiological safety is one of the main criteria for the acceptability of any processed food. The convenience foods should have a good shelf life and on storage the microbiological safety of these foods should be ensured. Microbiological criteria provide guidance on the acceptability of foodstuffs and their manufacturing processes.

According to Bryan (1974), several factors such as quality of raw materials, storage temperature, processing temperature, storage containers, processing technique, the environment in which it is processed, etc. will have an effect on microbial quality of processed foods. In the present study, before storage, the total bacterial count in different compositions of the soup mixes with the four tempeh flours ranged from 3.3 to 5.3 x 10^5 cfu/g. After storage, an increase in the total bacterial count was observed in all the compositions of soup mixes which varied from 4.6 to 8.6 x 10^5 cfu/g. Total yeast count in the soup mixes before storage ranged from 1.3 to 4.6 x 10^3 cfu/g and a slight increase in the yeast count was observed in the compositions of soup mixes on storage which varied from 3.0 to 6.6 x 10^3 cfu/g. The fungal count in the soup mixes was low and an increase in the fungal count was observed on storage which varied from 1.0 to 3.3 x10^3 cfu/g. The increase in the microbial load can be attributed to the increase in the moisture content of the soup mixes on storage. Frazier and Westhoff (1995) also reported that microorganisms have an absolute demand for water, for without water no growth can occur and hence a decrease in the moisture levels will bring down the total microbial count in foods. When the total count of bacteria (x 10^5), yeast (x 10^3) and fungi (x 10^3) in the soup mixes after storage were converted to total plate count (TPC), the count varied from 4.64 to 9.37 x 10^5 cfu/g. Thus in all the composition of soup mixes with the 4 tempeh flours after storage for six months in metalized polyester laminate pouches, the microbial load remained within the prescribed limit of < 10 x 10^5 cfu/g (ISI, 1988).

Hence all the soup mixes can be considered as microbiologically safe even after six months of storage.

5.9.5. Insect infestation in soup mixes during storage

Insect infestation was not observed in any of the compositions of soup mixes after the storage period. All the soup mixes were smooth flowing without any lumps after the storage period. Nasir *et al*. (2003) reported moisture as an important factor which affects the insect infestation of wheat flour. According to him, wheat flour having moisture less than 9 per cent after storage showed no insect infestation. Hence the absence of insect infestation in the soup mixes after storage can be attributed to low moisture content (< 9%) in the soup mixes even after storage.

5.9.6. Selection of soup mixes

From each tempeh flour (T_1, T_2, T_8 and T_9), one soup mix was selected with good overall acceptability and storage stability. Thus the soup mix composition $S_{2(55)}$ was selected from T_1 tempeh flour (100% soybean), $S_{1(50)}$ from T_2 tempeh flour (100% green gram), $S_{3(60)}$ from S_8 tempeh flour (green gram 75% + rice 25%) and $S_{2(55)}$ from T_9 tempeh flour (green gram 50% + rice 50%) was selected as the most acceptable soup mixes with good storage stability.

5.10.7. Cost of production of fresh tempeh, tempeh flours and the most acceptable soup mixes

The cost of 1 kg of fresh tempeh ranged from Rs. 57 to Rs. 68, with the highest cost for T_2 tempeh (100% green gram) and the lowest for T_9 tempeh (green gram 50% + rice 50%). All the selected fresh tempeh types were found to have a high BC ratio varying from 2.94 to 3.50.

The cost of production of 1 kg of tempeh flour from the selected tempeh types varied from Rs. 111.00 to Rs. 142.00, with the highest cost for T_2 tempeh flour (100% green gram) and the lowest for T_9 tempeh flour (green gram 50% + rice 50%).

The cost of production of 1 kg soup mix with the selected compositions each tempeh flour (T_1, T_2, T_8 and T_9) varied from Rs. 242.00 to Rs. 257.00. All the selected soup mixes were found to have a high BC ratio varying from 2.46 to 2.64.

Thus it is clear from the results that an alternative to soybean in tempeh fermentation (green gram and rice) at a replacement level that did not affect product quality provides a very attractive value proposition for the manufactures.

It is rarely this economical to produce any other plant food products with such nutritive value and good protein and mineral availability which can be used as delicious replacements for meat and dairy products as part of meatless or vegetarian diets. With animal foods becoming expensive, fermented foods like tempeh and tempeh products hold great promise to meet the demand for high quality proteins. Considering the nutritional potentials and acceptability of fresh tempeh and its second generation products, and being a low cost source of quality proteins and with high mineral availability, the necessity to commence its production on commercial basis and popularisation is imperative. Furthermore, this would create employment and improve the nutritional status of the people consuming these products.

Significant changes are evident in the supply of food to humans and in the technologies to prepare different food products in various ways, due to changes in food habits and hence the fast food markets have created a demand for processed foods in the form of convenience foods. Use of tempeh flours in making instant soup mixes not only improve its nutritive value and acceptability but also stretches its availability to average income consumers. Tempeh flours can also be considered for the fortification of widely consumed legume/cereal based food products.

Future line of work

In the present study, tempeh fermentation was carried out with pure culture of *Rhizopus oligosporus* - MTCC 556. Inspite of a fermented food, all the tempeh types were found to be very low in B vitamins. There are reports that certain strains of *Rhizopus* can produce thiamine, riboflavin, niacin, pyridoxine and pantothenic acid. *Rhizopus oryzae* another important fungus frequently found in tempeh products has also been reported to produce niacin, vitamin K, tochopherol, pyridoxine, riboflavin and biotin. Hence, investigations are to be conducted to increase the vitamin content of tempeh types by using such strains of *Rhizopu*s for tempeh fermentation and also co-inoculating *Rhizopus oryzae* along with *Rhizopus oligosporus*. It is also possible to use specific strains such as high folate producers to enhance the concentration of certain nutrient.

Vitamin B_{12} is one of the most frequently studied vitamin produced by bacteria. Bacteria belonging to *Klebsiella* spp. can produce tempeh rich in vitamin B_{12}. Such studies can be taken to improve the vitamin B_{12} content of different tempeh types.

Kiers *et al.* (2002) has reported that *Rhizopus oligosporus* can produce an antibacterial protein that interfere with the adhesion of *E.coli* in the intestine. In the present study no antibacterial effect was exhibited by the tempeh types fermented with *Rhizopus oligosporus* – MTCC 556. Such strains with specific antibacterial activity can be identified for tempeh fermentation.

The abilities of probiotic LAB to grow together with *Rhizopus oligosporus* during tempeh fermentation and their possible effects on tempeh quality can also be studied by co-inoculating LAB during tempeh fermentation. Probiotic bacteria can inhibit the growth of pathogenic organisms and this is particularly important in the manufacture of tempeh products with functional properties designated for special target groups.

Summary

6. SUMMARY

The study entitled "Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes" was undertaken with the objective to standardise the fermented food tempeh with green gram, cowpea, soybean, rice and wheat, and to evaluate their quality attributes. The study also aims to evaluate the nutritional and shelf life qualities of tempeh flour, to develop instant soup mixes with tempeh flour and quality evaluation of these products.

Tempeh fermentations were carried out with pure cultures of *Rhizopus oligosporus* – MTCC 556 and twenty different combinations of selected legumes and cereals such as soybean, green gram, cowpea, rice and wheat were used as substrates. Among the different fresh tempeh types prepared, tempeh prepared with 100 per cent soybeans (T_1) was found to be a thick firm cake with the white cottony mycelium distributed uniformly. All the other treatments had a comparatively less dense mycelium than the control. However, the combinations having soybeans as a constituent had more firm texture and more mycelial coverage. Binding of cotyledons with the fungal hyphae was found to be less in treatments having cowpea. The treatments with green gram had a comparable firmness with the control (T_1). Among the cereals, the tempeh types with rice had a more firm texture than that of wheat.

Fresh tempeh was used to prepare chips and roast and these products were evaluated for their organoleptic qualities by a selected panel of 10 judges using a nine point hedonic scale. Tempeh chips and roasts prepared with different fresh tempeh types were acceptable and the mean score for overall acceptability of chips ranged from 6.9 to 8.9 and the overall acceptability score of the roast ranged from 7.5 to 8.9. The IVSD and IVPD of the fresh tempeh types were found to be high in all the treatments. The IVSD of the fresh tempeh types ranged from 69.18 to 82.83 per cent with the highest in T_9 and lowest in T_1. The IVPD of different treatments were ranging from 71.43 to 88.98 per cent and the highest IVPD was for the treatment T_8. From the twenty different fresh tempeh types, three tempeh types which were most acceptable with maximum protein and starch digestibility was selected for further nutritional and shelf life studies with fresh tempeh and also for developing tempeh flour and instant soup mixes. The selected three treatments were T_2 (green gram

100%), T_8 (green gram 75% + rice 25%), and T_9 (green gram 50% + rice 50%). T_1 (100% soybean), was also included as the control.

Among the selected fresh tempeh types, the treatment T_1 (control) had the maximum moisture (55.85 g/100g), protein (21.09%), total fats (10.12 g/100g), β carotene (331.85 µg/100g), thiamine (0.31 mg/100g), calcium (149.10 mg/100g), iron (3.6 mg/100g), phosphorus (270.61 mg/100g) and zinc (2.17 mg/100g) content, whereas the treatment T_2 had the highest fibre (2.52 g/100g), reducing sugar (2.01 g/100g), total sugar (4.21 g/100g) riboflavin (0.30 mg/100g) and potassium (525.11 mg/100g) content. Starch content was highest (29.13 g/100g) in $T_{9.}$ None of the selected fresh tempeh types contained vitamin C. IVSD of the selected three fresh tempeh types varied between 80.00 to 82.83 per cent whereas it was only 69.18 in T_1 (control). IVPD of the three tempeh types were in the range between 86.72 to 88.98 per cent against 72.03 per cent in control (T_1). Mineral availability was found to be very high in the three tempeh types compared to the control. The availability of calcium (61.77%), iron (66.82%), phosphorus (65.72%), potassium (63.60%) and zinc (88.99%) was highest in $T_{9.}$

Probiotic characteristics such as *in vitro* acid and bile acid tolerance of the microorganisms in fresh tempeh and antimicrobial activities of tempeh against enteropathogenic bacteria were studied. Bacterial viability was not observed between pH 1.5 to 3.5 in all the treatments and maximum bacterial viability was observed between pH 5.5 to 6.0. Viability of yeast was not observed in tempeh at low pH levels of 1.5 to 2.5 and the maximum yeast growth was observed in pH 4.5. The biomass of the fungus *Rhizopus oligosporus* was found to be highest in pH 4.5 and there was no growth below pH 3.0. Bile acid tolerance (1 – 4 % level) was not exhibited by any of the microorganisms present in the fresh tempeh. Fresh tempeh types as such did not show antagonistic activity towards enteropathogens viz. *Escherichia coli, Salmonella enteritidis, Bacillus cereus* and *Staphylococcus aureus*. Hence no probiotic activity can be attributed to any of the selected fresh tempeh types.

Selected three fresh tempeh types along with the control (T_1) were packed in polythene bags of 250 gauge thickness and were kept under refrigerated condition (3 to 7^0C) for 12 days and in a deep freezer (-15 to -18^0C) for 1 month. Quality evaluation was done initially and during 3rd, 6th, 9th and 12th day in refrigerated samples and in frozen samples, evaluation was done initially, during the 15th and 30th day of storage.

The appearance, colour, flavour and texture of the fresh tempeh types stored under refrigerated condition were satisfactory up to 12 days in the case of T_1 (100% soybean tempeh) and T_2 (100% green gram). In T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%), the shelf life was only up to 6 days of refrigerated storage. Frozen tempeh types were comparable to the fresh tempeh even after the 30th day of storage.

A gradual reduction in the overall acceptability score of the chips and roasts prepared with the stored tempeh was observed when compared with the same products prepared with fresh tempeh types. The overall acceptability score for chips was highest for T_2 during all the storage period under refrigerated condition. With regard to tempeh roast, the overall acceptability score was comparatively higher in T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) tempeh types up to the 6th day of refrigerated storage. Only a slight decrease in the overall acceptability score was observed in the tempeh chips and roasts prepared from the frozen tempeh types. Chips prepared with all the frozen tempeh types after 15 days had an overall acceptability score of ≥ 8.0, and also after the 30th day except in T_9 (green gram 50% + rice 50%). Similarly roast prepared with all the frozen tempeh types after 15 and 30 days had an overall acceptability score of ≥ 8.0 except in the control T_1.

