STANDARDIZATION OF GREEN GRAM BASED MEAT ANALOGUES

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Home Science

(FOOD SCIENCE AND NUTRITION)

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2008

DECLARATION

I, hereby declare that this thesis entitled **"Standardization of green gram based meat analogues"** 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.

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ACKNOWLEDGEMENT

I humbly bow my head before the lord Almighty who blessed me with the power and courage to complete this endeavour successfully in spite of the most difficult times faced by me during the period of my study.

With deep respect I express my whole hearted gratitude and unforgettable indebtedness to Dr. V. Usha, Professor and Head, Dept. of Home Science and the chairperson of my advisory committee for her inimitable guidance, valuable suggestions, constructive criticism, unceasing interest, constant encouragement and above all the extreme patience, understanding and whole hearted co-operation rendered throughout the course of the study. I consider myself being fortunate in having the privilege of being guided by her.

I owe a great debt of gratitude to Dr. V. Indira, Professor, Dept. of Home Science and member of my advisory committee for her unwavering support during my academic career.

I am grateful to Dr. Sheela K.B., Professor, Dept. of Processing Technology and member of my advisory committee for her constructive suggestions and ever willing help rendered throughout this venture.

I am extremely thankful to Sri. S. Krishnan, Assistant Professor, Dept. of Agricultural Statistics and member of my advisory committee for his meticulous guidance and valuable suggestions given during the analysis of data.

I avail this opportunity to thank my loving friends Sangeeeth, Hemalata, Renu, Rashmi, Swapna, Deepa, Shiji, and Kimi for making my M.Sc. programme meaningful.

It is my pleasant privilege to express my utmost gratitude to all my seniors and juniors who had really helped me in every way. I am also thankful to Ms. Umaiba for her timely help and co-operation while working in the laboratory.

Words cannot really express the true friendship that I enjoyed with my friends whose mere being gave me the strength to move on. I'm really indebted to Nidhi, Ramya, Shifa, Neema, Swapna lata, Maalathi, Uma, Anu, Sweta, Ratish, Arun, Madan and Sudhakar. The relation I *shared and the help I received from Sambodh, Nina, Shashi, Nitu, Trilok and Navin is something which cannot be expressed in words.*

My profound thanks to Mr.Santhosh for all the help rendered during the preparation of the thesis. I also deeply thank those whose names have not been specified over here but who have helped me in accomplishing this task successfully.

Above all, I express my unreserved gratitude to my most beloved father, mother and my whole extended family members for their affection and encouragement throughout the study. I am forever indebted to the lady who showed me this world, who keeps on bestowing blessings in every path of my life.

Nisha.

Nisha

Dedicated to
My Beloved
Family

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Abbreviation

AOAC	Association of Official Analytical Chemists
^0C	Degree centigrade
cfu	Colony forming unit
cm	Centimeter
CRD	Complete Randomised Design
FAO	Food and Agricultural Organisation
g	Gram
mg	Milligram
ml	Milliliter
mm	Millimeter
nm	Nanometer
%	Percentage
ppm	Parts per million
WHO	World Health Organisation

Introduction

1. INTRODUCTION

India is well-known for its tradition of vegetarianism which has a history spanning more than two millenia. It has also been claimed that the Indian diet is still recognizably different from that of other cultures. The claim is based on the structure of the main meal and its food combinations. Vegetarianism was mainly seen among the people, mainly Hindus which changed over time. This change in the food habits was owing to several factors such as urbanization, increased purchasing power and imitating the western culture (which is primarily non vegetarian).

There has been a remarkable increase in the consumption of animal products in developing countries, although the levels are still well below the levels of consumption in most other industrialized countries. Diets in developing countries have changed as income increased. Increase in income in developing countries, is driving strong growth in *per capita* and total meat consumption. In 1961–94, the total and *per capita* meat consumption in the developing countries accelerated. The share of staples, such as cereals, roots and tubers, are declining, while that of meat, dairy products and oil crops are rising. Between 1964-1966 and 1997-1999, the per capita meat consumption in developing countries rose by 150 per cent. The domestic demand for meat is huge and the Indian domestic market is one of the biggest in the world (FAO, 2002a).

Indian states and regions are diverse in terms of economic factors affecting food demand, including population, income, and urbanization. High income and urbanization in the South are supportive of the region's rapid gains in meat demand, supply, and commercialization. Over 95 per cent of Keralites are meat consumers. The per capita consumption of meat is the highest in Kerala. There has been a switch in food preferences towards non-cereal items such as meat/fish in both rural and urban areas.

The changes in the food consumption patterns brought about by the people, are apparently contributing to the changing picture with respect to prevalence of degenerative diseases such as coronary heart disease and Type II diabetes. Meat can also transmit certain parasitic diseases such as trichinosis. Meat and meat products may also contribute to the hetero-cyclic amines and other carcinogenic substances, which are potent health hazards. The most common diseases caused due to meat consumption are heart diseases, high blood pressure, kidney problems, gall bladder problems, hypercholesterolaemia, wounds in arteries, eczema, paralysis, tuberculosis, constipation, pains, arthritis, hysteria etc. (Gupta, 2006).

Meat is generally eaten for its high protein and micronutrient content. In addition the taste, texture, flavour, feel and enjoyment it gives, are the other factors which motivate one to go for meat. Looking towards the negative aspects of meat, substitutes have been developed which could mimic meat for the traits it is preferred and at the same time overcomes all its negative aspects. A meat analogue is an engineered or fabricated protein food product (Ensminger and Ensminger, 1994). At the first glance, a meat substitute gives a clear focus of a food product designed to replace meat (Kuntz, 1995). Meat analogues or imitation meat typically refers to any vegetarian food product, designed to imitate the texture and flavour of processed meat. It also refers to meat produced by mincing a low quality protein to form an imitation of higher quality meat (Anon, 2004).

Meat analogues generally use naturally occurring plant ingredients. Earlier these products were developed for the consumers who avoided meat, owing to a variety of reasons like the ethical, social and value issues and obtained all their protein from other sources. These products were mainly designed to mimic the nutritional qualities of meat. But with the passage of time, as the people came to know about the harmful or side effects of consuming too much meat, the number of consumers for the meat analogues or the simulated meat products increased enormously. The more people became health conscious, the more they turned towards the products like this.

A number of attempts have thus been made in the past to provide meat substitutes. Accordingly, a clear need exists for a simple and economic process of obtaining synthetic food products resembling meat and fish, and exhibiting satisfactory flavor, appearance and textural characteristics. The appeal of imitation meat, poultry and fish analogue is partially due to their resemblance to the natural foods which are widely accepted as premium quality foods. Meat analogues have the additional advantage over meat that, the amount and type of dietary fat can be controlled and cholesterol can be excluded. Such products also have special appeal to consumers who do not eat meat or fish because of religious, health or philosophical reasons. Furthermore, meat and fish analogues can offer cost savings compared to the natural products, because plant protein can be produced much more efficiently than animal protein.

The extended products provide a mixture of proteins and other nutrients to the consumers, which are desirable from nutrition point of view, and it also satisfy the consumer's desire for meat particularly when they cannot afford the costly meat. This is also an effective way to utilize other agricultural produce. The number of extenders could be very large and it was felt necessary to find an effective method to select functionally compatible ones (Modi and Prakash, 2008).

People who now look forward for the meat analogues include not only the vegetarians but also the non-vegetarians. These included the consumers who loved the taste and texture of the meat and meat products and desired the analogues to have a similar taste and texture, with added health benefits.

Moreover, the relatively high cost of high protein foods, such as meat and fish and their scarcity in major areas of the world, have long constituted economic and nutritional problems. Protein malnutrition is prevalent in the underdeveloped and overpopulated areas of the world and the situation is forecasted to become progressively worse. Hence through product development and innovations, food scientists and food technologists are trying to produce vegetarian meat alternative products, which meet the expectations of not only the vegetarians but are also convincing to the meat eaters and at the same time, make it cost effective in terms of both production aspects and health benefits when compared to its meat counterparts.

There is a need to develop a product which has the nutritional and sensory qualities of meat and at the same time is cost effective and health beneficial with no side effects as that of meat and meat products. So, the present study entitled "Standardization of green gram based meat analogues" was undertaken with the following objectives.

1. To standardize meat analogues with green gram blended with soya and wheat.
2. To evaluate the quality attributes of meat analogues.
3. To evaluate the storage stability of the selected products.

Review of Literature

2. REVIEW OF LITERATURE

The relevant literature on the study entitled "Standardization of green gram based meat analogues" has been briefly reviewed here under the following subtitles.

2.1. Development of meat analogues and its constituents

2.2. Physical characteristics of meat analogues

2.3. Nutritive value of meat analogues

2.4. Negative effects of meat consumption on human health

2.5. Consumers of meat analogues

2.6. Acceptability of meat analogues

2.7. Health benefits of consuming meat analogues over meat.

2.1 Development of meat analogues and its constituents

An analogue is a compound similar in structure to another compound but differing in some slight structural details. It is something with a particular similarity in particular circumstances to something else, so that a comparison can be made. A meat analogue is a food product which is designed as a traditional animal meat protein. Meat analogue consists of those products that are primarily of vegetable origin but are meant to imitate meat or meat based products (Khader, 2001).

Generally, meat analogue is understood to mean a food made from non meat, sometimes with dairy products. Meat analogue may also refer to meat based healthier and / or less expensive alternatives to meat products (Raiz, 2006). It is a manufactured food product that looks and tastes like meat (Flynt, 2007). Meat analogue is an industrial term for meat substitute or synthetic meat made principally from plant protein (Wrick, 2007). A meat analogue, also called a meat substitute, mock meat, faux meat, imitation meat or veat, approximates the aesthetic qualities primarily texture, flavour, appearance and / or chemical characteristics of certain types of meat.

Steinkraus (1978) says that there has been an interesting trend in the west which included development of meat analogue mainly from soya. Since 1955, the pioneering work of Hartman and Robert, blended the protein from soya, wheat, yeast and egg albumin resulting in a product for Worthington Food Company (Thomas, 1979). The earliest meat analogue was developed by John Harvey Kellogg and presented to his patients in Battle Creek, Michnigan sanitarium, as early as 1898. These products were based exclusively on wheat gluten which was obtained by washing starch from high protein wheat flour (Khader, 2001).

Designing meat substitute involved countless options like marketing, nutritional and technical aspects. These considerations greatly influenced the formulation processes (Kuntz, 1995). Using proper ingredients in an analogue, mimics the texture. Analogues often require a combination of different protein sources for mouth feel and functionality and the ingredient choices are varied (Kobs, 1999). Plant protein can be obtained from a variety of sources but today vegetarian entrees rely mostly on soya and wheat derivatives to provide the protein matrix (Klahorst, 2001). Hegenbert (2002) also reported that various proteins are often found in meat analogue and in many combinations, but soya and wheat protein are the two that are commonly used.

Looking back 20 – 30 years ago, vegetarian convenience foods were few and were mainly based on texturized soya protein and nuts (Sadler, 2004). To recreate particularly realistic meat texture, formulations often used both wheat and soya protein. This is because, a laminar protein structure based solely on wheat can sometimes go from pleasant meaty to leathery. Seeding that structure with hydrated soya protein gels, balances wheat's tendency towards toughness and towards soy's tenderizing effects. This approach hits the target even better when soya protein is associated with a certain degree of fibre. More fibre means more disruption of wheat protein network and thus more softening (Decker, 2004).

Since products based on soya protein cannot fulfill the need for texture, mouthfeel and flavour, technologists are now focusing on the use of integrated textured particles made from combinations like wheat gluten, pea protein, soya protein etc. in the development of meat analogues (Bardic, 2006).

Flynt (2007) also reported that soya, wheat gluten, beans and / or nuts are used as the main protein source with other ingredients to provide texture and a meat like taste. Manipulation of the proportion of different protein ingredients such as soya, gluten etc. and the condition of temperature, pressure and moisture level determines the final product character. The growing market potential has stimulated the development of wide range of ingredients delivering improved taste and eating quality that simulates the texture of meat and meat products. These are based on wider range of ingredients such as soya, wheat derivatives, pulses, vegetables and mycoprotein (Wrick, 2007).

Bhatt (1985) says that soyabean is the best source of protein from vegetable kingdom and it is an established fact that soyabean contains more protein when compared to meat, egg, dhal and grams.

De Man (1990) suggested that soya protein is a major important component to fabricate the structure of meat analogue. The protein value of soya protein is comparable to that of meat protein. Bean (1993) indicated that soyabean can be effectively used as a protein supplement in the place of meat.

Roussel (1996) agrees that soya is capable of producing a wide range of cooked foods with high fibrous texture simulating meat, poultry and fish muscle.

Vittadini and Vodovotz (2003) revealed that soya is a popular component of Asian diet and has been successfully incorporated in various products including chicken – meat analogue. Texturized vegetable protein is made up of good quality soya flour which can be used to replace meat in a variety of recipe (Itapu, 2003). Fibrous vegetable protein is a soya based preparation. It is designed to simulate meat muscle thus providing a different eating texture to soya formulates. It is also suitable for the use in products such as burgers, schnitzels sausages, cold cuts and beef style, chicken style ingredients for home cooking (Sadler, 2003).

The 1990's FAO/WHO Protein Evaluation Committee put soya protein at par with egg and milk protein and ahead of beef protein (Itapu, 2004). Thus, it has been variously called "the miracle bean", "golden bean" "nugget of nutrition", "the meat that grows on vines", "protein hopes of future" etc. indicating its value and importance in human diet (Sharma and Kalia, 2005).

Soya is found most commonly as tofu, but other common sources include soya flour, soya nuts, soya milk, soyabean meat analogue products like sausages, burgers, franks and all of which are intended as substitutes for animal based counterparts

(Behar, 2006). It may also lower LDL cholesterol when it replaces dairy protein or a mixture of animal protein (Hoie *et. al.*, 2007).

Thermo – irreversible starch gels are also used in the preparation of meat analogue. Thermo – irreversible starch gels are gelling agents comprising of starch, a polymer having repeated carbohydrate units which occurs in abundance in many plants. These may be obtained from rice starch, wheat starch, corn starch etc. These when included in the preparation, provides lubricity and body to the food with characteristic juicy and tender texture commonly associated with meat foods having normal fat content (Lai, 1997).

Analogue currently marketed are primarily blends of soya and wheat protein. The technology which has been developed to-date is based on soya or soya – wheat combinations (Khader, 2001).

According to Kuntz (1995) wheat gluten is very effective in the role of a binding agent to make and keep products in a homogeneous mass and to be used in meat substitutes for replicating the texture of meat. Alexander (1997) reported that faux meat products are made from wheat gluten wherein the gluten's stretchy texture is more easily transformed into the chewiness of meat. When wheat gluten is used in food processing, it has the ability to add specific textural properties, making it a valuable ingredient in meat free diet (Lai, 1997).

Texturized wheat protein concentrates make excellent meat analogue. Wheat gluten products provide a porous cellular structure and tissue like striations. It becomes a soft meat like structure and has a low flavour profile. Wheat gluten is also used in analogues functioning as a binder for other protein sources and helps in improving texture and firmness (Kobs, 1999).

Midwest Grain Products, introduced texturized protein from wheat gluten and the texturized product looked and felt like meat due to a fibrous structure. So this can be adopted to mimic the looks and texture of beef, chicken, pork or fish (Klahorst, 2001). Wheat protein is essentially made up of gluten that has been processed and extruded to resemble the texture of meat (Sadler, 2003).

The high protein content and unique structural and adhesive characteristics of wheat gluten makes it a useful additive. The gluten based products ends up being more like meat (Kaur *et. al.*, 2004).

Inclusion of gluten results in a chewier product. When cooked in a broth, gluten absorbs some surrounding liquid and becomes firm to bite so is widely used in vegetarian, vegan and Buddhist cuisine as a meat substitute. In China, wheat gluten is the basis for imitation meat resembling chicken, fish, duck, pork and beef (Wrick, 2007).

2.2. Physical characteristics of meat analogues

Appearance, tenderness, juiciness and flavour of the meat decide the quality of meat. Flesh must be firm, the fescicute should be small and velvety in texture with the characteristic colour of the meat (Begum, 1991). Meat and meat products generally include skeletal muscles of animals which is composed of bundles of hair like muscle fibres. A major protein of muscle fibre is myosin. Fat penetrates the muscle fibre and this is called marbling which makes the fibre more tender (Srilakshmi, 2003). There are two major aspects of meat quality – nutritional quality which is objective and flavour, juiciness, tenderness and colour – which is highly subjective (Bhatt and Tomar, 2006).

Meat analogue is a major type of texturized plant protein which extensively imitates meat products (Sheard *et. al.*, 1984). Kuntz (1995) reported that in meat analogues, there is a need for the protein that produces a similar texture to that of meat. Many such products are formed from the combination of wheat and soya (Alexander, 1997).

For processed meat such as minced meat food, the mouth feel of the fat relates not only to the melting and juiciness characteristic of lard or tallow but also to the underlying adipose or fat tissue texture. Fat tissue texture is non liquid, smooth, springy and some what slippery and chewy at warm temperature. Numerous attempts have been made to mimic the physical attributes of fat in non meat food products. While these attempts have been reported to improve the delivery of juiciness at serving, they have failed to mimic the unique and appealing texture of fat tissue (Lai, 1997).

One of the important considerations in developing a meat analogue is that it should have many of the qualities of the product it is replacing (Kuntz, 1995). The technology is becoming quite accomplished at creating realistic analogues that equals their meat counterparts in terms of flavour, texture and most important, satiety (Kobs, 1999). He observed that soya and wheat protein in meat analogue provided the texture, mouth feel, chew and experience similar to that of ground beef. According to Kobs (1999) texture and flavour are the biggest challenges in developing a meat analogue.

Knox (2000) felt that meat analogues resembled in looks, taste and mouth feel to meat and can be used to replace meat in recipes. Khader (2001) also agreed that the greatest challenge to the food technologists in the design of meat analogues is in the area of taste and texture. Manufacture of meat analogue parallels very closely to those of processed meat. He observed that when raw materials are of vegetable origin they are extremely difficult to flavour. The most valued functional characteristic such as water retention, emulsification and texture enhancement through gelation are critically important to provide appearance of meat analogue, allowing it to achieve the mouth feel and juiciness of cooked meat products (Klahorst, 2001).

Decker (2004) opined that some soya based meat alternatives replicates the texture and appearance of whole meat. Admin (2007) agreed to this fact and suggested that meat alternatives are very tasty and many have a similar structure to meat. He also reported that some of these products are indistinguishable from real meat.

Meat analogues are manufactured to look like meat and closely approximate its nutritional and sensory qualities primarily texture, flavour and appearance (Wrick, 2007). The chewy texture refers to and includes the physical characteristic of protein material which gives a feeling of resilience, elasticity and resistance to shear when chewed. The quality and quantity of soya protein are important factors that affect the chemical and physical characteristics of meat analogues (Rareunrom *et. al.*, 2007).

2.3. Nutritive value of meat analogues

Meat protein is often considered as being nutritionally superior to plant protein with respect to protein quality but the difference is not very great (Young and Serimshav, 1974). Meat, including the organ meat are high in protein and B vitamins especially, vitamin B_1, Vitamin B_6, vitamin B_{12}, vitamin A, iron and zinc (Bender, 1975). Hegarty and Ahn (1976) advocates that meat analogue usually have a protein content that is higher than or comparable to the product it is replacing. They reported that meat analogue had a higher proportion of PUFA and a higher concentration of potassium, calcium, phosphorus and sodium than ground beef. However, Smith (1988) feels that biological value of meat analogue is lower than that of meat. But still he agrees that in the context of a mixed diet, the biological value of meat analogue is comparable to that of meat. While developing a meat analogue it is important to maintain its nutritional equivalence to the product it is replacing, if not better. If the protein level and the quantity are the key attributes in the finished products, it makes sense to formulate it with a high quality protein such as soya (Kuntz, 1995).

Kuntz (1995) felt that besides protein, there are other nutrients that should be considered if nutritional equivalence to meat is a concern. Nutritionally, meat analogues are often lower in saturated fat than real meat. The vegetarian products may also contain more fibre and carbohydrate since they are made from plant sources such as soya and wheat (Anon., 1998).

Granular meat analogues are an economical source of good quality protein containing 31 per cent protein (Anon, 2000). Khader (2001) also agrees that the nutritional equivalence of vegetable protein products are fundamental to product design.

Meat analogues are useful source of protein particularly for those adopting a vegetarian diet. Meat analogue can be a nutritious source of vegetable protein and add variety and good nutrition to vegetarian diet (Anon., 2002).

Soyabean, used in the preparation of meat analogue has high protein content (38 per cent approx). This protein has an excellent nutritional quality containing all the essential amino acids necessary for human growth and development (British Nutrition Foundation, 2003).

Osho (2003) compared the nutritional composition of beef and texturized vegetable protein and revealed that texturized vegetable protein has a higher protein content of 51.2 per cent than beef (20.6 per cent). Moisture content was higher in beef (67.8 per cent) than texturized vegetable protein (7.1 per cent). Fat was also higher in beef (13.2 per cent) than texturized vegetable protein (11.2 per cent). Minerals like calcium, iron etc. were higher in texturized vegetable protein than beef. Iron was found to be 12.7mg $100g^{-1}$ in texturized vegetable protein and 3.6 mg $100g^{-1}$ in beef while calcium was 250.9mg $100g^{-1}$ in texturized vegetable protein and 10.8mg $100g^{-1}$ in beef.

Filho *et.al.* (2005) evaluated the nutritional composition of a meat analogue and reported protein 20.69 $\pm$ 0.80 per cent, fat 1.92 $\pm$ 0.37 per cent, fibre 1.47 $\pm$ 0.18 per cent, iron 1.65 $\pm$ 0.12 mg per 100g and phosphorus 63.30 mg per 100g.

Patel (2006) suggested that protein in the diet can be increased through the use of plant protein products including tofu, temph, meat analogue etc. Admin (2007) observed that meat does not contain any fibre at all whereas meat alternatives do, so consuming meat alternatives instead of meat can help to reduce overall fat consumption.

Border (2007) agrees that meat analogues are cholesterol – free and are low in saturated fat and total fat while they contain some amount of fibre and micronutrients. When developing a product the key requirement is that the final product must have a protein content equivalent to that of the milk protein casein. This ensures that it will offer a similar balance and bioavailability of all the essential amino acids needed for human health. Banman (2008) revealed that vegetable products are similar to the meat products in terms of potassium and phosphorus content and are significantly lower than meat in terms of fat and saturated fat.

2.4. Negative effects of meat consumption on human health

Lai (1997) reported that animal fat is relatively high in saturated fat and cholestrol which have been linked to various health problems including heart diseases and arteriosclerosis. Meat and sausages contain relatively high number of saturated fatty acids. Several studies have shown that a high intake of saturated fatty acid reduces insulin sensitivity (Vessby, 2000; Vessby, 2001, Van Dam, 2002, Mann, 2002). The high level of meat and saturated fat consumption in USA and other high income countries exceeds nutritional needs and contributes to a high rate of chronic diseases such as cardio vascular diseases (CVD), diabetics mellitus (DM) and some cancers. Affluent citizens in the middle and low income countries are adopting similar high meat diet and are thus experiencing increased rates of same chronic diseases (Walker *et. al.,* 2005). One study suggests that the diet high in animal protein regardless of fat content increases the risk of cardio-vascular mortality (Kelemen *et. al.,* 2005).

Meat protein also constitutes a significant substrate for bacterial production of sulfide in the intestine. Sulfide compounds also contribute to the development of colitis (Magee, 2000). It is also found that the animal protein stimulates the release of insulin and activates HMG CoA reductase, the limiting enzyme for cholesterol synthesis. This also influences the metabolism of insulin and glucagon. Studies have shown that the high insulin glucagon ratio is associated with increased cholesterol concentration i.e. hypercholestremia (Ernahrung, 2006). Protein that comes from animal sources contains all essential amino acids that one needs but they contain large amounts of saturated fat which can not only make one fat, but also puts at the risk of heart disease and strokes (Behar, 2006). Everyone agrees to the fact that meat eater ingest excessive amount of cholesterol, making one dangerously susceptible to heart attack (Subramuniyaswami, 2006).

Meat products on an average have more phosphorus than calcium as compared to vegetable food. A raised dietary phosphorus calcium ratio leads to secondary hyperthyroidism (Calvo, 1990).

Cunnigham (1976) revealed that animal foods are a potential source of infection by oncogenic viruses. Meat can also transmit certain diseases such as trichinosis. The out break of bird flu has stimulated global concern over public health problems. In addition to this, hetero-cyclic amines and other carcinogenic substances are produced in the intestine during digestion of meat (Rieger, 1999).

Persistent organic pollutants such as dioxions, furans and polychlorinated bromides enter the human food chain through the diet of food animals, which undergoes bio-accumulation and bio-magnification resulting in potent human health hazards (Food and Nutrition Board, 2003). A regular consumption of meat promotes detrimental immune response and can make the body more susceptible to inflammation with all the secondary diseases that ensues (Anon, 2007).

2.5. Consumers of meat analogues

Many consumers are concerned about the amount of fat in their diet and limit the dietary intake of fat and calories (Yankelovich, 1985; Bruke, 1987). In addition, there is concern about the presence of pesticide residues, hormones and growth promoters in meat used to increase the yield and concerns about human diseases thought to be transmitted through meat. For all these reasons, the trend towards consumption of less meat and a greater interest in vegetarian foods and meat analogues is growing significantly. Moreover, in the developing countries meat is short in supply which can yet be a reason for replacement (FAO, 2002b). A vegetarian diet that keeps adults in good health is not necessarily appropriate for infants and young children as it is a time of rapid growth and development when a good supply of energy and nutrient is important, hence the need for meat analogue (British Nutrition Foundation, 2003).

Kuntz (1995) indicated that many people do not have the access to non vegetarian foods; in those situations a high protein meat substitute becomes a nutritional necessity. Low consumption of meat is associated with high poverty levels and protein deficiency. Hence, diets should include meat analogue not only as a protein source but also as a means of ending world hunger, preserving livestock's and land management. He also observed that the three major markets includes vegetarians, those looking for healthier alternatives to meat and those looking for low cost protein sources.

The public's insatiable demand for low cost fat food, in general, has fueled the search for methods of making meat analogues that do not leave meat bereft to texture, flavour and physical properties, normally associated with full fat meat food (Lai, 1997).

Today health benefits are a major reason why many people are eliminating or reducing meat from their diet. Fat and cholesterol ranks as the top concerns for consumers. Hence, meat analogues can be a part of healthy diet because they provide a source of vegetable protein, vitamins and minerals without saturated fat and cholesterol of animal products (Kobs, 1999). Moreover, concerns about environment has created a renewed interest in the vegetable protein foods in the world. Meat analogues are also chosen because they provide convenient and good tasting addition to daily meal. There are a variety of reasons for choosing meat alternatives such as health benefits, ecological benefits, philosophical reasons, convenience and taste (Anon, 2002).