The bacterial load in the fresh tempeh was found to be high and was in the range of 49.3 to 69.3 x 10^8 cfu/g. The high bacterial count in fresh tempeh types were mainly due to non pathogenic bacterias such as *Lactococci* and *Klebsiella*, the normal identified microflora found in tempeh and in other naturally fermented foods. A gradual decrease in the bacterial count up to the 6th day of refrigerated storage was observed in all the treatments and thereafter there was a slight increase in the bacterial count. Maximum bacterial count at the 12th day of refrigerated storage was observed in T_9 (70.6 x 10^8 cfu/g). The total yeast count in the fresh tempeh types was found in the range of 42.6 to 66.3 x10^5 cfu/g the maximum being T_9. A decrease in the yeast count was observed on storage in T_1 and T_2, but in T_8 and T_9, there was an increase in the total yeast count on the 9^{th} and 12^{th} day of refrigerated storage which resulted in the spoilage of these tempeh types. The total fungal count was in the range of 2.6 to 5.3 x $10^{6\ cfu}$/g in fresh tempeh types and a gradual reduction in the total fungal count was observed on refrigerated storage which varied from 1.0 to 2.3 x 10^6 cfu/g on the 12^{th} day.

There was a sharp decline in the microbial count of fresh tempeh types stored in deep freezer in all the treatments. In frozen tempeh types, the bacterial count was reduced to 0.17 to 0.49 x 10^8 cfu/g, yeast count to 0.46 to 0.86 x 10^5 cfu/g and fungal count to 0.06 to 0.1 x 10^6 cfu/g after 30 days of storage.

Thus, under refrigerated condition, fresh tempeh types T_1 (100% soybean) and T_2 (100% green gram) can be stored up to 12 days and T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%) can be stored up to 6 days. Frozen tempeh can be stored up to one month without any changes.

The flours prepared from the tempeh types were packed in metalised polyester laminate pouches and stored for a period of six months. The stored tempeh flours were evaluated for their chemical constituents, *in vitro* digestibility of proteins and starch and the shelf life attributes like total micro flora and insect infestation.

Moisture content of all the tempeh flours were found to be increasing during storage, but significant increase in the moisture content was observed only in T_1. Maximum moisture content was in T_2 (7.1%) after storage. The tempeh flour prepared with T_1 had the highest protein (43.15 g/100g), total fats (20.87 g/100g), calcium (331.25 mg/100g), iron (8.03 mg/100g), phosphorus (601.36 mg/100g) and zinc (4.82 mg/100g). Maximum fiber (3.30g/100g), riboflavin (0.03 mg/100g) and potassium (979.68 mg/100g) was observed in tempeh flour prepared with $T_{2.}$ A reduction in all the mineral content during storage was observed which was not significant. Among the treatments, starch content was found to highest in treatment T_9 (49.38 g/100g) and a significant reduction of starch content in tempeh flours were observed during storage. Thiamine was not detected in any of the tempeh flours. A decrease in the protein, total fats and riboflavin, was observed on storage. But in many of the cases, the decrease observed was not significant.

The IVPD of tempeh flours in the present study ranged between 75.12 to 90.86 per cent with the highest IVPD in T_8 (green gram 75% + rice 25%). A significant increase in IVPD was observed in tempeh flours after storage which ranged from 75.31 to 91.16 highest in T_8 followed by T_2 (91.02%). IVSD ranged from 72.18 to 83.86 per cent with the highest IVSD in T_9 (green gram 50% + rice 50%). A significant increase in the *in vitro* starch digestibility was observed in tempeh flours during storage which ranged from 72.89 to 84.78 per cent, the maximum IVSD being observed in T_9 (green gram 50% + rice 50%).

There was a drastic reduction in the total microbial count when fresh tempeh types were converted to tempeh flours. The total bacterial count of different tempeh flours ranged from 5.0 to 6.6 x 10^5 cfu/g with the highest bacterial count in treatment T_8 and the lowest in T_2 after storage. Total yeast count in the tempeh flours were found to be maximum in the treatment T_8 (3.3 x 10^3 cfu/g) and the least count in T_2 (1.00 x 10^3 cfu/g) after storage. The fungal count was also found to be very low which ranged from 0 to 0.66 x 10^3 cfu/g after storage of tempeh flours. Converting the microbial load to total plate count (TPC), the count varied from 5.01 to 6.63 x 10^5 cfu/g; the lowest in T_2 and highest in T_8 tempeh flour after storage. TPC of all the tempeh flours remained within the prescribed limit making them microbiologically safe even after 6 month of storage. Insect infestation was not observed in any of the treatments initially or during the 6th month of storage.

Tempeh flours prepared from the selected tempeh types were used for standardising instant soup mixes with suitable blending materials. For each of the selected tempeh flour (T_1, T_2, T_8 and T_9), four different compositions of soup mixes $S_{1(50)}$, $S_{2(55)}$, $S_{3(60)}$, $S_{4(65)}$, the number in brackets indicating the percentage of tempeh flour used. The soup mixes after blending with the prepared vegetable mix, spice mix and other ingredients were packed in metalised polyester laminate pouches and were stored for six months under ambient conditions. The quality evaluation of the soup mixes were studied initially, and during the 6th month of storage.

On storage, there was an increase in the moisture content of all the soup mixes which varied from 6.53 to 7.53 per cent, the maximum moisture content in $S_{2(55)}$ with

T_1 tempeh flour (100% soybean). A decrease in the protein content of soup mixes were observed on storage with a maximum protein content in composition $S_{4(65)}$ with 65 per cent tempeh flour in all the soup mixes prepared with T_1, T_2, T_8 and T_9 tempeh flours. The starch content of different compositions of soup mixes ranged from 16.96 to 53.33 g/100g and a significant decrease in the starch content was observed on storage which varied from 16.67 to 51.45 g/100g. The fiber content in different compositions of soup mixes ranged from 0.45 to 2.97 g/100g. A significant decrease in the fiber content was observed on storage, in some of the soup mixes. The fat content in different combinations of soup mixes varied from 0.67 to 17.93 g/100g without significant change in storage. The riboflavin content in the different compositions of soup mixes was very low which ranged from 0.0002 to 0.054 mg/100g. After storage a further reduction in the riboflavin content was observed and with T_8 and T_9 tempeh flours, there was no riboflavin content in any of the compositions of soup mixes after storage. Thiamine was not detected in any of the soup mixes. Calcium content in different compositions of soup mixes varied from 39.96 to 314.00 mg/100g; the highest calcium content in soup mixes prepared with T_1 tempeh flour. The iron content in different compositions of soup mixes ranged from 1.10 to 5.17 mg/100g. The phosphorus content ranged from 226.27 to 557.76 mg/100g in different compositions of soup mixes. Potassium content of the soup mixes varied from 432.93 to 965.57 mg/100g. All the different compositions of soup mixes had a good amount of zinc which ranged from 2.15 to 4.41 mg/100g. After storage, there was no significant variation in the mineral content of different combinations of soup mixes.

Water required to cook the instant soup mixes to a soup of acceptable consistency was found to be maximum in soup mixes with T_9 (green gram 50% + rice 50%) tempeh flour which varied from 1280 to 1530 ml/100g. The cooking time of the instant soup mixes varied from 3-6 minutes. The soup mixes prepared with tempeh flour T_9 (green gram 50% + rice 50%) took the least time to cook (3 minutes). The maximum time to cook was taken by the compositions of soup mixes prepared with tempeh flour T_1 (100% soybean).

The mean score for overall acceptability of the soups prepared with soup mixes ranged from 7.7 to 8.4 before storage. The overall acceptability score of the soups was lowest in compositions $S_{4(65)}$ with all the tempeh flours. There was a reduction in overall acceptability score of soups prepared with soup mixes stored for six months. However, the soup mixes with $S_{1(50)}$ and $S_{2(55)}$ compositions with all the tempeh flours were found to be highly acceptable with an overall acceptability score of > 8.0 even after storage.

The total bacterial count in different compositions of soup mixes ranged from 3.3 to 5.3 x 10^5 cfu/g. After storage, an increase in the total bacterial count was observed in all the compositions of soup mixes which varied from 4.6 to 8.6 x 10^5 cfu/g. Total yeast count in the soup mixes ranged from 1.3 to 4.6 x 10^3 cfu/g and a slight increase in the yeast count was observed in the compositions of soup mixes on storage which varied from 3.0 to 6.6 x 10^3 cfu/g. The fungal count in the soup mixes was low and an increase in the fungal count was observed on storage which varied from 1.0 to 3.3 x 10^3 cfu/g. Total plate count in the soup mixes after storage varied from 4.64 to 9.37 x 10^5 cfu/g. Since the microbial load in all the soup mixes after storage were within the prescribed limit, all the soup mixes can be considered as microbiologically safe even after storage for six months. Insect infestation was not observed in any of the different compositions of soup mixes after the storage period.

From each tempeh flour (T_1, T_2, T_8 and T_9), one soup mix was selected with good overall acceptability and storage stability. Thus the soup mix composition $S_{2(55)}$ was selected from T_1 tempeh flour (tempeh flour with 100% soybean; 55% tempeh flour in soup mix), $S_{1(50)}$ from T_2 tempeh flour (tempeh flour with 100% green gram; 50% tempeh flour in soup mix), $S_{3(60)}$ from S_8 tempeh flour (tempeh flour with 75% green gram + 25% rice; 60% tempeh flour in soup mix) and $S_{2(55)}$ from T_9 tempeh flour (tempeh flour with 50% green gram + 50% rice; 55% tempeh flour in soup mix) as the most acceptable soup mixes with good storage stability.

Cost of production of selected fresh tempeh (T_1, T_2, T_8 and T_9), tempeh flours and the selected soup mixes prepared with each of the selected tempeh flours were computed. The cost of production of 1 kg of fresh tempeh types varied from Rs. 57.00 to Rs. 68.00 with the highest cost for T_2 tempeh (100% green gram) and the lowest for T_9 tempeh (green gram 50% + rice 50%). All the selected fresh tempeh types were found to have a BC ratio varying from 2.94 to 3.5, the maximum for T_9 tempeh. The cost of production of 1 kg tempeh flour with the selected tempeh types varied from Rs. 111.00 to 142.00 with the lowest production cost for the tempeh flour prepared with T_9 (green gram 50% + rice 50%) and highest in T_2 (100% green gram). The cost of production of 1 kg soup mix with the selected compositions in each tempeh flour (T_1, T_2, T_8 and T_9) varied from Rs. 242.00 to Rs. 257.00. Cost of unit pack of soup mixes (50g) varied from Rs. 12.00 to 13.00 as against Rs. 32.00 for the commercially available vegetable soup mix in the market. All the selected soup mixes were found to have a high BC ratio varying from 2.46 to 2.64.

Thus the results of the present investigation clearly brought out that, besides T_1 tempeh (100% soybean), T_2 (100% green gram), T_8 (green gram 75% + rice 25%), and T_9 (green gram 50% + rice 50%) tempeh types had proved to be efficient for the preparation of tempeh and tempeh products with respect to nutritional qualities, digestibility of starch and proteins, availability of minerals, overall acceptability and with good storage stability.

Tempeh is a traditional fermented food that has received attention from all over the world, and different countries make tempeh products from locally available substrates. Tempeh fermentation represents a technological alternative for a great variety of legumes and cereals, or a combination of them to improve their nutritional quality and to obtain edible products with palatable sensorial characteristics.

The process to prepare tempeh requires a relatively simple infrastructure and can produce profound chemical changes that improve the nutritional quality. The tempeh manufacture could be an appropriate method for small and medium scale processing of locally available legumes and / or cereals into wholesome products of high nutritional value. With modern technology and hygienic conditions industrial production processes can be developed which make tempeh fermentation more popular and enhance their spread to new consumer groups, especially in developing countries where meat products are in poor supply due to its high cost.

References

REFERENCES

[Anonymous]. 2003. 8,000-year-old wine unearthed in Georgia. [Online]. Available: http://www.stonepages.com/news/archives/000498.html. [28 Dec. 2003].

[Anonymous]. 2009. *Benefits of Tempeh*. [Online] Available: http://www.lifestyle india.com/lounge/benefits-of-tempeh-6006.html. [25th Nov.2009].

A. O. A. C. 1955. *Official and Tentative Methods of Analysis.* Eighth edition. Association of Official Analytical Chemists, Washington, D.C, 1015p.

A. O. A. C. 1970. *Official and Tentative Methods of Analysis.* Eleventh edition. Association of Official Analytical Chemists, Washington, D.C, 1128 p.

A. O. A. C. 1980. *Official and Tentative Methods of Analysis.* Thirteenth edition. Association of Official Analytical Chemists, Washington, D.C, 1018 p.

Aba, M. H. 2003. Shelf life of dehydrated tomato powder. *Trends Food Sci. Technol.* 12: 23-31.

Abdel-Gawad, A. S. 1993. Effect of domestic processing on the oligosaccharide content of some dry legumes seeds. *Food Chem.* 46: 25–31.

Abeysinghe, C. P. and Illepurema, C. K. 2006. Formulation of an MSG (Monosodium Glutamate) free Instant vegetable soup mix *J. Nutr. Sci. Found. Srilanka.* 34(2): 91-95.

Abiose S. and Adedeji, A. A. 1992. The microflora associated with fermentation of plantain. *Niger. Food J.* 10: 136-143.

Achi, O. K. 2005. The potential for upgrading traditional fermented foods through biotechnology. *Afr. J. Biotech.* 4(5): 375-380.

Adams, M. R. 1990. Tropical aspects of fermented foods. *Trends Food Sci. Technol.* 1: 141-144.

Aderibigb, E. Y. and Osegboun, A. O. 2006. Acceptability of tempeh among health workers in Ado-Ekiti, Nigeria. *Pakist. J. Nutr.* 5(2): 122-124.

Afoakwa, E. 2004. Effects of cowpea fortification, dehydration method and storage time on some quality characteristics of maize-based traditional weaning foods. *Afr. J. Food Agric. Nutr. Dev.* 4 (1): 58-64.

Agarwal, G. P. and Hasija, S. K. 1986. *Microorganisms in the Laboratory*. Print House India Ltd., Lucknow, 155p.

Agerholm-Larsen, L., Raben, A., Haulrik, N., Hansen, A. S., Manders, M., and Astrup, A. 2000. Effect of 8 week intake of probiotic milk products on risk factors for cardiovascular diseases. *Eur. J. Clin. Nutr.* 54: 288–289.

Aguirre, M. and Collins, M. D. 1993. Lactic acid bacteria and human clinical infection. *J. Appl. Bacteriol*. 75: 95-107.

Ahmad, R. 1996. Physico-chemical and biological evaluation of protein enriched biscuits using wheat soy blends. M. Sc. thesis, Department of Food Technology, University of Agriculture, Faisalabad, Pakistan, 79p.

Aidoo, K. E. 1986. Lesser-known fermented plant foods. *Trop. Sci.* 26: 249-258.

Aidoo, K. E., Nout, M. J. R., and Sarkar, P. K. 2005. Occurrence and function of yeasts in Asian indigenous fermented foods. *Yeast Res.* 6: 30–39.

Akesowan, A. 2007. Effect of a *konjac* flour soy protein isolates mixture on reduced-fat, added water chiffon cakes. *Aus. J. Technol.* 11(1): 23-27.

Alekel, D. E., Germain. A.S., Peterson, C. T., and Hanson, K. B. 2000. Isoflavones-rich soy protein rich isoflavone attenuates bone loss in the lumbar spine of perimenopausal women. *Am. J. Clin. Nutr.* 72(3): 844-852.

Ali, A. A. and Mustafa, M. M. 2009. Use of starter cultures of lactic acid bacteria and yeasts in the preparation of *Kisra*, a Sudanese fermented food. *Pak. J. Nutr.* 8(9): 349-353.

Aliya, S. and Geervani, P. 1981. An assessment of the protein quality and vitamin B content of commonly used fermented products of legumes and millets. *J. Sci. Food Agric.* 32: 837-842.

Alm, L. 1982. Effect of fermentation on B - vitamin content of milk in Sweden. *J. Dairy Sci.* 65(3): 353-359.

Anderson, J. W. and Gilliland, S. E. 1999. Effect of fermented milk (yoghurt) containing *Lactobacillus acidophilus* liansuum cholesterol hyper cholesterolemia in humans. *J. Am. Clin. Nutr*. 18: 43-45.

Anjum, F. M., Rehman, S., Butt, M. S., and Huma, N. 2003. Stability and acceptability components of the project on iron fortification of wheat flour. *Cereal Chem.* 16: 45-51.

Annor, G. A., Dawson, E. S., Saalia, F. K., Dedeh, S. S., Afoakwa, E. O., Debrah, K. T., and Budu, A. S. 2010. Response surface methodology for studying the quality characteristics of cowpea *(*vigna unguiculata*)-based tempeh. J. Food Proc. Eng.* 33(4): 606-625.

Antai, S. and Obong, U.S. 1992. The effect of fermentation on the nutrient status and on some toxic components of Icacinia manni. *Plant Food Hum. Nutr*. 42 (3): 219-224.

Antony, U. and Chandra, T. S. 1998. Antinutrient reduction and enhancement in protein, starch, and mineral availability in fermented flour of finger Millet (*Eleusine coracana*). *J. Agric. Food Chem.* 46(7): 2578–2582.

Anukam, K. C. and Reid, G. 2009. African traditional fermented foods and probiotics. *J. Med. Food* 12(6): 1177- 1184.

Applewhite, T. H. 1988. Production and utilization of tempeh in Indonaesian foods. In: Storly, M.L. and Ray, J. (eds.), *Proceedings of the world congress on vegetable protein utilization in human foods and animal feedstuffs*; 19-23 October, 1988; Singapore. pp. 324-335.

Appukuttan, A. 2010. Bioavailability of minerals from pulses. M.Sc. (Home Science.) thesis, Kerala Agricultural University, Trichur, 170p.

Areekul, S. 1990. The source and content of vitamin B_{12} in tempeh. *J. Med. Assoc. Thai.* 73(3): 152-156.

Arora, S., Jood, S., Khetarpaul, N., and Goyal, R. 2009. Effect of germination and probiotic fermentation on antinutrients and *in vitro* digestibility of starch and protein and availability of minerals from barley based food mixtures. *J. Food Sci. Technol.* 46(4): 359-362.

Aryana, K. J., and McGrew, P. 2007. Quality attributes of yogurt with Lactobacillus casei and various prebiotics. *Food Sci. Technol.* 40: 1808–1814.

Aso, Y. and Akazan, H. 1992. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. *Urol. Int.* 49: 125–129.

Ashenafi, M. 1992. Growth of *Listeria monocytogenes* in fermenting tempeh made of various beans and its inhibition by *Lactobacillus plantarum*. *Food Microbiol.* 8: 303-310.

Ashenafi, M. and Busse, M. 1991. Development of microorganisms during cold storage of pea and chickpea tempeh and effect of *Lactobacillus plantarum* on storage microflora. *J. Sci. Food Agric.* 56: 71-78.

Astuti, M., Meliala, A., Dalais, F. S., and Wahlqvist, M. L. 2000. Tempe, a nutritious and healthy food from Indonesia. *Asia Pacific J. Clin. Nutr*. 9: 322-325.

Ataie-Jafari, A., Larijani, b., Majd, H. A., and Tahbaz, F. 2009. Cholesterol lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemic subjects. *Ann. Nutr. Metab*. 54: 22 – 27.

Awada, S. H., Hady, A., Hassan, A.B., Ali, M. I., and Babikar, E. I. 2005. Antinutritional factors content and availability of proteins, starch and minerals of maize and lentil as influenced by domestic processing. *J. Food Techol.* 3(4): 523–528.

Babu, D., Bhakyaraj, D., and Vidhyalakshmi, R. 2009. A low cost nutritious Food "Tempeh"- A review. *Wld J. Dairy Food Sci.* 4(1): 22-27.

Badger, T. M., Ronis, C. M., Simmen, R., and Simmen, F. A. 2005. Soy protein isolate and protection against cancer. *J. Am. Coll. Nutr.* 24(2): 146-149.

Badi, S. 1991. Partial characterization of starch and use of millet flour in breadmaking. *Cereal Chem.* 53: 718-724.

Beizadea, A. E. 2009. Fortification of wheat flour. *Rom. Biotechnol.* 14(2): 4300-4306.

Bejarano, P. A., Nadia, M., Montoya, V., Rodríguez E.A., and Cuauhtémoc, R. 2006. Tempeh flour from chickpea (*Cicer arietinum* L.), nutritional and physicochemical properties. *Food Chem.* 10(6): 106-112.

Benchimol, E. I. and Mack, D. R. 2004. Probiotics in relapsing and chronic diarrhoea. *J. Pediatr. Hematol. Oncol.* 26: 515–517.

Bengmark, S. 2000. Colonic food: pre- and probiotics. *Am. J. Gastroenterol*. 95: 5–7.

Bera, S. K., Singh, S. P., Dash, P., and Dash, M. M. 2001. Screening groundnut germplasm for pod yield under residual moisture conditions of paddy fallow. *Indian J. Plant Genet. Res*. 14: 337-338.

Berg, S., Olsson, J., Swanberg, M., Schnürer, J., and Eriksson, A. 2001. Method for the production of fermented cereal food products and products thereof. In: Olligon, A.B. (ed.), *World Intellectual Property Organization*. Longman, pp. 123-135.

Berghofer, E., Grzeskowiak, B., Mundigler, N., Sentall, W.B., and Walcak, J. 2001. Antioxidative properties of faba bean, soybean and oat tempeh. *Int. J. Food Sci. Nutr.* 49: 45-54.

Bergqvist, S. W., Andlid, T., and Sandberg, A. S. 2006. Lactic acid fermentation stimulated iron absorption by Caco-2 cells is associated with increased soluble iron content in carrot juice. *Brit. J. Nutr*. 96: 705-711.

Bhandal, A. 2008. Effect of fermentation on *in vitro* digestibilities and the level of antinutrients in moth bean (*Vigna aconitifolia*). *Int. J. Food Sci. Technol*. 43(11): 2090 – 2094.

Bhatia, A., Khetarpaul, N., and Neelam, A. 2009. Development of an indigenously fermented Indian bread - *doli ki roti.* Effect on phytic acid content and *in vitro* digestibility of starch and protein. *Nutr. Food Sci.* 39(4): 330-336.

Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., and Webb, C. 2003. Cereal-based fermented foods and beverages. *Food Res. Int.* 36(6): 527-543.

Bohra, A. and Parihar, P. 2006. *Food Microbiology*. Agrobios Publications, Jodhpur, 454p.

Boutran, M. 1996. Calcium, phosphorus, vitamin D, dairy products and colorectal carcinogenesis: A French case--control study. *Br. J. Cancer*. 74(1): 145-151.

Bozena, S., Anna, S., and Janiszewska, A. 2008. The influence of tempeh fermentation and conventional cooking on anti-nutrient level and protein bioavailability (*in vitro* test) of grass-pea seeds. *J. Food Sci. Agric.* 88(13): 2265-2270.

Brissenden, R. 2003. *Southeast Asian Food: Classic and Modern Dishes from Indonesia, Malaysia*. Berkely Books, Singapore. 556p.

Brown, A. C. and Valier, A. 2004. Probiotics and medical nutrition therapy. *Nutr. Clin. Care* 7: 56-68.

Bryan, G. W. 1974. Adaptation of an estuarine polychaete to sediments containing high concentrations of heavy metals. In: Vernberg, F.J. and Vernberg, W.B. (eds.), *Pollution and physiology of marine organisms*. Academic press, London, pp. 123-135.

Buchanan, R. L. 2007. Inherant safety of cheese and meat fermentations: A review of some toxicological considerations. *J. Food Safety*. 4(2): 125-137.

Buckenhuskes, H.J. 2001. Fermented vegetables. In: Doyle, M. P. and Montville, T. J. (eds.). *Food Microbiolology*. ASM press, Washington, DC, pp. 665-679.

Buckle, K. A. and Kartadarma, E. 2008. Inhibition of bongkrek acid and toxoflavin production in tempe bongkrek containing *Pseudomonas cocovenenans. J. Appl. Microbiol.* 68(6): 571 – 576.

Burdock, G. A. 2000. Organisms in fermented foods. *Int. J. Toxicol*. 19(3): 195-218.

Byun, J. W., Lee, H. S., Yook, H. Y., and Kim, L. M. 2002. Improvement of shelf stability and processing properties of meat products by gamma irradiation. *Radiation Phys. Chem.* 63(6): 361-364.

Cabrejas, M. A. M., Aguilara, Y., Pedrosa, M. M., Cuardado, C., Hernandez, C., and Esteban, R. M. 2004. The impact of dehydration process on antinutrients and protein digestibility of some legume flours. *Food Chem.* 114: 1063-106.

Campbell-Platt, G. 1987. *Fermented Foods of the World – A Dictionary and Guide*. Lanclet Publishers. New York, 234p.

Cavalier, D., McGovern, P. E., Hartl, D. L., Mortimer, R., and Polsinelli, M. 2003. Evidence for *S. cerevisiae* fermentation in ancient wine. *J. Mol. Evol.* 57(1): 226-232.

Chavan, J. K. and Kadam, S. S. 1989. Critical reviews in food science and nutrition. *Food Sci.* 28: 348-400.

Chaverrí, J. 2004. Soy protein diet ameliorates renal nitrotyrosine formation and chronic nephropathy induced by puromycin aminonucleoside. *Life Sci.* 79: 987-999.

Chen, Y., Sun, T., Wang, J., Airden, C., Bai, M., and Zhan, H. 2009. Comparison of nutrition and microbiological compositions between two types of fermented milk from Tibet in China. *Int. J. Food Sci. Nutr.* 60(7): 243-250.

Chmielewski, R. A. N. and Frank, J. F. 2006. Biofilm formation and control in food processing facilities. *Comprehensive Rev. Food Sci. Food Safety.* 2: 22-32.

Chompreeda, P. T. and Fields, M. L. 1984. Effects of heat and natural fermentation on amino acids, flatus producing compounds, lipids oxidation and trypisin inhibitor in bunds of soybean and corn meal. *J. Food Sci.* 49: 560-563.

Chopra, S. L. and Kanwar, J. S. 1978. *Analytical Agricultural Chemistry.* Kalyani Publishers, Ludhiana, 210p.

Chou, L. and Weimer, B. 1999. Isolation and characterisation of acid and bile tolerant isolates from strains of *L. acidophilus*. *J. Dairy Sci*. 82: 23-31.