Therefore, one needs to identify a substitute food that contains the nutrients previously provided by meat (British Nutrition Foundation, 2003). Osho (2003) revealed that the meat analogues are processed and flavoured as beef and compared to beef from animal sources, which lead to the search of an alternative source of inexpensive protein and has also lead to the promotion and utilization of soyabean in order to enhance food security.

Sadler (2004) observed that, the key drivers to the market includes the consumer's concerns over food safety, growth in the number of vegetarians, meat avoiders, meat reducers, meat eaters seeking variety from the diet, those having a desire for high quality meat alternative products, growing interest in healthy eating and growing trend towards the use of convenience food products. The market for meatless foods includes health conscious non-vegetarians, lactose intolerant people, people following the rules of Kashrut or who address ethical or nutritional issues for vegetarians and vegans and also include non vegetarians who enjoy the taste of meat. Moreover, people chose not to eat meat for a variety of reasons and obtain all the protein from plant sources. Buddhist cuisine features the oldest known use of meat analogue (Bodhipaksa, 2004).

Way back in 60's the market for the meat analogues was driven by the pure vegetarians but today it is also supported by the consumers who have a balanced life style. They eat meat on certain days and go meatless on other days (Bardic, 2006). Meat alternatives are not just for vegetarians, they are perfect for the meat eaters who want to cut down on their fat consumption but who like the taste and texture of meat (Admin, 2007). Flynt (2007) also agreed that the vegetarians and other health conscious individuals eat meat analogue because they are relatively high in protein. In addition, they contain no cholesterol and can therefore assist those who are on low cholesterol diet (Admin, 2007).

Many vegetarians welcome meat substitutes or meat analogues which are derived from various plant protein sources. Meat analogue products targeted to those (vegetarians) groups has seen flavour and texture improvement over time, winning fans both in and outside the community (Wrick, 2007). This is because meat alternatives are easy to prepare, require less time for cooking and can be used in meat recipes in same way as meat is used. In addition they are tasty and have similar texture to meat (Admin, 2007). Moreover, today consumers make food choices based on their own definition of good nutrition, a healthy life style, religious traditions and increased ecological sustainability (Wrick, 2007). Banman (2008) also agrees that meat substitutes are undeniably convenient to prepare as they provide both vegetarian and non-vegetarian alike an alternative to meat product.

2.6 Acceptability of meat analogues

It has been recognized that enjoyment of food is essential for good health. Enjoyment would mean choice and acceptance and not always nutrition and wholesomeness (Solms and Hall, 1981). Measuring the sensory properties and determining the acceptance of these properties and determining the importance of these properties as a basis of predicting acceptance by customers represents major accomplishments for sensory evaluation (Bodyfelt *et.al.*, 1988). For customers, the perceivable feel, aroma, flavor, taste and texture etc. are the deciding factors in food acceptance (Pal *et.al.*, 1995). Vengi (1997) opined that sensory methods can be used to evaluate the quality of foods as well as to determine consumers preferences among food items.

Meat analogue can generate a few developmental headache on the road of an acceptable product. They share some of the issues like health and nutritional concerns, to provide a flavor and texture that almost meets the target without the use of specific ingredients of animal origin. The meat analogue designers have the added burden to create a product that possess something it is not. If the product does not have good taste, one might take it for trial initially but the consumers certainly would not come back for more (Kuntz, 1995). Meat analogue may appear some what pricy at first glance, they compare quite well to meat and meat substitutes. Consumers go for the price quality relation when buying foods (Knox, 2000).

Klahorst (2001) observed that when developing meat analogue the product developer must add characteristic attributes inherently found in its counterparts. Since vegetables have a bland profile it is more acceptable as they are easy to flavour. Decker (2004) stated that meat analogues are vegan friendly blends of wheat protein grain, vegetables and legumes. They are vegetarian meat for the meat eater. Hence, one can measure an analogue's success by how closely it resembles the ideal.

According to British Nutrition Foundation (2004), sensory evaluation of meat analogues are critical to ensure that the sensations are as similar as possible to those experiences when eating meat. The meatless magic has increased the demand for all types of non-meat high protein alternatives. Therefore, one finds diverse assortment of meatless alteration designed to broaden consumers appeal and acceptance. There has been a notable shift in the profile of consumers buying meat substitutes. In terms of overall advancements in the meatless arena, the two main factors are, removing the beany flavor (from soya) and improving the texture. Mouth feel of meat analogues is also a very important characteristic and is extremely important to customers. It is clear that modern customers have moved forward and are demanding products of better quality such as texture, bite and flavor (Bardic, 2006).

Erbert and Border (2006) reported that, to be accepted as a simulated meat substitute, products must have the texture and mouth feel associated with meat. Wrick (2007) indicated that, the focus has been on designing a more whole muscle – like products for customers to prepare authentic style products that mimics the steak cuts and poultry cuts. The meat analogue market saw a considerable growth during 1990's. The introduction and success of meat alternative led to the further introduction of meatless entrée items. Peter Goldbitz of Soya tech. Inc. believes that meat analogue will see a more retail growth when the analogue would better resemble and taste like the products they are intended to substitute.

2.7. Health benefits of consuming meat analogues over meat

Numerous epidemiological and clinical studies have shown that vegetarians are nearly 50 per cent less likely to die from cancer than non – vegetarians. Also vegeterianism helps in lowering blood pressure and heart diseases (Robbins, 1987).

Meat analogues are concentrated sources of protein and very little fat (Anon, 1998). Nutrition is the driving force for both vegetarians and nonvegetarians for substituting soya protein to animal protein. The health benefit as well as the improved technology makes eating an analogue a pleasurable experience and aroused the customers interest in veggie meat (Klahorst, 2001).

Realeat (2001) conducted a survey and revealed the consumers perceptions that meat – free alternatives offer positive health benefits as well as meat alternative ingredients are nutritious. It is increasingly recognized that certain foods and their components can deliver health benefits beyond the nutritional value. In this regards meat alternatives offer a wider range of possibility as potential functional foods and have the potential to deliver public health benefits (Sadler, 2003).

It is reported that those who eat flesh are more likely to have cancer than those following a vegetarian diet. Reports suggest that, completely eliminating meat and meat products from one's diet reduces the risk of heart attack by 90 per cent (Subramuniyaswami, 2006). The Food and Drug Administration suggests that four serving of soya can lower LDL cholesterol by 10 per cent but to use this claim, a food product must contain 6.25g of soya per serving. Soya which is used as an ingredient in meat analogues have several benefits like the potential of lowering the risk of heart diseases, menopausal bone loss, osteoporosis, breast and prostrate cancer. Isoflavones and phytochemicals in them exert a physiological effect on the body and provide benefit to the body beyond nutrition (Bardic, 2006). Data also show that substitution of soya protein to animal protein is associated with significant decrease in the total cholesterol and triglyceride while maintaining the HDL concentration (Behar, 2006).

Soya protein found in meat analogue products is high in quality protein, low in saturated fat and high in other healthful compounds. It also contains genistein which is reported to block cancer development and reduces the risk of heart diseases (Bardic, 2006). Admin (2007) suggests that reducing the consumption of meat helps in reducing the development of heart diseases, high BP, and certain types of cancer. Meat alternatives are good, as they are cholesterol free or very low in cholesterol. They also contain fibre which helps to lower the cholesterol whereas meat does not contain any fibre.

Meat analogue is considered to be a health food which contains high protein and fibre (Rareunrom *et. al.,* 2007). Wrick (2007) also agrees that consumers turn to meat analogue because they are lower in saturated fat and cholesterol as well as the texturized soya protein concentrates used to prepare meat analogue are high in protein, rich in fibre, cholesterol free and low in sodium. Admin (2007) reported that along with the above, meat alternatives have a higher level of carbohydrates, magnesium, potassium, folate, antioxidants and phytochemicals. Some of the deadliest food borne illnesses enter the body through animal foods, but meat alternatives are less likely to be contaminated by bacteria than the real meat.

Current researches revealed that plant based diets lower the risk of chronic diseases (Rareunrom *et. al.,* 2007) and meat substitutes offer a lower fat, higher fiber and offer health benefits than meat products (Banman, 2008).

Materials and Methods

3. MATERIALS AND METHODS

The materials used and the methods followed for the development of meat analogues and quality evaluation of the products such as sensory qualities, chemical composition and shelf life qualities are given under the following headings.

3.1. Collection of raw ingredients and pre-treatments

3.2. Development of meat analogues

3.3. Acceptability of the meat analogues

3.4. Selection of most acceptable three treatments using statistical techniques

3.5. Storage of the selected products

3.6. Analysis of the chemical constituents

3.7. Microbial enumeration and insect infestation of the products during storage

3.8. Organoleptic evaluation of the recipes

3.9. Benefit cost analysis

3.10. Statistical analysis of data

3.1. Collection of raw ingredients and pre-treatments

Green gram, soya bean, whole wheat, spices such as ginger, garlic, pepper and cinnamon, wheat flour and all the other ingredients required were purchased in one lot from the local market.

3.1.1. Pre treatments given to the raw ingredients

Green gram was roasted for 15 minutes and then was soaked in water for 12 hours at ambient temperature. Soya bean was soaked in water containing 1 per cent sodium bicarbonate at ambient temperature for 12 hours. The soaked beans were then pressure cooked for 15 minutes. Whole wheat was also soaked in water for 12 hours at ambient temperature. Wheat gluten used for texturising the product was extracted from wheat flour by standard procedure (Kaur *et. al.,* 2004). Spices like cinnamon, garlic, ginger and pepper were used for flavouring the product.

3.2. Development of the meat analogues

Meat analogues were prepared using green gram as the base and were blended with soya bean and wheat in different combinations. The treatments and their composition are given in the table1.

Table1. Percentage of green gram, soyabean and wheat in meat analogues.

Treatments	Green gram (%)	Soya bean (%)	Wheat (%)
Control	100	-	-
T_1	70	-	30
T_2	80	-	20
T_3	90	-	10
T_4	60	10	30
T_5	70	10	20
T_6	80	10	10
T_7	50	20	30
T_8	60	20	20
T_9	70	20	10

A pilot study was conducted wherein the products were prepared with the above proposed compositions, spices and 80g of gluten. The acceptability of the product was studied after preparing a standard recipe wherein meat was replaced by the developed product. The product was found to be high in gluten which drastically affected the texture of the product rendering it unacceptable. Hence, the amount of gluten in the product was reduced to 40 g.

During the study, to all the above treatments and control (Table 1) 40g of gluten was added for texturising the product and 40g of spices (ginger – 5g, garlic – 20g, pepper – 10g, cinnamon – 5g) were used for flavouring the product.

The pre-treated grains were ground to a coarse paste along with the spices and gluten. The batter was then spread on trays and steamed for 40 minutes. After cooling, it was cut into half inch pieces and was dried in a cabinet drier at 65°C for 18 hours.

3.3.Acceptability of the meat analogues

Selection of judges

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

Preparation of score card

The score card used for the evaluation of meat analogue recipe is given in appendix 1.

Organoleptic evaluation

A standard procedure (Mathew, 1985) for preparing the meat curry was followed for preparing the curry by replacing the meat with the developed product. The dried products were soaked in hot water for one hour before preparing the curry. The procedure for the preparation of the recipe is given in appendix 2.

Organoleptic evaluation was carried out in the morning time using a 9 point hedonic scale by a panel of 10 selected judges. The quality attributes namely appearance, colour, flavour, texture, taste and overall acceptability were evaluated.

3.4 Selection of the most acceptable three treatments using statistical techniques

The acceptability scores were analysed by Kendall's coefficient of concordance to select the best three treatments (including control).

3.5 Storage of the selected meat analogues

The meat analogues selected on the basis of their acceptability were stored for a period of six months in metallised polyester polyethelene laminate pouches (100g per packet) under ambient storage conditions.

3.6 Analysis of the chemical constituents of the selected meat analogues

Meat analogues were stored for six months and evaluated for the following chemical constituents initially, during third and sixth months of storage. Analysis were carried out with six replications of each sample.

3.6.1. Moisture

3.6.2. Protein

3.6.3. Fat

3.6.4. Fibre

3.6.5. Calcium

3.6.6. Phosphorus

3.6.7. Iron

3.6.8. Potassium

3.6.1. Moisture

Moisture content of the meat analogues was estimated using the method of AOAC (1980).

To determine the moisture content, five gram of the powdered sample was taken in a petri dish and dried in a hot air oven at $110^0C \pm 5^0C$, then cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant weight was achieved. The moisture content was calculated from the loss in weight during drying and expressed in percentage.

3.6.2. Protein

The protein content was estimated using the method of AOAC (1980).

Powdered meat analogue (0.3g) was digested with 6 ml Con. H_2SO_4 after adding 0.4 g of $CuSO_4$ and 3.5 g K_2SO_4 in a digestion flask until the sample became colourless. After digestion, it was diluted with water and made upto 100ml, out of which 10 ml of the sample was pipetted out. 25 ml of 40 per cent NaOH was pumped. This distillate was collected in 20 per cent boric acid containing mixed indicator and then titrated with 0.2 N H_2SO_4. The nitrogen content obtained was multiplied with a factor of 6.25 to get the protein content and was expressed in percentage

3.6.3. Fat

The fat content of meat analogue was estimated using the method of AOAC (1955).

Five gram of powdered meat analogue was taken in a thimble and plugged with cotton. The material was extracted with petroleum ether for 6 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 in g 100 g^{-1} of the sample.