Clark, E. 2007. *Tempting Tempeh*. Aduki independent Press, Australia, 48p.

Clark, P. A., Cotton, L. N., and Martin, J.H. 1993. Selection of bifido bacteria for use as dietary adjunts in cultured diary foods: Tolerance to simulated pH of human stomach. *Cult. Dairy Prod. J.* 28: 11-14.

Collado, A. and Lilia, S. 1992. Comparative study of high amylose and low amylose rice for the preparation of *Puto*, a Filipino fermented rice cake. *Food Sci. Biotechnol.* 6: 129-132.

Correia, R. T. P., McCue, P., Magalhaes, M. M. A., Macedo, G.R., and Shetty, K. 2004. Production of phenolic antioxidants by the solid-state bioconversion of pineapple waste mixed with soy flour using *Rhizopus oligosporus*. *Process Biochem.* 39: 2167-2172.

Crittenden, R. G., Martinez, N. R., and Playne, M. J. 2003. Synthesis and utilisation of folate by yoghurt starter cultures and probiotic bacteria, *Int. J. Food Microbiol.* 80: 217-222.

Cross, M. L., Stevenson, L. M., and Gill, H. S. 2001. Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria. Int. *Immunopharmacol*. 1(5): 891-901.

Cuevas-Rodriguez, E. O., MiIan-Carrillo, J., Mora-Escobedo, R., Cardenas-Valenzuela, O. G., and Reyes-Moreno, C. 2004. Quality protein maize (Zea mays L.) tempeh flour through solid state fermentation process. Food Sci. Technol. 37: 59-67

Dahal, N., Rao, E. R., and Swamilingappa, B. 2003. Biochemical and nutritional evaluation of Masayura - A legume based traditional savoury of Nepal. J. Food Sci. Technol. 40(1): 17-22.

Dariy Council of California. 2000. Dairy Council of California home page [on line]. Available:http://www.dairycouncilofca.org/pdfs/probiotics.pdf. [5 Jan. 2009].

Dellatt, E. 1979. *General Microbiology*. Academic Press, London, 454p.

Devi, N. L. 2004. Technology of fermented cereal foods. *Proceedings of the Biotechnological Approaches to Food Processing*; 20 January - 9 February, 2004; Hyderabad. Post Graduate and Research Centre, Acharya N.G.Ranga University, pp. 45-49.

Dirar, H. 1993. The indigenous fermented foods of sudan: A Study in African food and nutrition. *Food Res. J.* 6: 23-34.

Djien, K. S. and Hesseltine, C. W. 1990. *Tempeh and Related Foods in Microbial Biomass*. Academic Press, London, 205p.

Donohue D. C., Deighton M., Ahokas J. T., and Salminen S. 1998. Toxicity of lactic acid bacteria. In: Saminen S. and Wright A. (eds.), *Lactic Acid Bacteria*. (3rd ed.). Marcel Dekker, New York, pp. 307–313.

Dubos, R. 1998. *Pasteur and Modern Science.* Liberty of Science Publications, New York, 325p.

Duhan, A. Khetarpaul, N., and Bisnoi, S. 2001. HCl extractability of zinc and copper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *J. Food. Sci. Technol.* 17(5): 279 – 304.

Edward, R. 2003. *Handbook of Fermented Functional Foods*. Elsiever Pub. London, 236p.

Egounlety, M., Aworh, O. C., Akingbala, J.O., Houben, J. H., and Nago, M. C. 2002. Nutritional and sensory evaluation of tempe-fortified maize-based weaning foods. *Int. J. Food Sci. Nutr.* 53: 15-27.

Eklund-Jonsson, C., Sandberg, A.S., and Alminger, M. 2006. Reduction of phytate content while preserving mineral content during whole grain cereal tempe fermentation. *J. Cereal Sci.* 44: 154-160.

Elkhalifa, O., Schiffler., and Bernhard, R. 2004. Effect of fermentation on the starch digestibility, resistant starch and some physicochemical properties of sorghum flour. 48(2): 91-94.

Ereifej, K. I. 1995. Preparation of acceptable traditional dehydrated lentil soup at small scale. *J. Food Sci. Technol.* 32(6): 486 – 488.

Esuoso, K. O. and Bamiro, F.O. 1995. Studies on the baking properties of non-wheat flours. *Int. J. Food Sci. Nutr*. 46: 267-273.

Farnworth, E. 2005. *Hand Book of Fermented Functional Foods.* CRC press, Florida, 379p.

Farooque, N. A. and Nautiyal, A. 1999. Traditional knowledge and practices of Bhotia pastoralists of Kumaun Himalayas: The need for value addition. *Int. J. Sustain. Dev. Wld. Ecol.* 6(2): 60-67.

Fellows, P. 1997. *Traditional Foods*. Intermediate Technology Publications, UK, 245p.

Feng, X. M., Eriksson, A. R. B., and Schnürer, J. 2005. Growth of lactic acid bacteria and *Rhizopus oligosporus* during barley tempeh fermentation. *Int. J. Food Microbiol.* 104(3): 249-256.

Feng, X. M. 2006. Microbial dynamics during tempeh fermentation. Ph.D thesis, Swedish University Agricultural Sciences, Uppsala, 52p.

Frazier, W. C. and Westhoff, D.C. 1995. *Food Microbilogy*. Tata McGraw Publishing Company, New Delhi, 537p.

Fujita, M. 1993. Purification and characterization of a strong fibrinolytic enzyme (nattokinase) in the vegetable cheese *natto*, a popular soybean fermented food in Japan. *Biochem. Biophysical Res. Commun.* 19(3): 1340–1347.

Gamer, F. 1994. Safety of lactic acid bacteria and their occurrence in human clinical infections. *Bull. Inst. Pasteur*. 92: 45-67.

Garcia, R. A., Hotchkiss, J. H., and Steinkraus, K. H. 1999. The effect of lipids on bongkrekic (Bongkrek) acid toxin production by *Burkholderia cocovenenans* in coconut media**.** *Food Addit. Contam.* 16: 63-69.

Gandjar, I. 1978. *Fermentation of Winged Bean Seeds.* Council for Agriculture and Resources, Philippines, 380p

Giannella, R. A., Broitman, S. A., and Zamcheck, N. 1972. Gastric acid barrier to ingested microorganisms in man: studies *in vivo* and *in vitro. Gut Health* 13: 251-256.

Gibson, R. S., Perlas, L., and Hotz, C. 2006. Improving the bioavailability of nutrients in plant foods at the household level. *Proc. Nutr. Soc.* 65: 160-168.

Gill, H. S., Cross, M. L., Ratherfurd, K. J., and Gopal, P. K. 2000. Dietary probiotic supplementation to enhance cellular immunity in the elderly. *Br. J. Biomed. Sci.* 57(2): 94-96.

Goldin, B. R. 1998. Health benefits of probiotics. *Br. J. Nutr*. 80(2): 203-207.

Gopalan, C., Sastri, B.V.R., and Balasubramanian, S. C. 1999. *Nutritive Value of Indian Foods.* NIN, Hyderabad, 117p.

Greshe, H. R. 2006. Fortification of widely consumed cereal based food products. *Food Sci. Technol*. 39: 1072-1079.

Hachmeister, K. A. and Fung, D. Y. C. 1993. Tempeh: a mold-modified indigenous fermented food made from soybeans and/or cereal-grains. *Crit. Rev. Microbiol.* 19: 137-148.

Hag, M., Abdullahi, H., Tinay, E. l., and Yousif, N. E. 2002. Effect of fermentation and dehulling on starch, total polyphenols, phytic acid content and *in vitro* protein digestibility of pearl millet. *Food Chem.* 77(2): 193-196**.**

Hahn, S. K. 1989. An overview of African traditional cassava processing utilization. *Outlook Agric.* 18: 110-118.

Halm, M., Lillie, A., Spreusen, A. K., and Jakobsen, M., 1993. Microbiological and aromatic characteristics of fermented maize doughs for *kenkey* production in Ghana. *Int. J. Food Microbiol.* 19: 135-143.

Hansen, A. 2002. Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. *Trends Food Sci Technol.* 16: 85-95.

Haron, H., Ismail, E., Azlan, A., Suzana, S., and Peng, L. S. 2008. Daidzein and genestein contents in tempeh and selected soy products. *Food Chem.* 115(4): 1350-1356.

Hata, Y., Yamamoto, M., Ohni, M., Nakajima, K., Nakamura, Y., and Takano, T. 1996. A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. *Am. J. Clin.Nutr*. 64: 767-771.

Heller, K. J. 2001. Probiotic bacteria in fermented foods: product characteristics and starter organisms. *Am. J. Clin. Nutr.* 73(2): 374-379.

Henderson, P. J. F. and Lardy, H. A. 1970. Bongkrekic acid: An inhibitor of adenine nucleotide translocase of mitochondria. *J. Biol. Chem.* 245: 1319-1326.

Herken, E. N., Senol, I., Oner, M. D., and Esra, I. 2006. The *in vitro* protein digestibility, microbiological quality and gelatinization behaviour of macaroni as affected by cowpea flour addition. *Food Chem.* 98(4): 664-669.

Hesseltine, C. W. and Wang, H. L. 1979. Fermented Foods. *Chem. Ind.* 23: 393-399.

Hesseltine, C. W., Smith, M., Bradle, B., and Djien, K. S. 1963. Investigations of tempeh, an Indonesian food. *Dev. Ind. Microbiol.* 4: 275-287.

Hesseltine, C. W. 1983. The future of fermented foods. *Nutr. Rev.* 41(10): 293-301.

Hesseltine, C. W. 1995. A millennium of fungi, food and fermentation. *Mycologia*, 57(4): 149-197.

Holzapfel, W. H. 1998. Overview of gut flora and probiotics. *Int. J. Food Microbiol*. 41: 85-101.

Homma, N. 1988. On effects of lactic acid bacteria, Part 1- Biological significance. *New Med. Clin*. 35(12): 2687-2695.

Hotz, C. and Gibson, R.S. 2007. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *J. Nutr.* 137: 1097-1100.

Hsu, C. K., Yu, Y. P., and Chung, Y. C. 2009. Effect of tempeh on the intestinal microbiota and colon cancer in rats. *New Biotechnol.* 25(1): 205-206.

Huis, J. H. I. and Havenaar, R. 1992. Probiotics: A general view. In: Wood, B.J.B (ed.), The *Lactic Acid Bacteria in Health and Disease*. Chapman and Hall, New York, pp. 209-224.

Hutkins, R. W. 2006. *Microbiology and Technology of Fermented Foods*. Blackwell publishing. New York, 441p.

ISI. 1988. IS: 7463 *Wheat Flour for use in Biscuit Industry*. Bureau of Indian Standards, New Delhi, 76p.

Isolauri, E., Joensuu, J., and Suomaainen, H. 1991. Improved immunogenecity of oral DX RRV reassortant rotavirus vaccine by L.casei GG vaccine. *Pediatr. Infect. Dis. J.* 14: 107-11.

Jackson, M. L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi, 299p.

James, W., Anderson, M. D., and Stanley, E. 1999. Effect of fermented milk (Yogurt) containing lactobacillus acidophillus L1 on serum cholesterol hypercholestrolemic humans. *J. Am. Coll. Nutr*. 18: 43-50.

Jay, J. M. 1996. *Modern Food Microbiology*. Chapman and Hall, New York, 410p.

Jellinek, G. 1985. *Sensory Evaluation of Food - Theory and Practice.* Ellis Horwood Ltd. Chichester England and VCH Verlagsgerellschaft mbh, Weinhein, Germany, 218p.

Jennessen, J., Nielsen, K. F., Houbraken, J., Lyhne, E. K., Schnürer, J., Frisvad, J. C., and Samson, R.A. 2005. Secondary metabolite and mycotoxin production by the *Rhizopus microsporus* group. *J. Agrl. Food Chem.* 53: 1833-1840.

Jens, H. 2008. The transcription factor ETS-1: Its role in tumour development and strategies for its inhibition. *Mini Rev. Med. Chem.* 8(11): 1095 -1105.

Jiao, Z., Kawamura, Y., Mishima, N., Yang, R., and Ezaki, T. 2003. Need to differentiate lethal toxin-producing strains of *Burkholderia gladioli*, which cause severe food poisoning: description of *B. gladioli* pathovar *cocovenenans* and an emended description of *B. gladioli*. *Microbiol. Immunol*. 47: 915-25.

Jood, S. and Khetarpaul, N. 2005. Improving nutritional quality of coarse cereals through probiotic fermentation. *Processed Food Ind*. 23: 17-25.

Jujin, W., Hanhu, J., and Zhenli, L. 2000. Study on tempeh sausage. *Meat Ind.* 8: 17-19.

JuQin, W., Mingshen, D., and Hanhu, J. 2001. Study of tempeh ice cream. *Sci. Technol. Food Ind.* 22: 47-48.

Jurus, M. A. and Sundberg, J. W. 1976. Penetration of *Rhizopus oligosporus* into soybeans in Tempeh. *Appl. Envion. Microbiol*. 32(2): 284-287.

Kada, S., Yabusaki, M., Kaga, T., Ashida, H., and Yoshida, K. 2008. Identification of two major ammonia-releasing reactions involved in secondary natto fermentation. *Biosci. Biotechnol. Biochem.* 72(7): 1869-1876.

Kariluoto, S., Aittamaa, M., Korhola, M., Salovaara, H., Vahteristo, L., and Piironen, V. 2006. Effects of yeasts and bacteria on the levels of folates in rye sourdoughs. *Int. J. Food Microbiol.* 106: 137-143.

Karlson, P. 1963. *Introduction to Modern Biochemistry*. Academic Press, London, 433p.

Karyadi, D. and Lukito, W. 1996. Beneficial effects of tempeh in disease prevention and treatment. *Nutr. Rev.* 54: 94–98.

Kearney, L., Mary Upton, M., and Mcloughlin, A. 1990. Meat fermentations with immobilized lactic acid bacteria. *Appl. Microbiol. Biotechnol.* 33(6): 648-651.

Ken, A. 2007. *Beans: a History*. Berg Publishers. New Jersey, 216p.

Kennedy, A. R. 1995. The evidence of soybean products in controlling diabetis. *J. Nutr*. 125: 733-738.

Keuth, S. and Bisping, B. 1993. Formation of vitamins by pure cultures of tempeh moulds and bacteria during the tempeh solid substrate fermentation. *J. Appl. Bacteriol*. 75: 427-434.

Khader, V. 2001. *Text Book of Food Science and Technology*. Indian council of Agricultural Research, New Delhi, 449p.

Khedkar, J. N., Dave, J. M., and Sannabhadti, S. S. 1998. Antibacterial activity associated with *Bifidobacterium adolescentis*. *J. Food Sci. Technol*. 35(6): 527-529.

Khetarpaul, N. 2005. *Food Microbiology*. Daya publication house, New Delhi, 469p.

Khetarpaul, N., and Chauhan, B. M. 1990. Fermentation of pearl millet flour with yeasts and lactobacilli: *in vitro* digestibility and utilisation of fermented flour for weaning mixtures. *Plant. Food Hum. Nutri.* 40: 167-173.

Kiers, J. L. 2001. Effects of fermented soya bean on digestion, absorption and diarrhoea. Ph.D thesis, Wageningen University, Netherlands, 110p.

Kiers, J. L., Nout, M. J. R., Rombouts, F.M., Nabuurs, M. J. A., and Van der Meulen, J. 2002. Inhibition of adhesion of enterotoxigenic *Escherichia coli* K88 by soya bean *tempe*. *Appl. Microbiol.* 35: 311-315.

Kilcast, D. and Subramanian, P. 2000. *The Stability and Shelf life of Foods*. Woodhead Publishers, England, 333p

Kim, H. S. and Gilliland, S. E. 1983. *Lactobacillus acidophilus* as dietary milk adjunct aid lactose digestion in humans. *J. Dairy Sci*. 66: 959-66.

Kim, L. and Zemel, M. B. 1986. *In vitro* estimation of the potential bioavailability of calcium from sea mustard, milk and spinach under simulated normal and reduced gastric conditions. *J. Food. Sci.* 51(3): 957 – 963.

Kim, M. and Chun, J. 2005. Bacterial community structure in *kimchi*, a Korean fermented vegetable food, as revealed by 16S rRNA gene analysis. *Int. J. Food Microbiol.* 15(1): 91-96.

Kingsley, C., Anukam, G. H., and Reid, G. 2009. Fermented foods - A review. *J. Med. Food* 12(6): 1177-1184.

Klein, D. W., Prescott, A., Lansing, M. K., Harley, J. H., and John, J. 2005. *Microbiology*. McGraw-Hill Publishers, New York, 342p.

Kneifel, W., and Mayer, H. K. 2007. Vitamin profiles of kefirs made from milks of different species. *Int. J. Food Sci Technol.* 26(4): 423-428.

Kobayasi, S. Y., Okazaki, N., and Koseki, T. 1992. Purification and characterization of an antibiotic substance produced from *Rhizopus oligosporus* IFO 8631. *Biosci. Biotechnol. Biochem.* 56: 94-98.

Koop, H. L. 2001. Prophylatic and therapeutic uses of probiotics. *J. Am. Dietetic Assoc.* 56: 98-104.

Koswan, D. and Hesseltine, C. W. 1979. Tempe and related foods. *Econ. Microbiol.* 4: 115-140.

Kozlowska, H. 1996. Natural fermentation of lentils: Influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphates. *J. Food. Sci. Agric*.71 (3): 367-375.

Krumhar, H. and Carleton, K. 2005. Removal of flatulence, treatment of inflammation and pain in mammals. Principles from legumes by mold fermentation. U S Patent No. 424/601.

Krusong, W., and Yongsmith, B. 1996. Factors affecting acid formation in soymilk from high vitamin B_{12}-tempeh. *Proceedings of the 2nd International Soybean and Utilization Conference;* 8-13 January, Bangkok, Thailand, pp 403-407.

Lakshmy, P. S. 2003. Evaluation of fruit quality in banana ‘Nendran’ (*Musa* AAB). M.Sc. (Home Science) thesis, Kerala Agricultural University, Trichur, 71p.

Lancaster, P. A., Ingram, J. S., Lim, M. Y., and Coursey, D. G. 1982. Traditional cassava based foods: Survey of processing techniques. *Econ. Bot*. 36(1): 12-48.

Lankahaputhra, W. E. V. and Shah, N. P. 1995. Survival of *Lactic acidophilus* and *Bifidobacterium Spp* in the presence of acid and bile salts. *Cult. Dairy Prod. J*. 30: 2-7.

LeBlanc, J. G., Burgess, C., Sesma, F., Savoy, G., and Sinderen, D. 2007. *J. Dairy Sci.* 88(10): 3435-3437.

Lei, V., Friis, H., and Michaelsen, K. F. 2006. Spontaneously fermented millet product - a natural probiotic treatment for diarrhoea in young children: An intervention study in Northern Ghana. *Int. J. Food Microbiol.* 110: 246-253.

Leroy, F. and Vuyst, L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci. Technol.* 15: 67-78.

Lin, S., Francis, A. K., Rocheford, T., and White, W. S. 2007. Retention of provitamin A carotenoids in high beta-carotene maize (*Zea mays*) during traditional African household processing. *J. Agric. Food Chem*. 55(26): 10744-10750.

Liem, I. T. H., Steinkraus, K.H., and Cronk, T. C. 1977. Production of vitamin $B\text{-}_{12}$ in tempeh fermented soybean food. *Appl. Environ. Microbiol.* 34: 773-776.

Lilieberg, H. G. M., Lonner, C. S., and Bjork, I. M. E. 1995. Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *J. Nutr.* 125: 1503-1511.

Liu, L., Shi-Yi, O., Cai-Huan, H., and Ai-Hua, Y. 2007. Analysis of nutrients in fermented by-products by *Aspergillus niger*. *Food Sci. Technol.* 9(3): 145-148.

Liya, S. 2001. Value addition and evaluation of nutritional quality in taro. M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 103p.

Ljarotimi, O. S., and Esho, R. 2009. Comparison of nutritional composition and anti- nutrient status of fermented, germinated and roasted 'bambara' groundnut seeds (*Vigna subterranean*). *Brit. Food J.* 111: 376-386.

Lopez, O. P. and Harry, G. I. 1990. Changes in selected chemical and antinutritional components during tempeh preparation using fresh and hardened common beans. *J. Food Sci.* 55: 123-126.

Lorri, W. and Svanberg, U. 1994. Lower prevalence of diarrhoea in young children fed lactic acid-fermented cereal gruels. *Food Nutr. Bull.* 15: 57-63.

Lothony, N. 1992. *Seed Innoculums and Their Production Technology*. Funny Publishing. Bangkok. 231p.

Mack, D. R. 1999. Probiotics inhibit enteropathogenic *E.coli* adhesence *in vitro* by inducing intestinal muccin gene expression. *Am. J. Physiol*. 276: 941-950.

Mahajan, S. and Chauhan, B. M. 2006. A research note- Effect of natural fermentation on the extractability of minerals from pearl millet flour. *J. Food Sci.* 53(5):1576-1577.

Man, M. D. and Jones, A. 2000. *Shelf Life Evaluation of Foods.* Aspen publishers, New York, 264p.

Mann, G. V. 1977. A factor in yogurt which lowers cholesterol in man. *Atherosclerosis*. 26: 335-337.

Mann, G. V. and Spoerry, A. 1974. Studies of a surfactant and cholesteremia in the *Maasi*. *Am. J. Clin. Nutr*. 27: 464 - 466.

Mark, W. 2002. Food and Nutrition. [on line]. Healthy eating club, Australlia. Available:http://healthyeatingclub.com/info/articles/func foods/probiotics.htm. [20 June 2009].

Marshall A., Barbara., F., Pfaue, B., and Thomas, H. B. 2007. Nutritional value of African yambean (Sphenostylis stenocarpa, L): improvement by solid substrate fermentation using the tempeh fungus *Rhizopus oligosporus. J. Sci. Food Agric.* 87(2): 297 – 304.

Marteau, P., Flourie, B., Pochart, F., Chastang, C., Desjeux, J. F., and Rambaud, J. C. 1990. Effect of the microbial lactase (EC 3.2.1.23) activity in yoghurt on the intestinal absorption of lactose: an *in vivo* study in lactase-deficient humans. *Br. J. Nutr*. 64: 71–79.

Marteau, P., Pochart, P., Flourie, B., Pellier, P., Santos, L., Desjeux, J. F., and Rambaud, J. C. 1993. Effect of chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am. J. Clin. Nutr.* 52**:** 685–688.

Martens, 2007. Microbial production of vitamin B_{12}. *Appl. Microbiol. Biotechnol.* 58: 275-285.

Masako, M. 2006. Suitability of quinoa fermented with *Rhizopus oligosporus* as an ingredient of biscuit. *J. Jpn. Soc. Food Sci. Technol.* 53(1): 62-69.

Matsuo, M. 2006. Suitability of 'okara tempe' as foodstuff. *Nippon Nogeikagaku Kaishi* 64: 1235-1236.

Mayra-Makein, A. and Bigret, M. 1993. Industrial use and production of lactic acid bacteria. In: Salminen, S. and Von –Wright, A. (eds.). *Lactic Acid Bacteria*. Marcel Dekker, New York, pp. 65–95.

McCue, P., Lin, Y. T., Labbe, R. G., and Shetty, K. 2004. Sprouting and solid-state bioprocessing by *Rhizopus oligosporus* increase the *in vitro* antibacterial activity of aqueous soybean extracts against *Helicobacter pylori*. *Food Biotechnol.* 18: 229-249.

McKay, L. L. and Baldwin, K. A. 1990. Applications for biotechnology: present and future improvements in lactic acid bacteria. *Microbiol. Rev.* 87: 3-14.

Mejias, E., Perez, W., Duarte, C., Garcia, I., Perez, O., Silveira, I., and Batte, R. 2006. Application of powdered tempeh. *Ciencia Tecnologia de Alimentos*. 15(1): 40-44.

Melissa, K. 2007. The joy of soy, *The Rotarian*. 180(10): 16-17.

Mensah, P. P. A., Tomkins A. M., Drasar, B. S., and Hamson, T. J. 1990. Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. *Lancet* 36: 140-143.

Merk, K., Borelli, C., and Korting, H. C. 2005. Lactobacilli - bacteria-host interactions with special regard to the urogenital tract. *Int. J. Med. Microbiol.* 29: 9-18.

Messina, M. and Messina, V. 2000. Soybean isoflavones and bone health. *J. Renal. Nutr*. 10: 63-68.

Misra, A. and Kulshrestha, K. 2003. Effect of storage on nutritional value of potato flour made from three potato varieties. *Plant Food Hum. Nutr*. 58(3): 1-10.

Mittal, B. K. and Garg, S. K. 1990. Tempeh - technology and food value. *Food Rev. Int.* 6(2): 213 – 224.

Mohammed, N. A., Ahmed, I. A. M., and Babiker, E. E. 2010. Nutritional evaluation of sorghum flour (*Sorghum bicolor L. Moench)* during processing of *Injera. Int. J. Bio. Life Sci.* 6: 113-117.

Molins, R. A. 2000. Quality evaluation of fermented soy product. *J. Nutr.* 36: 303-305.

Morcos, S. R. 1993. Egyptian *kishk*. *Indigenous Fermented Foods*, Marcel Dekker, New York, 354p.

Morteau, P., Vaerman, J. P., Bord, J. P., Brassart, D., Pochart, P., Desjeux, J.F., and Ramabaud, J. C. 1997. Effects of intrajejunal perfusion and chronic ingestion of *lactobacillus Johnsonii strain* on serum concentrations and jejunal secretions of immunoglobulins and serum proteins in healthy humans. *Gasteroentrol. Clin. Biol*. 21: 293-298.

Mugula, J. K. 1992. Evaluation of the nutritive value of maize-soybean *tempe* as a potential weaning food in Tanzania. *Int. J. Food Sci. Nutr.* 43: 113-119.