3.6.4. Fibre

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

Two gram of dried and powdered sample was boiled with 200 ml of 1.25 per cent sulphuric acid for thirty 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 thirty minutes. Again, it was filtered through a muslin cloth and washed with 1.25 per cent 25ml sulphuric acid, three times with 50 ml water and finally with 25ml alcohol. The residue was transferred to a pre-weighed ashing dish, dried, cooled and weighed. The residue was then ignited for 30 minutes in a muffle furnace at 600^0C, cooled in a desiccator and reweighed. The fibre content of the sample was calculated from the loss in weight on ignition and expressed in percentage.

3.6.5. Calcium

The calcium content was estimated using titration method with EDTA as suggested by Page (1982).

Two gram of dried and powdered sample was pre-digested with 20 ml of 9:4 mixture of nitric acid and perchloric acid and volume was made upto 100 ml. To five ml of diacid extract, 10 ml water, 10 drops of hydroxylamine hydrochloride, 10 ml triethanolamine, 2.5 ml sodium hydroxide and 10 drops of calcone were added. Then, it was titrated with 0.02 N EDTA till the appearance of permanent blue colour. Calcium content was expressed in mg 100 g^{-1} of the sample.

3.6.6. Phosphorus

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

To 5 ml of pre-digested aliquot, 5 ml of nitric acid vandate molybdate reagent was added and made upto 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 100 g^{-1}.

3.6.7. Iron

The iron content was analyzed colorimetrically using ferric iron, which gives a blood red color with potassium thiocyanate (Raghuramulu *et al.*, 2003).

To an aliquot of 6.5 ml diacid solution, one ml of 30 per cent sulphuric acid, one ml of 7 per cent potassium persulphate solution and 1.5 ml of 40 per cent potassium thiocyanate solution were added. The intensity of the red color was measured within twenty minutes at 540 nm.

A standard graph was prepared using serial dilution of standard iron solution. The iron content of the sample was estimated from the standard graph and expressed in mg 100 g^{-1}.

3.6.8. Potassium

The potassium content was estimated using flame photometer as suggested by Jackson (1973).

One ml of the digested solution was made upto 25 ml and read directly in flame photometer. The potassium content was expressed in mg 100 g^{-1}.

3.7. Microbial enumeration and insect infestation of the products during storage

The total microbial count of the meat analogues were enumerated initially, during the third month and sixth month of storage. The method used for evaluation was serial dilution and plate count method as described by Agarwal and Hasija (1986). Ten gram of the powdered 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} and 10^{-6} dilution 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 bacteria was 10^{-6} whereas for fungi and yeast 10^{-3} dilution was used.

By visual observation of the product and also by examining the powdered products under the microscope, presence of storage insects were assessed. The powder was sieved first with 60 BL sieve and followed by 100 BL sieve and observed under microscope.

3.8. Organoleptic evaluation of recipes with the stored products

Organoleptic evaluation of the selected meat analogues were carried out, by preparing the standard recipe with the stored products. Evaluation was conducted by the selected panel of ten judges. The selected treatments were evaluated initially, during the third month and sixth month of storage.

The quality attributes of the product like appearance, colour, flavor, texture and taste were evaluated. Each of the above mentioned quality attributes were assessed by a nine point hedonic scale. The score card used for evaluation is given in appendix 1.

3.9. Benefit cost analysis of developed products

Benefit cost analysis of the products was done to assess the extent of expense incurred to prepare the products.

The cost was worked out based on the amount incurred on food materials in the preparation of the product. The market price of similar popularly available processed product, fresh meat and mutton was compared with the developed products.

3.10. Statistical analysis of data

The observations recorded were tabulated and the data was analyzed statistically using Complete Randomised Design (CRD). The microbial count was analyzed statistically by two factor CRD. The scores of organoleptic evaluation of the products were assessed by Kendall's coefficient of concordance (W).

Results

4. RESULT

The results pertaining to the study entitled "Standardization of green gram based meat analogues" are presented in this section under the following headings.

4.1. Organoleptic evaluation and selection of the most acceptable three treatments

4.2. Chemical composition of the selected meat analogues

4.3. Enumeration of microbial population and insect infestation of the meat analogues during storage

4.4. Organoleptic evaluation of the preparation made with stored meat analogues

4.5. Benefit cost analysis

4.1. Organoleptic evaluation and selection of the most acceptable three treatments

The meat analogues were prepared with different proportions of green gram, wheat and soya (Table.1) and were evaluated organoleptically using score card for different quality attributes like appearance, colour, flavour, texture, taste and overall acceptability. The results are presented in table 2 and the meat analogue recipe is presented in plate 1. Each treatment was ranked for all these quality attributes based on mean rank score using Kendall's (W) test. Using the mean ranks obtained by the Kendall's (W) test for all the five parameters, an index was worked out say $w_1x_1 + w_2x_2 + w_3x_3 + w_4x_4+w_5x_5$, were w_1, w_2.... w_5 are the weights designated to the different ranks as texture (x_1), taste (x_2), flavour (x_3), colour (x_4) and appearance (x_5). Here w_1 was assigned 5, w_2 4, w_3 3, w_4 and w_5 1.5. The weights were given logically.

Table 2. Mean score and index for the organoleptic evaluation of the meat analogues

Treatments	Quality attributes					
	Appearance	Colour	Flavour	Texture	Taste	Total
Control	7.43 (4.95)	9.45 (6.30)	19.95 (6.65)	32.5 (6.50)	24.4 (6.10)	93.73
T_1	9.53 (6.35)	8.33 (5.55)	16.05 (5.35)	13.25 (2.65)	19.60 (4.90)	66.75
T_2	8.85 (5.90)	6.30 (4.20)	10.2 (3.40)	29.75 (5.95)	26.6 (6.65)	81.70
T_3	8.1 (5.40)	7.65 (5.10)	15.6 (5.20)	30.0 (6.00)	21.8 (5.45)	83.15
T_4	13.58 (9.05)	13.88 (9.25)	25.05 (8.35)	27.25 (5.45)	28.0 (7.00)	107.75
T_5	4.28 (2.85)	6.53 (4.35)	12.6 (4.20)	28.75 (5.75)	19.0 (4.75)	71.15
T_6	10.13 (6.75)	11.85 (7.90)	20.1 (6.70)	23.25 (4.65)	23.6 (5.90)	88.93
T_7	7.5 (5.00)	6.08 (4.05)	12.9 (4.30)	22.75 (4.55)	20.2 (5.05)	69.43
T_8	5.63 (3.75)	4.65 (3.10)	13.95 (4.65)	39.0 (7.80)	20.2 (5.05)	83.43
T_9	7.5 (5.00)	7.8 (5.20)	18.6 (6.20)	28.5 (5.70)	16.6 (4.15)	79.00

Figures in parenthesis are mean rank scores

Plate 1. Meat analogue recipe.

Plate 2. Meat analogue (Control, 100 % green gram)

Plate 3. Meat analogue (T_4, 60 % green gram + 30 % wheat + 10 % soya)

Plate 4. Meat analogue (T_6, 80 % green gram + 10 % wheat + 10 % soya)

After calculating the index the score obtained by the control was 93.73 while that of the different treatments i.e. T_1, T_2, T_3, T_4, T_5, T_6, T_7, T_8, and T_9 were 66.75, 81.70, 83.15, 107.75, 71.15, 88.93, 69.43, 83.43 and 79.00 respectively. Therefore the three treatments selected for further study were the control, T_4 and T_6 (Plate 2, 3 and 4).

4.2. Chemical composition of selected meat analogues

The chemical composition of meat analogues stored in metallised polyester polyethylene laminate pouches (Plate 5) under ambient storage condition was estimated initially, during the third and sixth months of storage. The constituents like moisture, protein, fat, fibre, calcium, phosphorus, iron and potassium were estimated. The different chemical constituents of meat analogues and changes in the constituents during storage are presented in the following tables. Statistical significance was found out using Duncan's Multiple Range Test and is also presented in the tables.

Moisture

The initial moisture content of meat analogues ranged from 8.3 and 8.8g $100g^{-1}$, the maximum being in control and minimum in T_4 (Table 3). This increased to 9.73 to 10.86g $100g^{-1}$ during the third month. During this period, maximum moisture was found in T_6. There was a further increase to a range of 11.13 to 12.28g $100g^{-1}$ during the sixth month of storage, the maximum being in the control (Fig.1). Initially there was no significant difference between T_4 (8.3 %) and T_6 (8.6 %) but the moisture content was significantly high for control. During the third month T_4 (9.73 %) and control (9.76 %) was found to have no significant difference but T_6 (10.86 %) was found to be significantly high in moisture content when compared to the other two treatments. During the sixth month, there was no significant difference in the moisture content in T_4 (11.13 %) and T_6 (11.88 %) but the control had a significantly high moisture content of 12.28 per cent. There was a significant increase in the moisture

Table 3. Moisture content of the meat analogues during storage (g $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	8.8 ^{bx}	9.76 ^{ay}	12.28 ^{bz}
T_4	8.3 ^{ax}	9.73 ^{ay}	11.13 ^{az}
T_6	8.4 ^{ax}	10.86 ^{by}	11.88 ^{az}

Figure with the same super script have no significant difference (a,b represent the difference within treatments, while x,y,z represent the difference during the storage period)

Table 4. Protein content of the meat analogues during storage ($g100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	23.22 ^{bx}	21.88 ^{ay}	18.20 ^{az}
T_4	24.84 ^{ax}	21.06 ^{ay}	18.84 ^{az}
T_6	26.00 ^{ax}	21.35 ^{ay}	18.50 ^{az}

Figure with the same super script have no significant difference (a,b represent the difference within treatments, while x,y,z represent the difference during the storage period

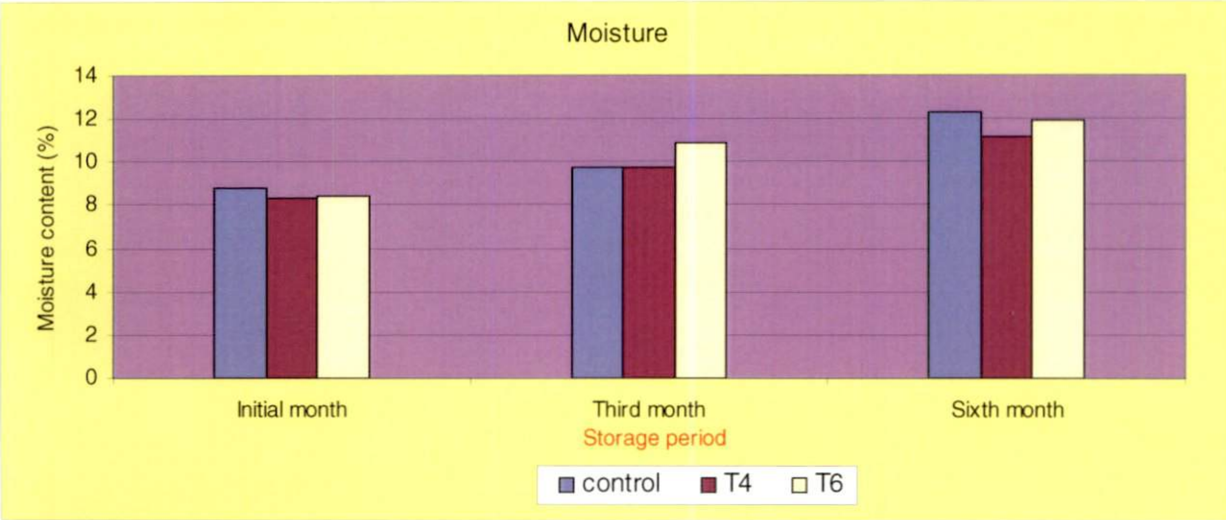

Fig.1. Moisture content of meat analogues during the storage period

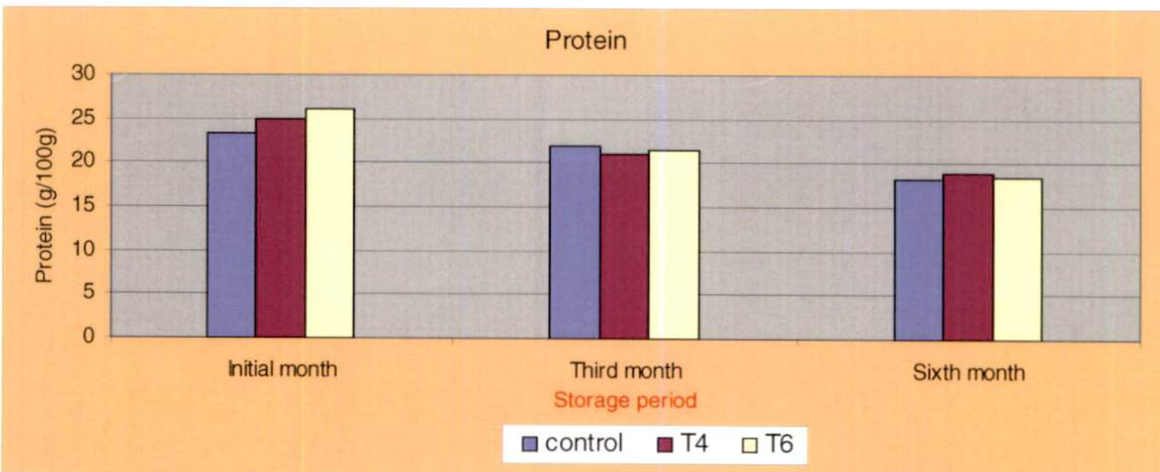

Fig. 2. Protein content of meat analogues during the storage period

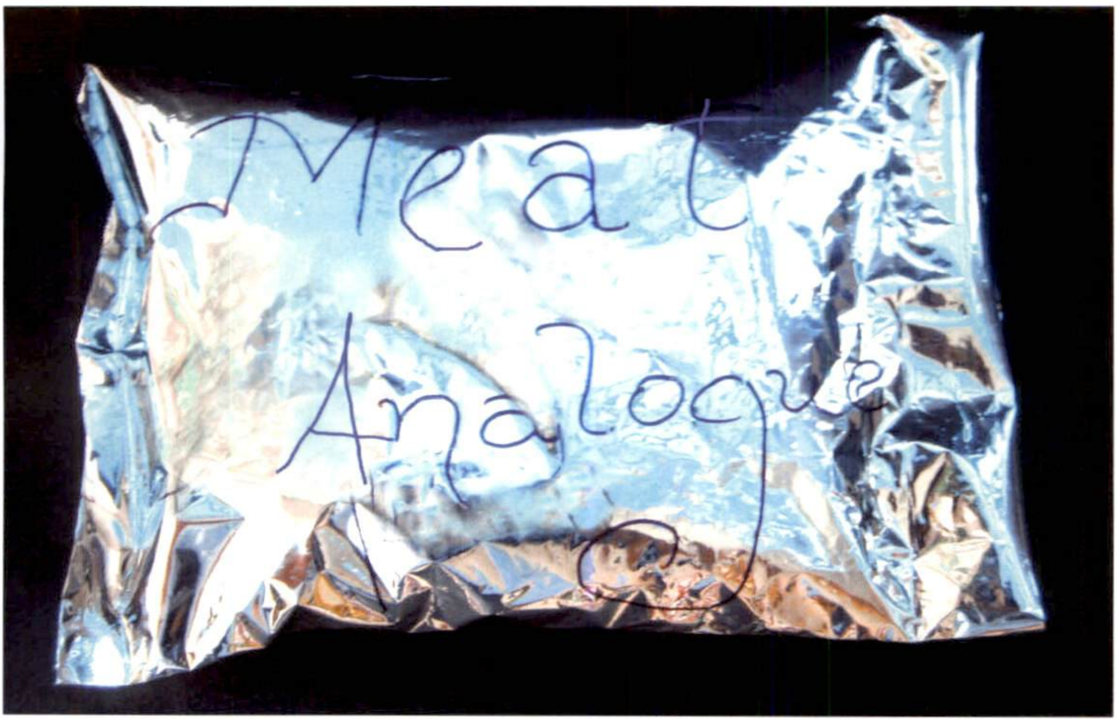

Plate 5. Packaging and storage of meat analogues

content in each treatment during the storage with maximum moisture content during the sixth month of storage for all the treatments.

Protein

Initially the protein content of the meat analogues ranged from 23.22g $100g^{-1}$ to 26.00g $100g^{-1}$, the maximum being in T_6 and minimum in control (Table 4). This decreased to a range of 21.06g to 21.88g $100g^{-1}$ during the third month with minimum in T_4. This decreased further to a range of 18.20g to 18.84g $100g^{-1}$during the sixth month of storage period. During this period, the minimum protein content was in control and maximum in T_4 (Fig.2). Initially between treatments, protein content was significantly low in control (23.22 %). During third and sixth month of storage, no significant difference was found in the protein content between the three treatments. For each treatment, the protein content decreased significantly during the entire period of storage. By the end of the storage period, control had minimum protein content (18.20 %) while T_4 had a maximum protein content of 18.84 per cent.

Fat

Initially the fat content of the meat analogues were in a range of 1.23g to 2.67g $100g^{-1}$ with a maximum fat content in T_4 and minimum in control (Table 5). This gradually decreased to a range of 0.93g to 1.87g $100g^{-1}$ during third month with a maximum in T_6 and minimum in control. This again decreased to a range of 0.70g to 1.57g $100g^{-1}$ during sixth month of storage with a minimum in the control (Fig.3). Between treatments, during initial and third month, there was no significant difference in fat content of T_4 and T_6, but control was found to have significantly low fat content (1.23 %). But during the sixth month, all the treatments varied significantly, in which T_6 (1.57 %) showed significantly high value for fat content followed by T_4 (1.33 %). On storage, the fat content decreased significantly for all the treatments during the entire period of storage with a maximum decrease in the control and minimum in T_6.

Table 5. Fat content of the meat analogues during storage (g $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	1.23^{bx}	0.93^{by}	0.70^{cz}
T_4	2.67^{ax}	1.73^{ay}	1.33^{bz}
T_6	2.20^{ax}	1.87^{ay}	1.57^{az}

Figure with the same super script have no significant difference (a,b,c represent the difference within treatments, while x,y,z represent the difference during the storage period)

Table 6. Fibre content of the meat analogues during storage (g $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	1.33^{bx}	1.10^{cy}	0.87^{bz}
T_4	1.76^{ax}	1.26^{by}	0.996^{az}
T_6	1.82^{ax}	1.36^{ay}	1.035^{az}

Figure with the same super script have no significant difference (a,b,c represent the difference within treatments, while x,y,z represent the difference during the storage period)

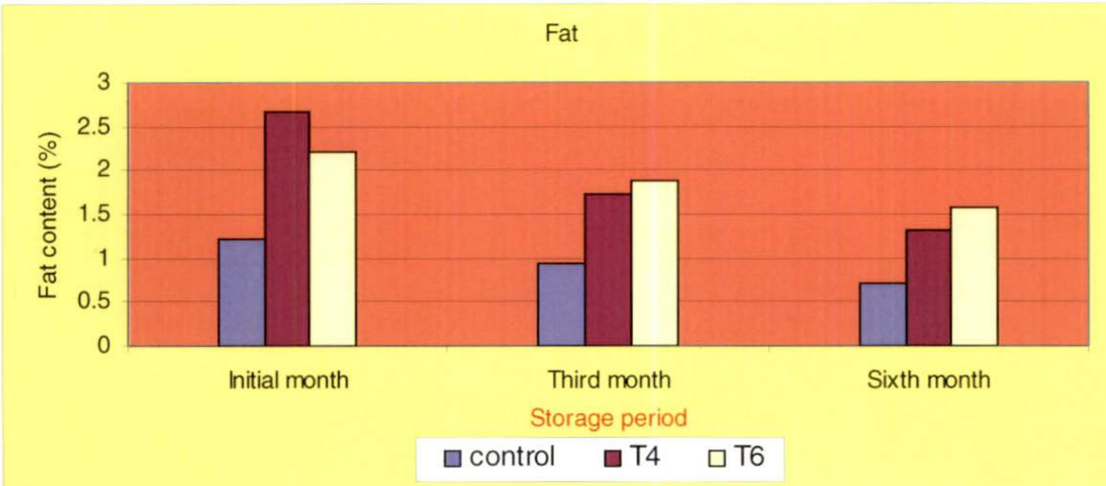

Fig. 3. Fat content of meat analogues during the storage period

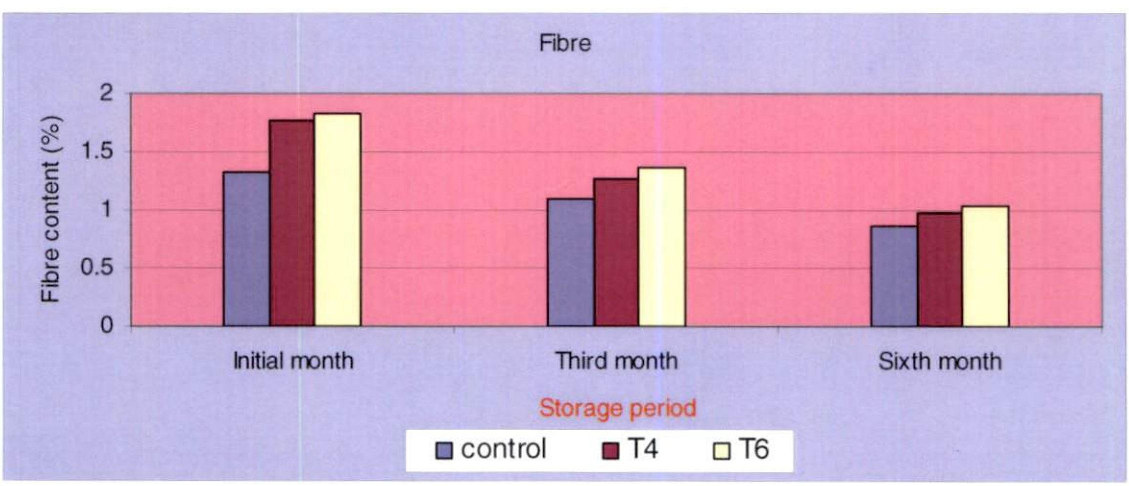

Fig. 4. Fibre content of meat analogues during the storage period

Fibre

The fibre content in the meat analogues ranged from 1.33g to 1.82g $100g^{-1}$ during the initial period and there was no significant variation in the fibre content of T_4 (1.76 %) and T_6 (1.82 %) (Table 6). Control showed significantly low fibre content (1.33 %). A gradual decrease in the fibre content was seen during the third month which was in the range of 1.10g to 1.36g $100g^{-1}$. During the sixth month of storage the fibre content decreased even further ranging from 0.87g to 1.035g $100g^{-1}$ with a minimum in the control and maximum in T_6 (Fig.4). During the third month, T_6 (1.36 %) had significantly high fibre content. By the end of the storage period, fibre content of T_4 (0.996 %) and T_6 (1.035 %) were found to be at par with control (0.87 %) while they (T_4 and T_6) showed no significant difference. On storage, the fibre content decreased significantly for all the treatments during the entire period of storage.

Calcium

The calcium content ranged from 253mg to 276mg $100g^{-1}$ for the entire storage period (Table 7). The calcium content of the meat analogues was 276mg $100g^{-1}$ for all the three treatments initially, which decreased slightly for the control to 253mg $100g^{-1}$, during the third month of storage. There was no change during the sixth month of storage. There was no change in the calcium content of T_4 and T_6 during storage (Fig.5).

Phosphorus

The initial phosphorus content of the meat analogues was in a range of 177.94mg to 190.85mg 100 g^{-1} (Table 8). The maximum phosphorus content was in T_6 and minimum in control. During the third month of storage, the phosphorus content decreased to a range of 122.96mg to 134.07mg 100 g^{-1}. A further decrease in the phosphorus content was noted during the sixth month of storage ranging from 81.19mg to 89.71mg $100g^{-1}$ with a minimum in control and maximum in T_6 (Fig.6).

Table 7. Calcium content of the meat analogues during storage (mg $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	276 ^{ax}	253 ^{ax}	253 ^{ax}
T_4	276 ^{ax}	276 ^{ax}	276 ^{ax}
T_6	276 ^{ax}	276 ^{ax}	276 ^{ax}

Figure with the same super script have no significant difference (a represent the difference within treatments, while x represent the difference during the storage period)

Table 8. Phosphorus content of the meat analogues during storage (mg $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	177.94 ^{bx}	122.96 ^{by}	81.19 ^{az}
T_4	190.82 ^{ax}	132.50 ^{ay}	84.15 ^{az}
T_6	190.85 ^{ax}	134.07 ^{ay}	89.71 ^{az}

Figure with the same super script have no significant difference (a,b represent the difference within treatments, while x,y,z represent the difference during the storage period)

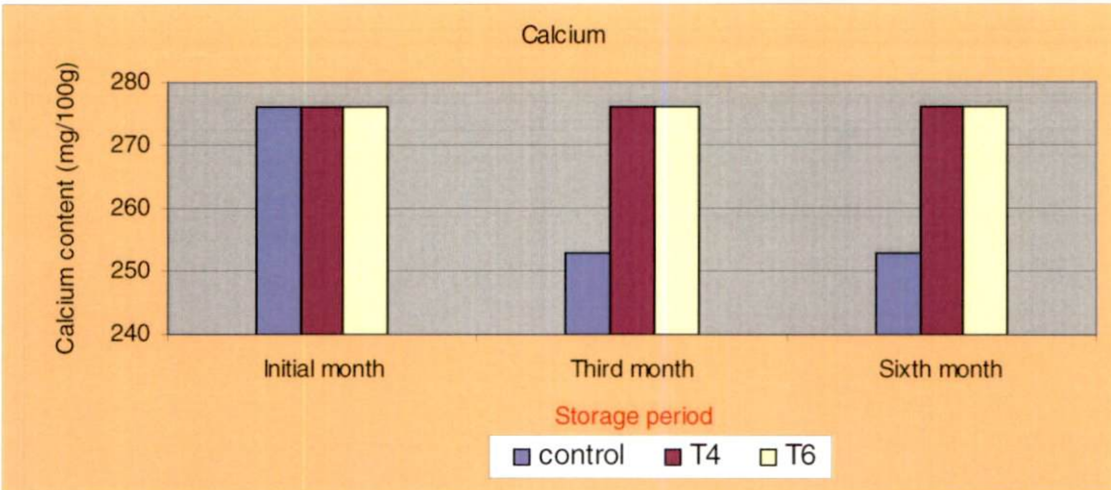

Fig. 5. Calcium content of meat analogues during the storage period

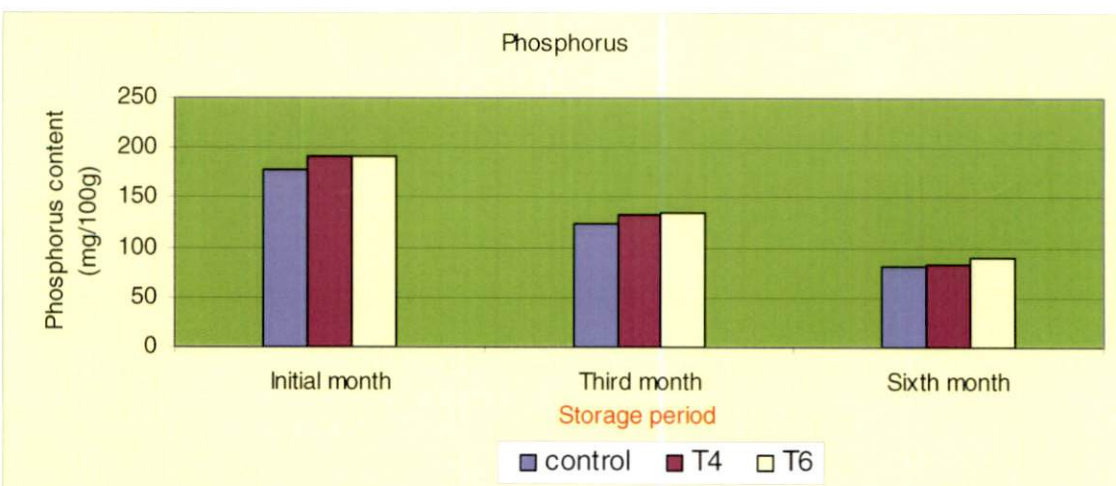

Fig.6. Phosphorus content of meat analogues during the storage period

Between treatments there was no significant change in the phosphorus content of T_4 and T_6 throughout the storage period. But for control, phosphorus content was significantly low during initial and third month, but by sixth month there was no significant variation between control, T_4 and T_6. On storage, the phosphorus content decreased significantly in each treatment during the entire period of storage with a maximum decrease in the control (81.19mg /100g).