Muhammad N., Butt, M. S., Anjum, F. M., Sharif, K., and Nasir, R. M. 2003. Effect of moisture on the shelf life of wheat flour. *Int. J. Agri. Biol.* 5(4): 234-241.

Mukherjee, P. S., Sinha, K., and Bera, H. G. 1992. Solid state fermentation and their application. *Appl. Mycopathol. Mycol.* 45: 211-222.

Mulyowidarso, R. K., Fleet, G. H., and Buckle K. A. 1990. Association of bacteria with the fungal fermentation of soy bean tempe. *J. Appl. Bacteriol.* 68: 43-47.

Murata, K., Ikehata,H., and Miyamata,T. 2006. Studies on the nutritional value of tempeh. *J. Food Sci.* 32(5): 580-586.

Murch, S. H. 2001. Toll of allergy reduced by probiotics. *Lancet* 357: 1057–1059.

Murphy, S. P. and Allen, L. H. 2003. Nutritional importance of animal source foods. *J. Nutr*. 133: 3932-3935.

Murugkhar, A. D. and Jha, K. 2011. Influence of storage and packaging conditions on the quality of soy flour from sprouted soybean. *J. Food Sci. Technol.* 48(3): 325-328.

Nagaraju, V. D. and Manohar, B. 2000. Rheology and particle size changes during *Idli* fermentation. *J. Food Eng.* 43: 167- 171.

Nakamichi, W., Hideyuki, A., and Kenshiro, F. 2008. Fermentation of soybean by *Rhizopus* promotes the calcium absorption ratio in rats. *J. Sci. Food Agric.* 88: 2749 – 2752.

Nakamura, Y., Masuda, O., and Takano, T. 1996. Decrease of tissue angiotensin-1-converting enzyme activity upon feeding sour milk in spontaneously hypertensive rats. *Bio. Sci. Biotechnol. Biochem*. 60: 488–489.

Nanri, A., Mizoue, T., Takahashi, Y., Kirii., K. M. Inoue, M., and Tsugane, S. 2010. Soy product and isoflavone intakes are associated with a lower risk of type 2 diabetes in overweight Japanese women**.** *J. Nutr.* 140(3): 580 - 586.

Nasir, M., Butt, M., Anjum, F. M., Sharif, K., and Minhas, R. 2003. Effect of moisture on the shelf life of wheat flour. *Int. J. Agric. Biol.* 5: 458-459.

Newman, R. K., Newman, C. W., and Eslick, R. F. 1985. Effect of fungal fermentation and other treatments on nutritional value of waxy barley fed to chicks. *Poultry Sci.* 64: 1514-1518.

Nnam, N. M. 2000. Chemical evaluation of multimixes formulated from some local staples for use as complementary foods in Nigeria. *Plant Food Hum. Nutr.* 55:255-263.

Nout, M. J. R. 1989. Effect of *Rhizopus* and *Neurospora* spp. on growth of *Aspergillus flavus* and *A. parasiticus* and accumulation of aflatoxin B1 in groundnut. *Mycol. Res.* 93: 518-523.

Nout, M. J. R. 1994. Mycotoxins in foods. *Food Res. Int*. 27(3): 291-298.

Nout, M.J.R. and Kiers, J.L. 2005. Tempe fermentation, innovation and functionality: update into the third millenium. *J. Appl. Microbiol.* 98: 789-805.

Nout, M. J. R. and Ngoddy, P. O. 1997. Technological aspects of preparing affordable fermented complementary foods. *Food Control* 8: 279-287.

Nout, M. J. R. and Rombouts, F. M. 1990. Recent developments in tempe research. *J. Appl. Bacteriol.* 69: 609-633.

Nowak, J. 1992. Oats tempeh. *Acta Biotechnol.* 12: 345-348.

Oakey, H. J., Harty, D. W. S., and Knox, K. W. 1995. Enzyme production by lactobacilli and the potential link with endocarditis. *J. Appl. Bacteriol*. 78: 142-148.

Obadina, A. O., Oyewole, O. B., and Awojobi, T. M. 2008. Effect of steeping time of milled grains on the quality of *Kunnu-zaki* (A Nigerian beverage). *Afr J. Food Sci*. 2: 33-36.

Odugbemi, T., Odujinrin, O. M. T., Akitoye, C. O., Oyerinde, J. P. O., and Esumeh F. I. 1991. Study on the pH of *ogi,* Nigerian fermented weaning food, and its effect on enteropathogenic *Escherichia coli, Salmonella typhi* and *Salmonella paratyphi. J. Trop. Med. Hygiene.* 94: 219-223.

Odunfa, S. A. 1987. African fermented foods - From arts to science. *J. Fd Agric.* 1: 179-183.

Okada, N., Hariantono, J., Hadioetomo, R. S., Nikkuni, S., and Itoh, H. 1985. Survey of vitamin B_{12}-producing bacteria isolated from Indonesian tempeh. *Rep. Nat. Food Res. Inst.* 47: 49-56.

Osawa, T. 1998. Functional foods for disease prevention. In: Osawa, T., Shibamoto, T. and Terao, J.(eds.), *Recent progress on functional foods research in Japan*. American chemical society, Washington, pp.2-9.

Ouwehand, A. C. and Salminen, S. J. 1998. The health effects of cultured milk products with viable and non-viable bacteria. *Int. Dairy J.* 8: 749-758.

Ouwehand, A. C., Salminen, S., and Isolauri, E. 2002. Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek* 82: 279-289.

Oyewole, O. B. and Sanni, L. O. 1995. Constraints in traditional cassava processing: A case of '*fufu*' production. In: Egbe, T.M., Brauman, A., Griffon, D., and Treche, S. (eds.), *Cassava Food Processing*. Scientific Development Cooperation, Paris, pp. 523-529.

Padmaja, G., George, M., Moorthy, S. N., Bainbridge, Z., Plumb, V., Wood, J. F., and Powell, C. J. 1994. Nutritional evaluation of the starchy flour obtained from cassava tubers on fermentation with a mixed-culture inoculum. *J. Agric. Food Chem.* 42: 766-770.

Pariza, M. W. and Johnson, E. A. 2001. Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. *Reg. Toxicol. Pharmacol.* 33(2): 173-186.

Parvez , S., Malik, K. A., Kang, S. A., and Kim, H. Y. 2006. Probiotics and their fermented food products are beneficial for health. *J. Appl. Microbiol.* 100(6): 1171–1185.

Patel, P., Parekh, T., and Subhash, R. 2008. Develpment of probiotic and symbiotic chocolate mousse: A functional food. *Biotechnol*. 7(4): 769-774.

Pedersen, C. S. 1979. *Microbiology of Food Fermentations*. AVI Publishers, NewYork, 256p.

Pelletier, X., Laure, S.B., and Donazzolo, Y. 2001. Hydrogen excretion upon ingestion of dairy products in lactose intolerant male subjects- importance of live flora. *Eur. J. Clin. Nutr*. 55: 509-512.

Perdigon, G. and Alvarez, S. 1992. Probiotics and the immune state. In: Fuller, R. (ed.), *Probiotics - The Scientific Basis* (2nd Ed). Chapman and Hall, London, pp. 146-180.

Perkin-Elmer. 1982. *Analytical Methods for Atomic Absorption Spectrophotometry*. Perkin-Elmer Corporation, USA, 114p.

Philip, T. E. 1993. *Modern Cookery for Teaching Trade*. Novena Publishers, Chenni, 567p.

Pillai, K. C. 2001. Shelf life of breadfruit (*Artocarpus altilis* (Park) Fosberg.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Trichur, 85p.

Platt, G. C. 2004. *Encyclopedia of Food Microbiology*. Lanclet pub. New York, 765p.

Posati, L. R. 2000. Quality evaluation of traditional Indonesian foods. *Nutr. Sci.* 28: 103-106.

Pszezola, E. 2002. Ingredients from fermentation. *Food Technol.* 56(2): 52-65.

Purushothaman, D., Dhanapal, N., and Rangaswami, G. 1993. Indian *idli, dosa, dhokla, khaman*, and related fermentations. In: Steinkraus, K.H., (ed.). *Handbook of Indigenous Fermented Foods* (4th Ed). Marcel Dekker, New York, pp. 149-165.

Racker, E. 1974. History of Pasteur effect and its pathobiology. *Mol.Cell. Biochem.* 5(1): 17-23.

Ragaee, S. M., El-Banna, A. A., and Damir, A. A. 1986. Effect of natural lactic acid fermentation on amino acids content and *in-vitro* digestibility of lentils. *Alex. Sci. Exch*. 7(1): 217-224.

Rakin, M., Vukasinovic, M., Marenkovic, S., and Maksimovic, M. 2007. Contribution of lactic acid fermentation to improve nutritive quality of vegetable juices enriched by brewers yeast autolysat. *Food Chem.* 100: 599-602.

Ramakrishnan, C. V. 1993. Indian *idli, dosa, dhokla, khaman*, and related fermentations. In: Steinkraus, K. H., (ed.). *Handbook of Indigenous Fermented Foods* (4th Ed). Marcel Dekker, New York, pp. 115-134.

Ranganna, S. 1986. *Handbook of Analysis and Quality Control of Fruit Vegetable Products*. Second edition, Tata McGraw Hill Pubishing Company Ltd., New Delhi, 594p.

Rao, N., Prabhakara, P. G., Balaswamy, K., and Rao, D. G. 2011. Preparation of instant tomato pickle mix and evaluation of its storage stability. *Int. Food Res. J.* 18: 583-587.

Rao, D. R., Chawan, C. B., and Pulsani, S. R. 1981. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterogenesis in rats. *J. Food Sci*. 46: 1339-1341.

Reddy, R. M. 2006. Value addition to minor millets by fungal fermentation (*Rhizopus oligosporus*). MSc. (Ag) thesis, University of Agricultural Sciences, Dharward, 43p.

Reddy, N. R. and Salunkhe, D. K. 2006. Effects of fermentation on phytate, phosphorus and mineral content in black gram, rice, and black gram and rice blends. *J. Food Sci.* 45(6): 1708-1712.

Reddy, N. R., Sathe, S. K., Pierson, M. D., and Salunkhe, D. K. 1982. *Idli,* an Indian fermented food: a review. *J. Food Qual.* 5(2): 89-101.

Reed, G. 1981. Use of microbial cultures: yeast products. *Food Technol.* 35: 89-94.

Rehman, Z. U. and Shah, W. H. 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. *Food Chem.* 91(2): 327-331.

Reyes-Moreno, C., Cuevas-Rodriguez, E. O., Milan-Carrillo, J., Cardenas-Valenzuela, O.G., and Barron-Hoyos, J. 2004. Solid state fermentation process for producing chickpea (*Cicer arietinum L*) tempeh flour. Physicochemical and nutritional characteristics of the product. *J. Sci. Food Agric*. 84: 271-278.

Roest, K. D. and Menghi, A. 2002. Reconsidering 'Traditional' food: the case of parmigiano reggiano cheese. *Eur. Soc. Rural Sociol.* 40(4): 439 – 451.

Rosado, J. L., Cassís, L., Solano, L., and Duarte-Vázquez, M. A. 2005. Nutrient addition to corn masa flour: effect on corn flour stability, nutrient loss, and acceptability of fortified corn tortillas. *Food Nutr. Bull.* 26(3): 266-272.

Rose, A. H. 1983. *Economic Microbiology*. Academic press, London, 228p.

Ross, R. P., Fitzgerald, G., Collins, K., and Stanton, C. 2002. Cheese delivering biocultures - probiotic cheese. *Aus. J Dairy Technol.* 57: 71–78.

Rowland, I. 2004. Probiotics and colorectal cancer risk. *Br. J. Nutr.* 91: 805–807.

Sachie, I., Yoshida, K., Tsurushin, S., Furusho, T., and Otobe, K. 2009. Antihypertensive effects of *natto*, a traditional Japanese fermented food, in spontaneously hypertensive rats. *Food Sci. Technol. Res*. 15(2): 199-202.

Sadasivam, S. and Manikam, A. 1992. *Biochemical Methods for Agriculture Sciences*. Eighth edition. Wiley Eastern Ltd., New Delhi and Tamil Nadu Agricultural University, Coimbatore, 422p.

Salminen. S., Von–Wright, A., Morelli, L., Marteau, P., Brassart, D., Fonden, R., Saxelin, M., Collins, K., Mogensen, G., Birkeland, S. E., and Mattila-Sandholm, T. 1998. Demonstration of safety of probiotics: A review. *Int. J. Food Microbiol.* 44(2): 93-106.

Samson, R. A. 1985. *Taxonomic Considerations of Beneficial and Harmful Moulds in Food.* Japan Scientific Society Press, Tokyo, 433p.

Samson, R. A., Kooij, J. A. V., and Boer, E. D. 1987. Microbiological quality of commercial tempeh in the Netherlands. *J. Food Prot.* 50: 92-94.

Sanna, M. G., Mangia, N.P., Garau, G., Murgia, M. A., Massa, T., Franco, A., and Deiana, P. 2005. Selection of folate-producing lactic acid bacteria for improving fermented goat milk. *Ital. J. Food Sci.* 17: 143-154.

Saono, S., Karsono, H., and D. Suseno, D. 1976. *Tempe. Ann. Microbiol*. 6: 83-95.

Satterlee, L. D., Marshall, H. F., and Tennyson, J. M. 1979. *J. Am Soc. Cereal Chemists* 56: 103-104.

Saxelin, M., Rautelin, H., Salminen, S., and Makela, P. H. 1996. The safety of commercial products with viable *Lactobacillus* strains. *Infect. Dis. Clin. Pract.* 5(5): 331-335.

Semwal, A. D., Sharma, G. K., Patki, P. E., Padmashree, A., and Arya, S. S . 2001. Studies on development and storage stability of instant vegetable pulav Mix. *J. Food Sci. Technol.* 38(3): 343-345.

Scalabrini, P., Rossi, M., Spettoli, P. and Matteuzzi, D. 1998. Characterization of *Bifidobacterium* strains for use in soymilk fermentation. *Int. J. Food Microbiol.* 39: 213-219.

Schaafsma, G., Meulig, W. J. A., Van- Dokkum, W., and Bouley, C. 1998. Effects of a milk product, fermented by *Lactobacillus acidophilus* and with fructo-oligosaccarides added, on blood lipids in male volunteers. *Eur. J. Clin. Nutr.* 52: 436-440.

Schipper, M. A. A. and Stalpers, J. A. 1984. A revision of the genus *Rhizopus*. *Mycol.* 25: 1-34.

Sekar, S. and Mariappan, S. 2007. Traditional fermented products by Indian rural folks and IPR. *Ind. J. Tradit. Knowl*. 6(1): 111-120.

Seppo, L., Jauhiainen, T., Poussa, T., and Korpela, R. 2003. A fermented milk high in bioactive peptides has a blood pressure–lowering effect in hypertensive subjects. *Am.J. Clin. Nutr.* 77(2): 326-330.

Shah, N. P. 2007. Functional cultures and health benefits. *Int. Dairy J. Sci.* 17(11): 1262-1277.

Shah, N. P., Fedorak, R.N., and Jelen, D. 1992. Food consistency effects of Quarg in lactose absorption by lactose intolerant individuals. *Intl. Dairy J.* 2: 257-269.

Shahzad, N., Butt, M. S., Rehman, S., and Sharif. K. 2005. Chemical characteristics of various composite flours. *Int. J. Agri. Biol*. 7(1): 105-108.

Shambuyi, M., Beuchat, L. M., Hung, Y. L., and Nakayama, T. 1992. Evaluation of substrates and storage conditions for preparing and maintaining starter cultures for tempeh fermentation. *J. Food Sci*. 15(2): 77-85.

Sharada, D. 2004. Nutritional and health benefits of fermented foods. *Proceedings of the Biotechnological Approaches to Food Processing*; 20 January - 9 February, 2004; Hyderabad. Post Graduate and Research Centre, Acharya N.G. Ranga University, pp. 85-89.

Sharma, A. and Kapoor, A. C. 1996. Levels of antinutritional factors in pearl millet as affected by processing treatments and various types of fermentation. *Plant Food Hum. Nutr.* 49: 241-252.

Sharma, R. and Sarbhoy, A. K. 1984. Tempeh-a fermented food from soybean. *Curr. Sci.* 53: 325-326.

Sharon, C. L. 2003. Nutritional and organoleptic qualities of value added products from breadfruit (*Artocarpus altilis* (Park) Fosberg.). M.Sc. (Home Science) thesis, Kerala Agricultural University, Trichur, 55p.

Sharon, C. L. 2010. Standardisation and quality evaluation of banana based probiotic fermented food mixtures. Ph.D. (Home Science) thesis, Kerala Agricultural University, Trichur, 230p.

Shermak, M. A., Saavedra, J. M., Jackson, T. L., Huang, S. S., Baylers, T. M., and Perman, J. A. 1995. Effect of yoghurt on symptoms and kinetic of hydrogen production in lactose malabsorbing children. *Am. J. Clin. Nutr*. 62: 1003-1006.

Shimelis, E. A. and Rakshit, S. K. 2007. Effect of processing on antinutrients and *in vitro* protein digestibility of kidney bean (*Phaseolus vulgaris* L.) varieties grown in East Africa. *Food Chem*. 103(1): 161-172.

Shimosato, T., Kimura, T., Tohno, M., Iliev, I. D., Katoh, S., Ito, Y., Kawai, Y., Sasaki, T., Saito, T., and Kitazawa, H. 2006. Strong immunostimulatory activity of AT-oligodeoxynucleotide requires a six-base loop with a self-stabilized 5 '-C. G-3 ' stem structure. *Cell. Microbiol.* 8: 485-495.

Shortt, C. 1998. Living it up for dinner. *Chem. Ind.* 8: 300-303.

Shurtleff, W. and Aoyagi, A. 2001. *The Book of Tempeh*. Ten Speed Press. Berk, California, 172p.

Silverstein, D. 2002. Usual dietary isoflavone intake, bone mineral density, and bone metabolism in postmenopausal women. *J. Womens Health Gen. Based Med*. 11(1): 69-78.

Sindhu, S. C. and Khetarpaul, N. 2002. Effect of probiotic fermentation on antinutrients and *in vitro* protein and starch digestibilities of indigenously developed RWGT food mixture. *Nutr. Heallth.* 16: 173-181.

Sindhu, S. C., Khetarpaul, N., and Sindhu, A. 2005. Effect of probiotic fermentation on carbohydrate and mineral profile of an indigenously developed food blend. *Acta Alimentaria.* 34: 41-47.

Singh, B. R. and Sharma, V. D. 1999. Characterization of broad-spectrum klebocin produced by *Klebsiella pneumoniae* subspecies *aerogenes. Indian J. Comp. Microbial. Immunol. Infect. Dis*. 20: 116-120.

Solis, P. and Lemonnier, D. 1996. Induction of human cytokines by bacteria used in dairy foods. *Nutr. Res*. 13: 1127-1140.

Somoygi, N. 1952. Notes on sugar determination. *J. Biol. Chem*. 195: 19-23.

Sotomayor , C. 1999. Lentil starch content and its microscopical structure as influenced by natural fermentation. *Starch Stärke*. 51(5): 152–156**.**

Srapinkornburee, W., Tassanaudom, U., and Nipornra, S. 2009. Commercial development of red kidney bean tempeh. *J. Food Agric*. 2(3): 362-372.

Srilakshmi, B. 1999. *Food Science.* 2nd edition. New Age International Pvt. Ltd., New Delhi.

Srivastava, S., Thathola, A., and Batra, A. 2001. Development and nutritional evaluation of proso millet based convenience mix for infants and children. *J. Food Technol.* 38 (5): 480- 483.

Stanton, C. R., Ross, P., Fitzgerald, G. F., and Sinderen, D. V. 2005. Fermented functional foods based on probiotics and their biogenic metabolites. *Curr. Opinion Biotechnol.* 16(2): 198-203.

Stanton, R. W. 1985. *Food Fermentation in the Tropics*. Elsevier Applied Science Publishers, UK, 356p.

Steinkraus, K. H. 1996. *Handbook of Indigenous Fermented Foods*. Mercel Dekker Inc., New York, 408p.

Steinkraus, K. H. 2004. *Industrialization of Indigenous Fermented Foods*. Mercel Dekker, Inc., New York, 652p.

Steinkraus, K. H., Cullen,R. E., Pederson, C. S., Nellis, N. F., and Gavitt, B. K.. 1983. Indonesian tempeh and related fermentations. In: .Steinkraus, K. H., Cullen, R. E., Pederson, C. S. , Nellis, L. F. and. Gavitt, B. K. .(eds.), *Handbook of Indigenous Fermented Foods*. Marcel Dekker. New York, pp. 19-94.

Steinkraus, K. H., Van Buren, J. P., Hackler, L. R., and Hand, D. B. 1965. A pilot-plant process for the Production of dehydrated tempeh. *Food Technol.* 19: 63-68.

Stien, G., Blanchard, F., Rondags, E., and Marc, I. 1999. An on-line method for the measurement of diacetyl and acetaldehyde in yoghurt, fermented milk, butter and margarine. *Lait.* 79: 615-624.

Sudermadji, S. and Suparmo, R. 1997. Re-inventing the hidden miracle of tempeh. In: Hedger, J.N. (ed.). *Proceedings of International Tempeh Symposium*; 13-15 July, Bali, pp. 54-61.

Sugihara, T. F. 1985. Microbiology of breadmaking. In: Wood, B. J. B. (ed.), *Microbiology of Fermented Foods* (9th ed.). Elsevier Applied Science Publishers, pp. 54-62.

Suparmo, D. 1989. Vitamin B-12 formation in tempeh fermented by mixed-culture. *Abs. Int.* 49: 4621-4622.

Sutardi, D. and Buckle, K. A. 2008. Phytic acid changes in soybeans fermented by traditional inoculum and six strains of *Rhizopus oligosporus. J. Appl. Microbiol.* 58(6): 539-543.

Swan, D. and Hesseltine, C. W. 1979. Tempe and related foods. *Econ. Microbiol.* 4: 115-140.

Tabera, J., Frias, J., Estrella, I., Villa, R., and Vidal-Valverde, C. 1995. Natural fermentation of lentils. Influence of time, concentration and temperature on protein content, trypsin inhibitor activity and phenolic compounds. *Lebensm Unters. Forsch.* 201 : 587-591.

Tamang J. P. 1998. *Sinki* a traditional lactic acid fermented product. *J. Gen. Appl. Microbiol*. 39: 395-405.

Taranto, M. P., Vera, J. L., Hugenholtz, J., De Valdez, G. F., and Sesma, F. 2003. *Lactobacillus reuteri* CRL1098 produces cobalamine. *J. Bacteriol.* 185: 5643-5647.

Tee, E. S., Ismail, M. N., Nasir, A., and Khatijah, I. 1997. Nutrient composition of Malaysian Foods (4th ed.). *Malaysian Food Composition Database Programme*. Institute for Medical Research, Kuala Lumpur 231p.

Thapa, N., Pal, J., and Tamang, J. P. 2004. Microbial diversity of *Nagri* – fermented fish product of South Eastern India. *Wld. J. Microbiol. Biotechnol*. 20: 599-607.

Thompson, T. L. and Marth, E. H. 1986. Changes in parmesan cheese during ripening: Microflora – coliforms, enterococci, anaerobes, propionibacteria and staphylococci. *Milchwiss.* 41: 201-204.

Tripathi, A. K. and Misra, A. K. 2005. Soybean – a consummate functional food: A review. *J. Food Sci. Technol*. 42(2): 111-119.

Tripathi, R. N. and Nath, N. 2002. Trypsin inhibitor in legume flour. *J. Food Sci. Technol*. 39(2): 85-86.

Urooj, A. and Puttaraj, S. 1994. Effect of processing on starch digestibility in some legumes – an *in vitro* study. *Nahrung.* 38: 38-46.

Vaidehi, M. P. 1993. *Tempeh - A Unique Food for Nutrition and Health Benefits.* Biotechnology in Foods. Series No.1, Agricultural University Press, Bangalore, 65p.

Vaidehi, M. P. and Rathnamani, A. 1990. The shelf-life of soy-sunflower tempeh and its acceptability to Indian children. *Food Nutr. Bull.* 12(1): 88-90.

Vaidehi, M.P., Annapurna, M.L., and Viswanath, N.R. 1985. Nutritional and sensory evaluation of tempeh products made with soybean, groundnut and sunflower seed combinations. *Food Nutr. Bull.* 7(1): 54-57.

Vaidehi, M. P., Sumangala, S. G., and Vijayakumari, J. 1996. Tempe-based ready-to-prepare food mixes of high nutritional value. *J. Food Sci. Technol.* 32: 1122-1155.

Vaishali, V. A., Medha, K. G., and Shashi, A. C. 1997. Effect of natural fermentation on in vitro bioavailability in cereal-legume mixtures. *Int. J. Food Sci. Technol.* 32: 29-32.

Valverde, C., Prodanov, M., and Sierra, I. 1997. Natural fermentation of lentils. Influence of time, temperature and flour concentration on the kinetics of thiamine, riboflavin and niacin*. Z. Lebensm. Unters. Forsch.* 205: 464-469.

Vandenplas, Y., Salvatore, S., Viera, T., Devreker, W., and Hauser, B. 2007. Probiotics in infectious diarrhoea in children: are they indicated? *Eur. J. Peadiatr.* 166(12): 1211-1218.

Vattem, D. A., Lin, Y. T., Labbe, R. G., and Shetty, K. 2004. Antimicrobial activity against select food-borne pathogens by phenolic antioxidants enriched in cranberry pomace by solid-state bioprocessing using the food grade fungus *Rhizopus oligosporus*. *Process Biochem.* 39: 1939-1946.