Iron

Initially, iron content in the meat analogues ranged from 1.77mg to 1.89mg 100 g^{-1} with a minimum in control and maximum in T_6 (Table 9). The iron content of the analogues reduced to a range of 1.18mg to 1.48mg 100 g^{-1} during the third month of storage. A further reduction in the iron content was observed ranging from 0.93mg to 1.05mg 100g^{-1} during sixth month of storage with a minimum in the control and maximum in T_4 (Fig.7). Initially there was no significant difference was found in the iron content between the three treatments, but during the third and sixth month of storage, iron content was significantly low for control. On storage, the iron content reduced significantly for all the treatments during the entire period of storage wherein the maximum reduction was found in the control (0.93mg /100g).

Potassium

Initially potassium content in the meat analogues ranged from 72.00mg to 73.00mg 100g^{-1} with minimum in the control and maximum in T_6 (Table 10). This gradually decreased with storage. During the third month of storage, potassium content reduced to a range of 62.66mg to 66.00mg 100 g^{-1} with a minimum in control. During the sixth month, it reduced even further to a range of 56.33mg to 58.66mg 100 g^{-1}. During this period, maximum potassium content was in T_6 and minimum in control (Fig.8). No significant difference was found between treatments during each period of storage. But potassium content decreased significantly on storage in each

Table 9. Iron content of the meat analogues during storage (mg $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	1.77 ^{ax}	1.18 ^{by}	0.93 ^{bz}
T_4	1.87 ^{ax}	1.46 ^{ay}	1.05 ^{az}
T_6	1.89 ^{ax}	1.48 ^{ay}	1.02 ^{az}

Figure with the same super script have no significant difference (a,b represent the difference within treatments, while x,y,z represent the difference during the storage period)

Table 10. Potassium content of the meat analogues during storage (mg $100g^{-1}$)

Treatments	Storage period in months		
	0	3	6
control	72.00 ^{ax}	62.66 ^{ay}	56.33 ^{az}
T_4	72.33 ^{ax}	66.00 ^{ay}	57.33 ^{az}
T_6	73.00 ^{ax}	64.33 ^{ay}	58.66 ^{az}

Figure with the same super script have no significant difference (a represent the difference within treatments, while x,y,z represent the difference during the storage period)

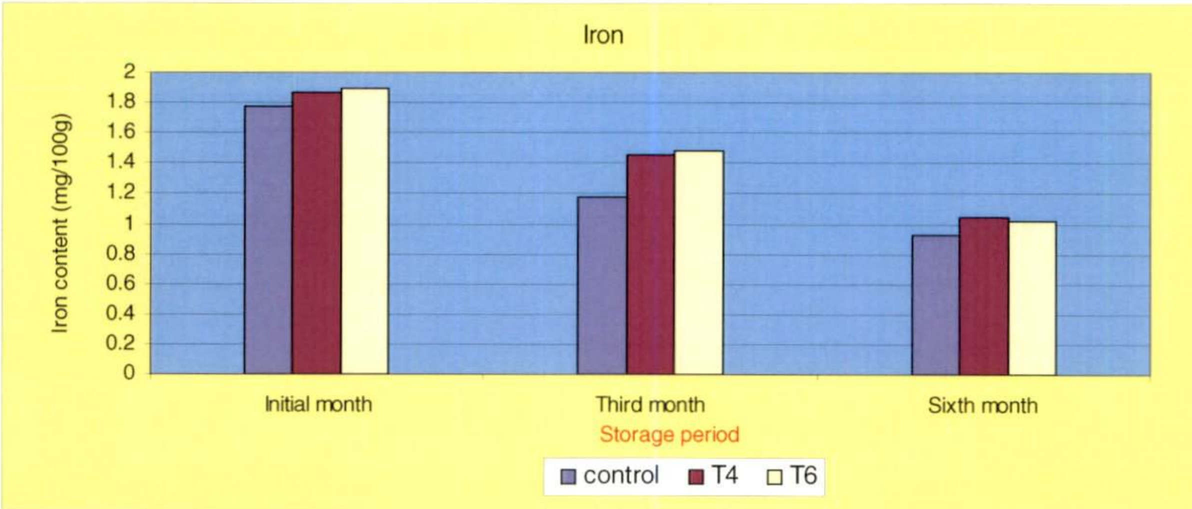

Fig. 7. Iron content of meat analogues during the storage period

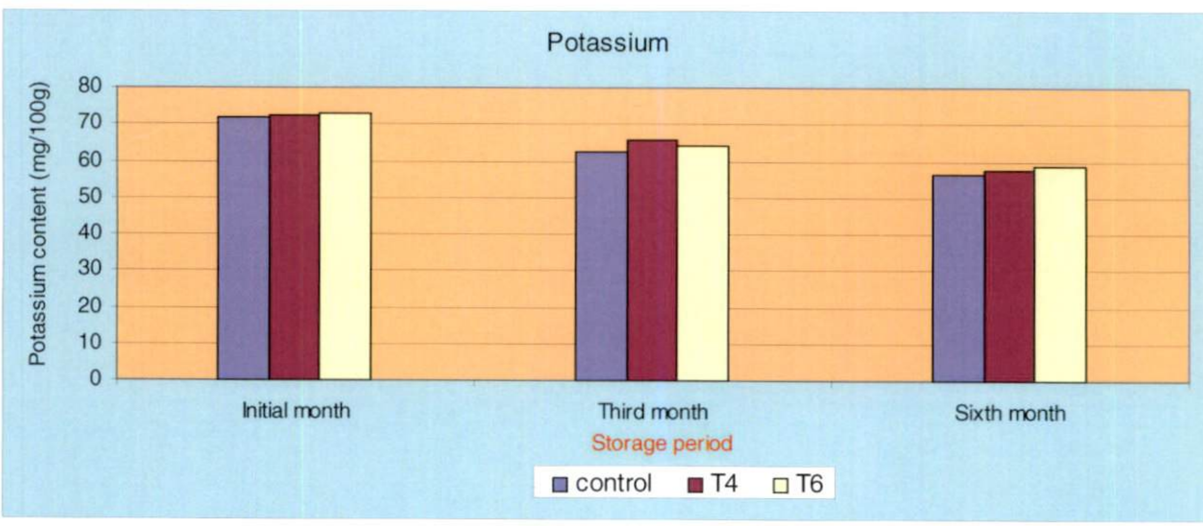

Fig. 8. Potassium content of meat analogues during the storage period

treatment with a maximum decrease in the control (56.33mg /100g) and minimum in T_6 (58.66mg /100g).

4.3. Enumeration of microbial population and insect infestation of the meat analogues during storage

The meat analogues were evaluated for bacteria, yeast and fungi initially and then during the third and sixth month of storage. Results pertaining to microbial enumeration are presented below.

Bacterial load of the stored samples are presented in table 11.The bacterial load was evaluated using two factor CRD. Square root transformation was performed where ever necessary. During the initial period bacterial count was maximum for T_4 and T_6 (0.333 x 10^6 cfu g^{-1}) while it was low in control (0.167 x 10^6cfu g^{-1}). The same pattern was observed in the third month of storage with a maximum bacterial load of 1.33 x 10^6 cfu g^{-1} for T_4 and T_6 and the lowest load for control (1.167 x 10^6 cfu g^{-1}). But during the sixth month of storage, maximum bacterial load was observed for control (2.5 x 10^6 cfu g^{-1}) while it was 2.167x10^6cfu g^{-1} for T_4 and T_6. Even though difference was observed in the bacterial load between treatments during each storage period, the difference was not significant between the treatments, since the difference was not more than the critical difference. Hence the bacterial load for all the treatments during the initial period can be expressed as a mean value of 0.278 x 10^6 cfu g^{-1}, during the third month a mean value of 1.278 x 10^6 cfu g^{-1}, and during the sixth month a mean value of 2.278 x 10^6 cfu g^{-1}.

With storage period, for control the bacterial load of 0.167 x 10^6 cfu g^{-1} increased to 1.67 x 10^6 cfu g^{-1}during the third month and to a maximum of 2.5 x 10^6 cfu g^{-1} during the sixth month of storage. For samples T_4 and T_6 also, there was an increase in the bacterial load from the initial value of 0.333 x 10^6 cfu g^{-1} to 1.333 x 10^6 cfu g^{-1} during the third month, to a maximum load of 2.167 x 10^6 cfu g^{-1}during the sixth month of storage. The increase in the bacterial load in each treatment with respect to storage period was found to be significant since difference between the periods was more than the critical difference (Fig.9). The bacterial load in the control, T_4 and T_6 during the sixth month of storage is shown in plate 6, 7 and 8 respectively.

Table 11. Bacterial count of the meat analogues during storage (x 10^6 cfu g^{-1})

Period of storage in months	control	T_4	T_6	Mean
0	0.167 (0.793)	0.333 (0.880)	0.333 (0.880)	0.278 (0.851)
3	1.167 (1.257)	1.333 (1.317)	1.333 (1.317)	1.278 (1.297)
6	2.5 (1.708)	2.167 (1.601)	2.167 (1.601)	2.278 (1.637)

Figures in parentheses indicate the square root mean transformation

Table 12. Fungal count of the meat analogues during storage (x 10^3 cfu g^{-1})

Period of storage in months	control	T_4	T_6	Mean
0	Nil (Nil)	Nil (Nil)	Nil (Nil)	Nil (Nil)
3	0.667 (1.025)	0.833 (1.112)	0.667 (1.025)	0.722 (1.054)
6	1.5 (1.403)	1.5 (1.365)	1.667 (1.451)	1.556 (1.406)

Figures in parentheses indicate the square root mean transformation

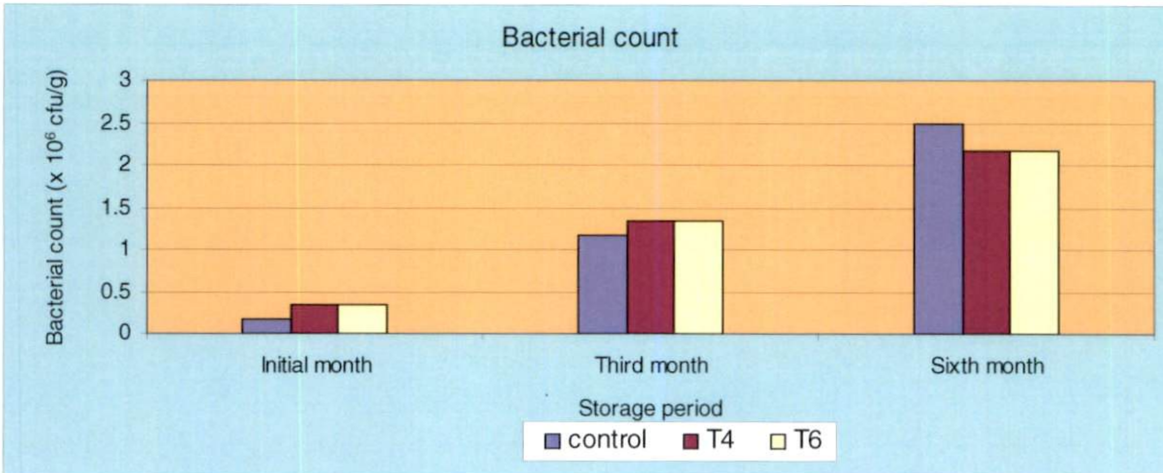

Fig. 9. Bacterial count of meat analogues during the storage period

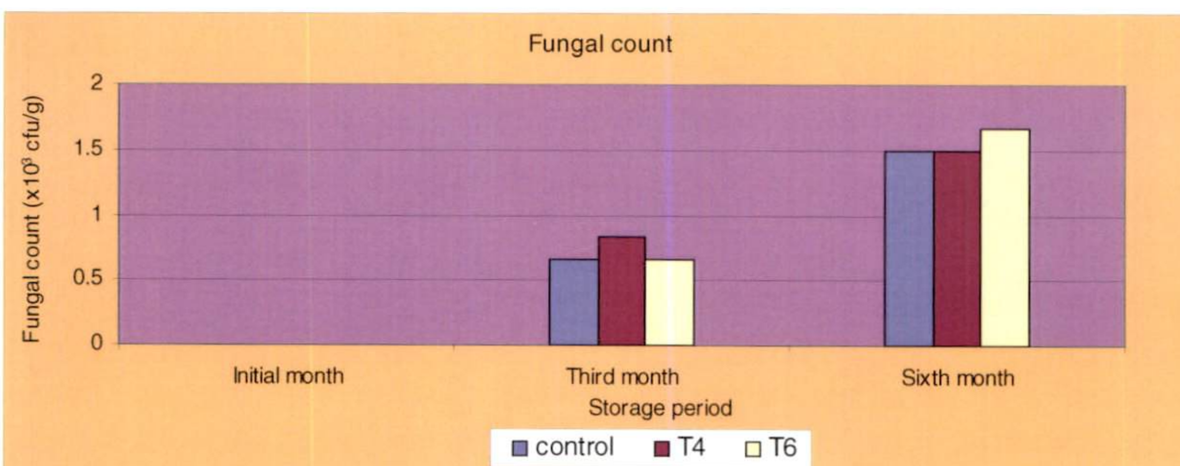

Fig. 10. Fungal count of meat analogues during the storage period

Plate 6. Bacterial growth in control during sixth month of storage

Plate 7. Bacterial growth in T_4 during sixth month of storage

Fungal count of the stored samples are presented in table 12. Fungal count was also evaluated on the basis of two factor CRD. Square root mean transformation was performed where ever necessary. Initially there was no fungal growth in the meat analogues for all the three treatments. Fungal contamination was observed from the third month of storage with 0.667 x 10^3 cfu g^{-1} in control and T_6, and 0.833 x 10^3 cfu g^{-1} in T_4. During sixth month of storage, maximum fungal load was in T_6 (1.667 x 10^3 cfu g^{-1}) and in control and T_4 the same value (1.5 x 10^3 cfu g^{-1}). But the difference observed in the fungal load of treatments during each storage period was not significant, since the difference in the mean fungal transformation was less than the critical difference. Hence the fungal load for all the treatments during the third month of storage can be expressed as a mean value of 0.722 x 10^3 cfu $g^{-1}$and a mean value of 1.556 x 10^3 cfu g^{-1} for the sixth month of storage.

With storage period, the fungal load of 0.667 x 10^3 cfu g^{-1} in third month increased to 1.5 x 10^3 cfu g^{-1}in control, 0.883 x 10^3 cfu g^{-1} to 1.5x10^3cfu g^{-1} in T_4 and 0.667 x 10^3 cfu g^{-1} to 1.667 x 10^3 cfu g^{-1} in T_6. This increase in fungal load in each treatment with storage period was found to be significant since the difference in the mean fungal transformation was more than the critical difference (Fig.10).). The fungal load in the control, T_4 and T_6 during the sixth month of storage is shown in plate 9, 10 and 11 respectively.

The yeast load in the products during storage is presented in table 13. The yeast load in the products were also analyzed via two factor CRD. Square root mean transformation was performed where ever necessary. The initial yeast count for all the three treatments was nil. During the third month of storage, the yeast count was 0.500 x 10^3 cfu g^{-1} in control and T_4 and 0.667 x 10^3 cfu g^{-1} in T_6. During sixth month of storage the yeast count was 0.667 x 10^3 cfu g^{-1} in control and T_4 and 0.833x10^3cfu g^{-1} in T_6. The difference observed in the yeast load between treatments in each storage period was not significant, since the difference in mean yeast transformation was less than the critical difference. Hence the yeast load of the samples during the third month and sixth month of storage can be expressed as a mean value of 0.533 x 10^3 cfu g^{-1} and 0.718 x 10^3 cfu g^{-1} respectively.

During storage, yeast load of control and T_4 increased from 0.500 x 10cfu g^{-1} in the third month to 0.667 x 10^3 cfu g^{-1} in sixth month, and in T_6 it increased from 0.667 x 10^3 cfu g^{-1} to 0.833 x 10^3 cfu g^{-1}. But the increase in yeast load in each

Table 13. Yeast count of the meat analogues during storage (x 10^3 cfu g^{-1})

Period of storage in months	control	T_4	T_6	Mean
0	Nil (Nil)	Nil (Nil)	Nil (Nil)	Nil (Nil)
3	0.500 (0.996)	0.500 (0.939)	0.667 (1.025)	0.553 (0.977)
6	0.667 (1.025)	0.667 (1.025)	0.833 (1.085)	0.718 (1.045)
Mean	0.583 (0.996)	0.583 (0.982)	0.750 (1.055)	

Figures in parentheses indicate the square root mean transformation

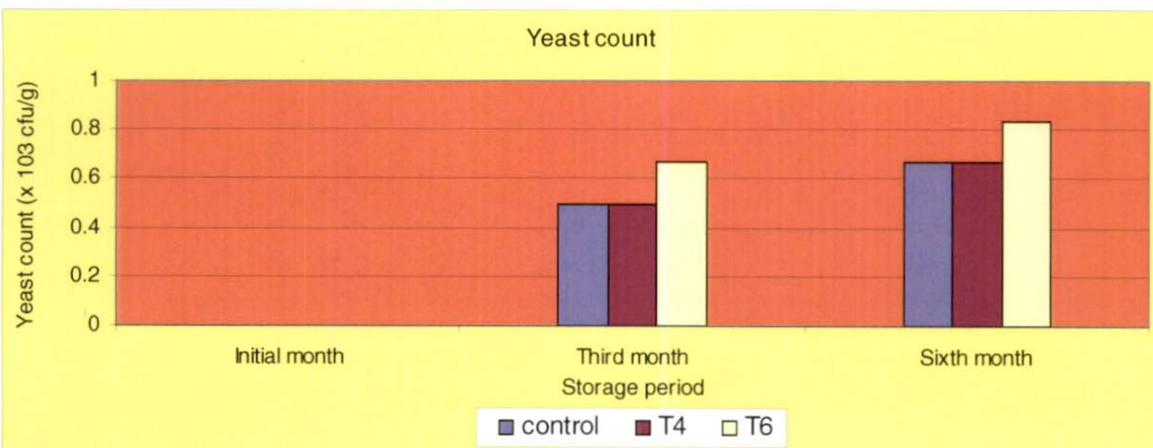

Fig. 11. Yeast count of meat analogues during the storage period

Plate 8. Bacterial growth in T_6 during sixth month of storage

Plate 9. Fungal growth in control during the sixth month of storage

Plate 10. Fungal growth in T_4 during the sixth month of storage

Plate 11. Fungal growth in T_6 during the sixth month of storage

treatment with respect to storage period was not significant, since the difference in mean yeast transformation was less than the critical difference. Hence the yeast load of control and T_4 for entire storage period can be expressed as a mean value of 0.583 x 10^3 cfu g^{-1} and that of T_6 as 0.750×10^3cfu g^{-1} (Fig. 11).

Insect infestation of the meat analogues was also evaluated and no insect infestation was observed in the samples upto a period of six months.

4.4. Organoleptic evaluation of the preparation made with stored meat analogues

The preparations with meat analogues stored for different periods were evaluated organoleptically using a score card with different quality attributes like appearance, colour, flavour, texture, taste and overall acceptability. Each treatment was ranked for all these quality attributes based on mean rank score using Kendall's (W) test. The results of the organoleptic evaluations are presented in this section. When the value of significance between treatments for each quality attribute under each storage period, multiplied by 100, is more than 10, it can be stated that significant difference existed between the treatments in that quality attribute during each storage period.

Appearance

The appearance of the meat analogue preparation initially scored a maximum of 9.05 for T_4, and a minimum of 4.95 for control (Table 14). Then a decreasing trend was noticed during storage. During the third month, there was an increase in the mean score for control (5.40) but for T_4 and T_6 the mean score decreased to 6.90 and 6.00 respectively. During the sixth month of storage, there was a decreasing trend when compared to third month except for T_6, which showed a mean score of 6.60. The appearance score during the sixth month of storage decreased for T_4 (6.00) and T_6 (6.60) compared to the initial scores (9.05 and 6.75 respectively) but for control, appearance score was more (5.10) than the initial score (4.95). There was no significant difference between treatments during each storage period but for the sixth

Table 14. Mean score for appearance of preparation with meat analogues during storage

Treatments	Storage period in months		
	0	3	6
control	4.95	5.40	5.10
T_4	9.05	6.90	6.00
T_6	6.75	6.00	6.60
Kendall's W	0.353	0.240	0.062
Percentage Significance	00.00	9.10	54.0

Table 15. Mean score for colour of preparation with meat analogues during storage

Treatments	Storage period in months		
	0	3	6
control	6.30	5.85	5.25
T_4	9.25	6.60	5.85
T_6	7.90	6.30	6.15
Kendall's W	0.430	0.175	0.025
Percentage Significance	00.00	17.4	77.9

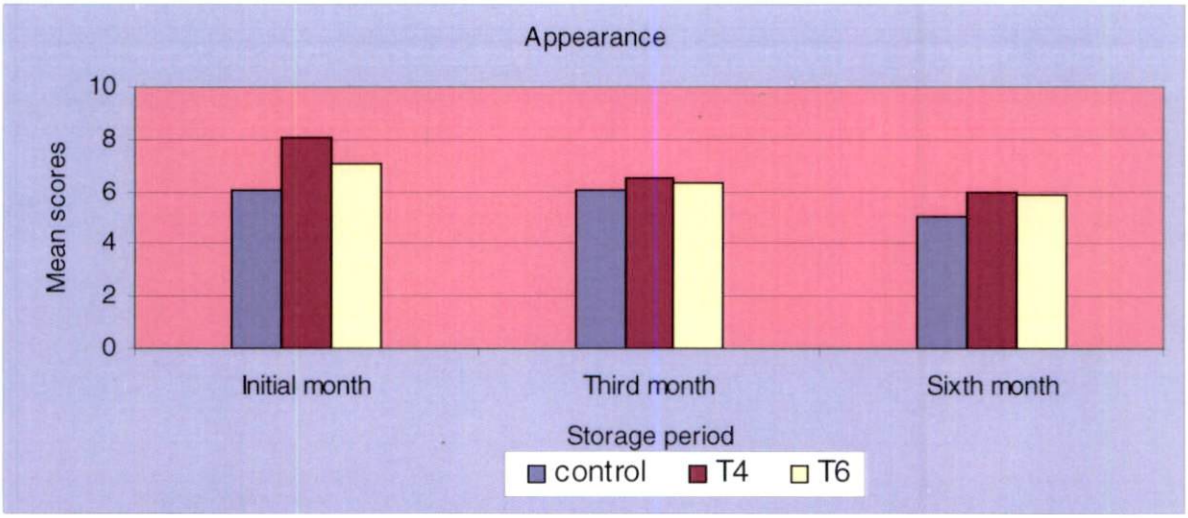

Fig. 12. Mean score for appearance of preparation with meat analogues during storage

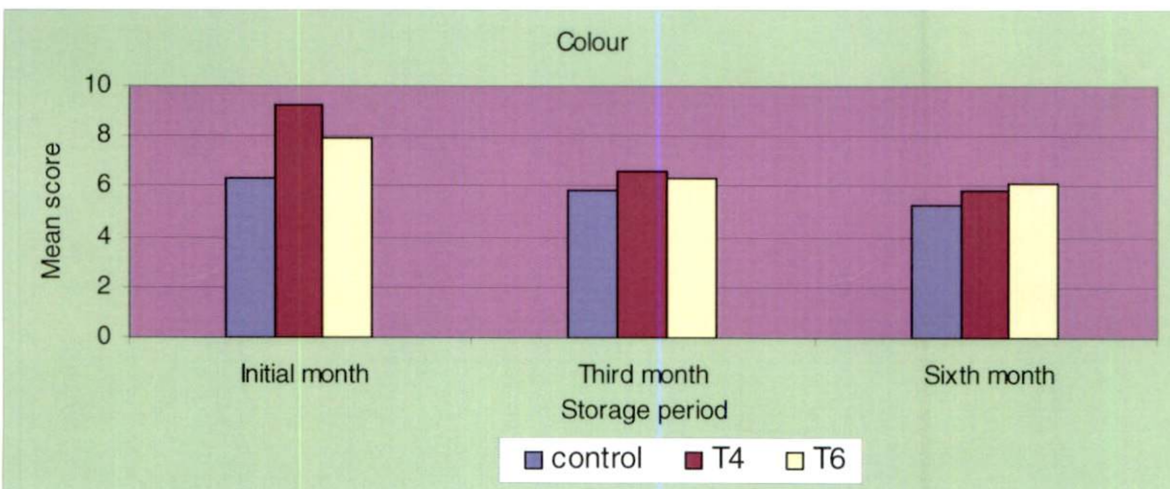

Fig. 13. Mean score for colour of preparation with meat analogues during storage

month of storage, significant difference in the mean score for appearance was observed between treatments (Fig.12).

Colour

The colour of the meat analogues preparation scored maximum during the initial period ranging from 6.30 to 9.25 with a maximum score for T_4 (Table 15). The scores decreased to a range of 5.85 to 6.6 during the third month of storage, and the maximum score was for T_4. The score finally reached to a range of 5.25 to 6.15 at the end of the storage period. During this period the maximum score was for T_6. There was no significant difference between treatments initially but for third and sixth month of storage, significant difference in the mean score for colour was observed between treatments (Fig.13).