Verma, J. and David, I. M. 1981. Modification of tempeh with the addition of bakla (*Vicia faba* Linn). *Int. J. Food Sci. Technol*. 16 (1): 39-50.

Wanink, J. F., Van-liet, T., and Nout, M. J. R. 1994. Effect of roasting and fermentation on viscosity of cereal-legume based food formulas. *Plant Food Hum. Nutr.* 46(2): 117-126.

Watanabe, F. 2007. Vitamin B12 Sources and bioavailability. *Exp. Biol. Med.* 232:1266-1274.

Wang, H. L. and Hesseltine, C. W. 1981. Protein quality of wheat and soybeans after *Rhizopus oligosporus* fermentation. *J. Nutr*. 96: 109-114.

Wang, H. L., Swain, W., and Hesseltine, C. W. 1975. Mass production of *Rhizopus oligosporus* spores and their application in tempeh fermentation. *J. Food Sci*. 15(2): 77-85.

Watanabe, N., Yasushi, E., Kenshiro F., and Aoki, H. 2006. Tempeh-like fermented soybean (GABA-tempeh) has an effective influence on lipid metabolism in rats. *J. Oleo Sci.* 55(8): 391 – 396.

Wee, Y. J., Kim, J. N. and Ryu, H. W. 2006. Biotechnological production of lactic acid and its recent applications. *Food Technol. Biotechnol.* 44: 163-172.

Wei, S. I., Hsiung, L. M., and Ying, H. 2005. Ankaflavin from monascus-fermented red rice exhibits selective cytotoxic effect and induces cell death on hep G2 cells. *J. Agri. Food Chem.* 53: 1949-1954.

Wiesel, I., Rehm, H. J., and Bisping, B. 1997. Improvement of *tempe* fermentations by application of mixed cultures consisting of *Rhizopus sp*. and bacterial strains. *Appl. Microbiol. Biotechnol*. 47: 218-225.

Wokoma, E. C. and Aziagba, G. C. 2001. Sensory evaluation of *Dawa Dawa* produced by the traditional fermentation of African yam bean (*Sphenostylis Stenocarpa* Harms) seeds. *J. Appl. Sci. Environ. Manag*. 5(1): 85-91.

Yamamoto, N. and Takano, T. 1999. Antihypertensive peptides derived from milk proteins. *Nahrung* 43: 159–164.

Yan, L. and Spitznagel, E. L. 2009. Soy consumption and prostrate cancer risk in men – a revised meta analysis. *Am. J. Clin. Nutr.* 89(4): 1155-1163.

Yokotsuka, T. 1985. Fermented Protein Foods in the Orient, with Emphasis on *Shoyu* and *Miso* in Japan. In: Wood, B.J.B (ed.), *Microbiology of Fermented Foods*. Elsevier Applied Science Publishers, UK, pp. 89-112.

Yonzan, T. and Tamang, J. P. 1998. Consumption pattern of traditional fermented foods. *J. Hill Res.* 11: 112-115.

Yousif, N. E. 2001. Effect of fermentation on protein fractions in food grains and *in vitro* protein digestibility. *Plant Food Hum. Nutr.* 56: 175-182.

Yueh, M. H., George V., Fred R. J., and Herman, M. W. 1972. Process for producing a fried snack food containing tempeh, US Patent No. 4151307.

APPENDIX - I

Tempeh roast was prepared by the standard procedure as suggested by Philip (1993).

Recipe for the preparation of tempeh roast

Ingredients

Tempeh cut into cubes (replacing meat)	: 250gm
Coriander powder	: 10gm
Chilli powder	: 5gm
Turmeric powder	: 2.5gm
Big onions	: 3 numbers
Green gram	: 4 numbers
Ginger	: 4 cm piece
Garlic	: 1 pod
Aniseed	: 5gm
Tomato	: 2 numbers
Garam masala powder	: 5gm
Coriander leaves	: 20gm
Oil	: 50gm
Sugar	: 5gm
Salt	: As required
Lime	: ½ lime

Procedure

Shallow fried the tempeh cubes in half the quantity of oil and kept aside. Green chillies, ginger, garlic, coriander powder and aniseed were ground to a fine paste. Sautéed sliced onion for three minutes and added the ground ingredients, chilli powder, *garam masala* powder and turmeric powder and fried for two minutes. Sliced tomatoes were added and sautéed for five minutes. To that fried tempeh, salt and 100 ml of water was added and cooked in an open pan till the moisture was completely absorbed. Finally added sugar, lime juice and coriander leaves.

APPENDIX - II

Score card for organoleptic evaluation of tempeh chips

Characteristics	Score		
	1	2	3
Appearance			
Taste			
Flavour			
Colour			
Texture			
Overall acceptability			

9 point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Name of the judge: **Signature:**

Date:

APPENDIX - III

Score card for organoleptic evaluation of tempeh roast

Characteristics	Score		
	1	2	3
Appearance			
Taste			
Flavour			
Colour			
Texture			
Overall acceptability			

9 point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Name of the judge: **Signature:**

Date:

APPENDIX - IV

Score card for organoleptic evaluation of soups prepared with tempeh based instant soup mixes

Characteristics	Score		
	1	2	3
Appearance			
Taste			
Flavour			
Colour			
Consistency			
Overall acceptability			

9 point Hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Name of the judge: **Signature:**

Date:

STANDARDISATION AND QUALITY EVALUATION OF 'TEMPEH' AND TEMPEH BASED INSTANT SOUP MIXES

By

LAKSHMY. P. S.

ABSTRACT OF THE THESIS

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ABSTRACT

The study entitled "Standardisation and quality evaluation of 'Tempeh' and tempeh based instant soup mixes" was undertaken with the objectives of standardising the fermented food tempeh with green gram, cowpea, soybean, rice and wheat, and to evaluate its quality attributes. The study also aims to evaluate the nutritional and shelf life qualities of tempeh flour, to develop instant soup mixes with tempeh flour and quality evaluation of these products.

Tempeh fermentations were carried out with pure cultures of *Rhizopus oligosporus* – MTCC 556 and twenty different combinations of selected legumes and cereals were used as substrates. Among the different fresh tempeh types prepared, T_1 (100% soybean) had good appearance and texture. The treatments with green gram had a comparable texture and appearance with the control (T_1). Tempeh types with rice had better appearance and texture than that of wheat.

The overall acceptability of tempeh chips prepared with different combinations of fresh tempeh types ranged from 6.9 to 8.9 with the highest overall acceptability score for T_2 (100% green gram) and the highest overall acceptability of roast ranged from 7.5 to 8.9 with the highest score for T_{11} (cowpea 50% + rice 50%). The highest IVSD of 82.83 per cent was for T_9 (green gram 50% + rice 50%) and the highest IVPD of 88.98 per cent was for T_8 (green gram 75% + rice 25%).

Fresh tempeh types T_2 (green gram 100%), T_8 (green gram 75% + rice 25%), and T_9 (green gram 50% + rice 50%) were selected based on their acceptability, IVSD and IVPD for further studies. T_1 (100% soybean) was selected as control.

Among the selected fresh tempeh types, the treatment T_1 (control) had the maximum moisture (55.85%), protein (21.09%), total fats (10.12 g/100g), β carotene (331.85 µg/100g), thiamine (0.31 mg/100g), calcium (149.10 mg/100g), iron (3.6 mg/100g), phosphorus (270.61 mg/100g) and zinc (2.17 mg/100g) content, whereas the treatment T_2 (100% green gram) had the highest fiber (2.52 g/100g), reducing sugar (2.01 g/100g), total sugar (4.21 g/100g) riboflavin (0.30 mg/100g) and potassium (525.11 mg/100g) content. Starch content was highest (29.13

g/100g) in T_9 (green gram 50% + rice 50%). None of the selected fresh tempeh types had vitamin C. Mineral availability was found to be very high in the three tempeh types (T_2, T_8 and T_9) compared to the control (T_1). The availability of calcium (61.77%), iron (66.82%), phosphorus (65.72%), potassium (63.60%) and zinc (88.99%) was highest in T_9 (green gram 50% + rice 50%).

None of the tempeh types showed viability at low pH (1.5 to 2.5), bile acid tolerance (1-4 % level) or antibacterial activity against enteropathogens and hence no probiotic activity can be attributed to any of the selected fresh tempeh types.

The appearance, colour, flavour and texture of the fresh tempeh types stored under refrigerated condition were satisfactory up to 12 days for T_1 (100% soybean tempeh) and T_2 (100% green gram). In T_8 (green gram 75% + rice 25%) and T_9 (green gram 50% + rice 50%), the shelf life was only up to 6 days of refrigerated storage. Frozen tempeh types were comparable to the fresh tempeh even after 30th day of storage.

A gradual reduction in the overall acceptability score of the chips and roasts was observed with the stored tempeh types. The bacterial load in the fresh tempeh was found to be high, in the range of 49.3 to 69.3 x 10^8 cfu/g. A gradual decrease in the bacterial count was observed in all the treatments up to the 6th day of refrigerated storage and thereafter there was a slight increase in the bacterial count. No pathogenic bacteria were identified in fresh tempeh types. The total yeast count in the fresh tempeh types was in the range of 42.6 to 66.3 x10^5 cfu/g with the maximum in T_9 (green gram 50% + rice 50%). A decrease in the yeast count was observed on storage in T_1 and T_2, but in T_8 and T_9, there was an increase in the total yeast count on the 9th and 12th day of refrigerated storage which resulted in the spoilage of these tempeh types. The only identified fungus in fresh tempeh types were *Rhizopus oligosporus*. The total fungal count was in the range of 2.6 to 5.3 x 10^6 cfu/g in fresh tempeh types which was reduced to 1.0 to 2.3 x 10^6 cfu/g on the 12th day of refrigerated storage. In frozen tempeh types, the bacterial count was reduced to 0.17 to 0.49 x 10^8 cfu/g, yeast count to 0.46 to 0.86 x 10^5 cfu/g and fungal count to 0.06 to 0.1 x 10^6 cfu/g after 30 days of storage.

The flours prepared from the tempeh types were packed in metalised polyester laminate pouches and stored for a period of six months under ambient conditions. The tempeh flours were evaluated for its quality and shelf life attributes. The tempeh flour prepared with T_1 had the highest protein (43.15 g/100g), total fats (20.87 g/100g), calcium (331.25 mg/100g), iron (8.03 mg/100g), phosphorus (601.36 mg/100g) and zinc (4.82 mg/100g). Maximum fiber (3.30 g/100g), riboflavin (0.03 mg/100g) and potassium (979.68 mg/100g) were observed in tempeh flour prepared with $T_{2.}$ Starch content was found to be highest (49.38 g/100g) in T_9 (green gram 50% + rice 50%). Thiamine was not detected in any of the tempeh flours. A decrease in the protein, total fats, riboflavin, and minerals was observed on storage but were not significant.

The highest IVPD of 90.86 per cent after storage was for $T_{8\,(}$green gram 75% + rice 25%) and highest IVSD of 83.86 per cent was for T_9 (green gram 50% + rice 50%).

All the tempeh flours remained within the prescribed limit of microbial load making them microbiologically safe even after six months of storage. Insect infestation was not observed in tempeh flours during storage.

Tempeh flours were used for standardising instant soup mixes with suitable blending materials. For each of the selected tempeh flour (T_1, T_2, T_8 and T_9), four different compositions of soup mixes viz. $S_{1(50)}$, $S_{2(55)}$, $S_{3(60)}$, $S_{4(65)}$ were developed, the number in brackets indicating the percentage of tempeh flour used.

The soup mixes (16 numbers) were packed in metalised polyester laminate pouches and stored for a period of six months under ambient conditions. The soup mixes were evaluated for quality and shelf life attributes.

After storage, the moisture content of soup mixes varied from 6.53 to 7.53 per cent. The nutrients ranged from 5.53 to 34.65 g/100g (protein), 16.67 to 51.45 g/100g (starch), 0.40 to 2.83 g/100g (fiber), 0.67 to 17.93 g/100g (total fats), 0.00 to 0.028 mg/100g (riboflavin), 39.96 to 298.60 mg/100g (calcium), 1.12 to 5.17 mg/100g (iron), 227.61 to 551.66 mg/100g (phosphorus), 432.31 to 961.51 mg/100g (potassium), and 2.11 to 4.42 mg/100g (zinc) in different compositions of soup mixes.

Water required for cooking the instant soup mixes to a soup of acceptable consistency varied from 1280 to 1530 ml/100g and the cooking time of the instant soup mixes varied from 3-6 minutes. The mean score for overall acceptability of the soups prepared with the stored soup mixes ranged from 7.5 to 8.2. The overall acceptability score of the soups were lowest in compositions $S_{4(65)}$ with all the tempeh flours.

The microbial load in all the soup mixes after storage was within the prescribed limit and all the soup mixes can be considered as microbiologically safe even after storage of six months. Insect infestation was not observed in any of the soup mixes.

The cost of production of 1 kg of fresh tempeh types varied from Rs. 57.00 to Rs. 68.00, 1 kg tempeh flour varied from Rs. 111.00 to 142.00 and that of soup mixes (50g) varied from Rs. 12.00 to 13.00.