Flavour

The attribute flavour scored a maximum value of 8.35 for T_4 and a minimum of 6.65 for control initially (Table 16). This decreased during the third month and ranged from 6.00 to 6.45 wherein the maximum score was for T_6. During the sixth month of storage, there was no change in the score for T_4 (6.00) but decreased to 5.55 and 6.00 in control and T_6 respectively. There was no significant difference between treatments initially but for third and sixth month of storage significant difference in the mean score for flavour was observed between treatments (Fig.14).

Texture

The texture of the meat analogues also showed a decreasing trend during the storage period of six months (Table 17). The maximum initial score was 7.35 for T_6 followed by T_4 (6.80). The score decreased during the third month, with the same mean score of 6.60 for T_4 and T_6 whereas for control it was 4.80. During the sixth month there was a further reduction in texture as observed by the mean score.

Table 16. Mean score for flavour of preparation with meat analogues during storage

Treatments	Storage period in months		
	0	3	6
control	6.65	6.00	5.55
T_4	8.35	6.00	6.00
T_6	6.70	6.45	6.00
Kendall's W	0.280	0.045	0.000
Percentage Significance	0.30	63.60	100.0

Table 17. Mean score for texture of preparation with meat analogues during storage

Treatments	Storage period in months		
	0	3	6
control	6.50	4.80	4.65
T_4	6.80	6.60	6.00
T_6	7.35	6.60	4.65
Kendall's W	0.228	0.386	0.150
Percentage Significance	1.50	2.10	22.30

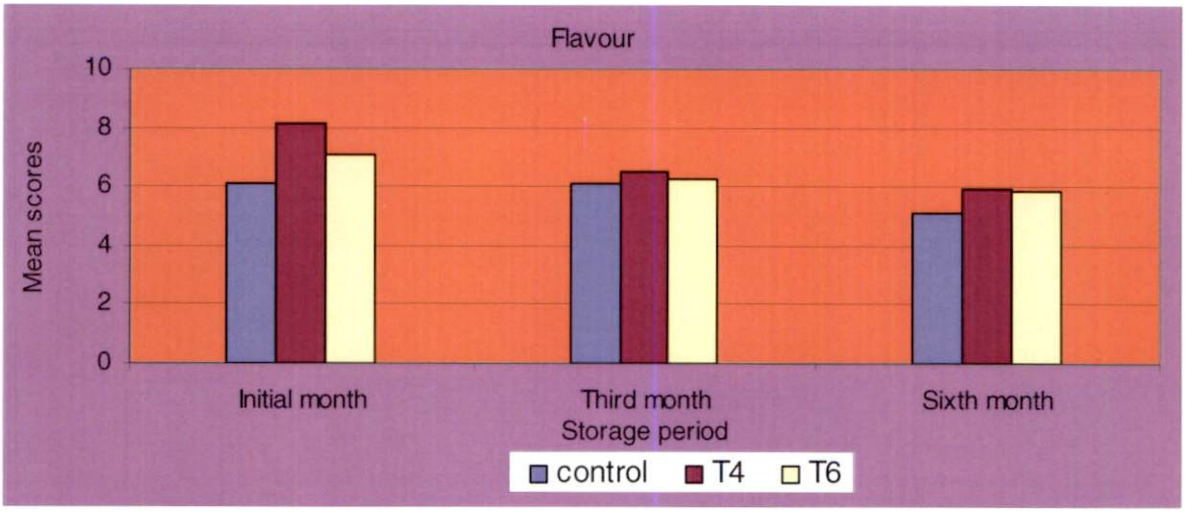

Fig. 14. Mean score for flavour of preparation with meat analogues during storage

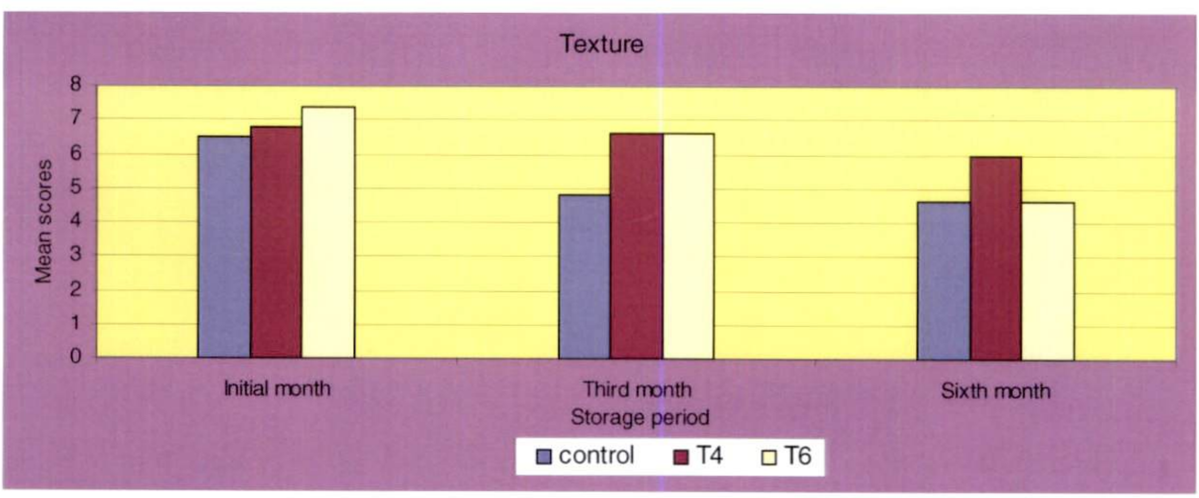

Fig. 15. Mean score for texture of preparation with meat analogues during storage

Maximum score during the sixth month was for T_4 (6.00) whereas control and T_6 had a mean score of 4.65. There was no significant difference between treatments during each storage period but for sixth month of storage, the mean score for texture was significantly high in T_4 compared to control and T_6 (Fig.15).

Taste

The initial mean scores obtained for taste of the meat analogue preparation was maximum for T_4 (7.00) followed by T_6 (6.75) (Table 18). The control had a least score of 6.10. This decreased and ranged from 4.95 to 6.30 during the third month with a maximum score for T_4. The same trend was followed till the end of the storage period with a maximum score for T_4 (6.00) followed by T_6 (5.90). There was significant difference in the mean score for taste between treatments during each storage period (Fig.16).

Overall acceptability

The overall acceptability of the meat analogue preparations was also high initially ranging from 6.10 to 8.09 with a maximum acceptability score for T_4 (Table 19). Control was least acceptable. During the third month of storage, the overall acceptability scores decreased which ranged from 6.10 to 6.48 with a maximum score for T_4. This again decreased and ranged from 5.09 to 5.97 during sixth month of storage but again T_4 obtained the maximum acceptability score. There was no significant difference between treatments initially but for third and sixth month of storage significant difference in the mean score for overall acceptability was observed between treatments (Fig.17).

Table 18. Mean score for taste of preparation with meat analogues during storage

Treatments	Storage period in months		
	0	3	6
control	6.10	4.95	4.95
T_4	7.00	6.30	6.00
T_6	6.75	6.15	5.90
Kendall's W	0.097	0.144	0.188
Percentage Significance	46.70	23.60	15.20

Table 19. Mean score for overall acceptability of preparation with meat analogues during storage

Treatments	Storage period in months		
	0	3	6
control	6.10	6.10	5.09
T_4	8.09	6.48	5.97
T_6	7.09	6.30	5.86
Kendall's W	0.278	0.198	0.085
Percentage Significance	9.70	23.20	53.9

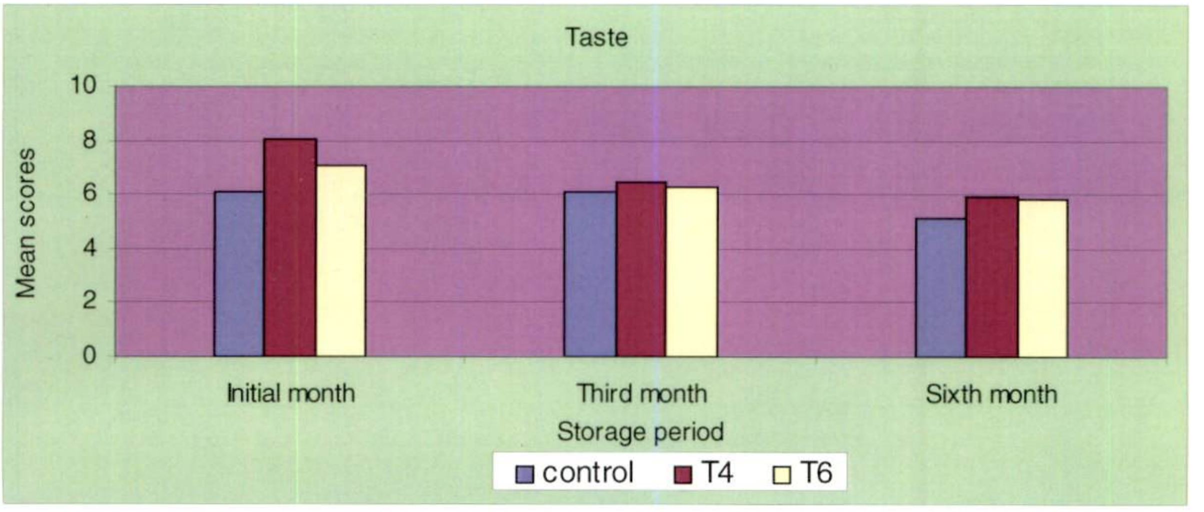

Fig. 16. Mean score for taste of preparation with meat analogues during storage

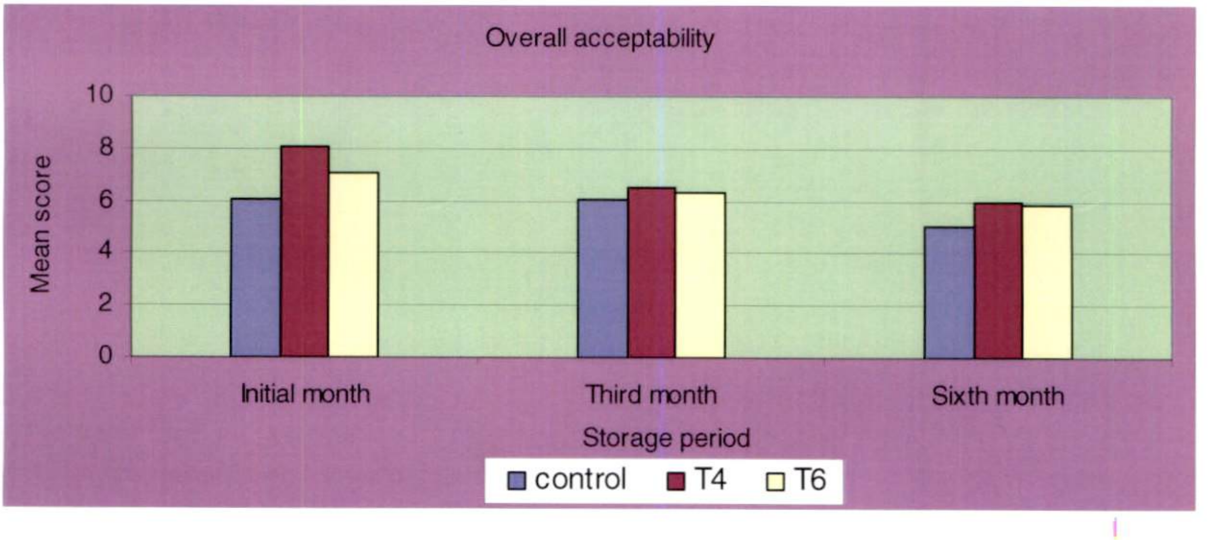

Fig. 17. Mean score for overall acceptability of preparation with meat analogues during storage

4.5. Benefit cost analysis

The cost of the food materials used for the selected meat analogues per kg were worked out and is presented in table 20.

In all the products, the amount of spices and gluten used was fixed and changes were only in the proportion of the grains. Hence, price variation was observed only in the grain ingredient. The cost of food materials ranged from approximately Rs 152 – 155 per kg of the product, the maximum being for T_6 (Rs 155 / kg).

The major contribution to the cost of the product is spices i.e. Rs 71.20 per kg in each product followed by gluten (Rs 46.25 / kg) which was extracted from whole wheat flour.

Table 20. Cost of the food materials used for the selected meat analogue (per kg)

Ingredients	Amounts used (in grams) {Amount spent (in Rs)}		
	Control	T_4	T_6
GRAINS			
1. Green gram	1000 (36.00)	600 (21.60)	800 (28.80)
2. Soya bean	-	100 (7.20)	100 (7.20)
3. Wheat (whole)	-	300 (5.70)	100 (1.9)
Total	1000 (36.00)	1000 (34.50)	1000 (37.90)
SPICES			
1. Ginger	50 (2.60)	50 (2.60)	50 (2.60)
2. Garlic	200 (17.10)	200 (17.10)	200 (17.10)
3. Pepper	100 (19.00)	100 (19.00)	100 (19.00)
4. Cinnamon	50 (32.50)	50 (32.50)	50 (32.50)
Total	400 (71.20)	400 (71.20)	400 (71.20)
GLUTEN			
400g gluten extracted from 2.5 kg wheat flour	400 (46.25)	400 (46.25)	400 (46.25)
TOTAL COST (Rs)	153.45	151.95	155.35

Figures in parenthesis indicates cost in Rupees

Discussion

5. DISCUSSION

The results of the present investigation "Standardization of green gram based meat analogues" are discussed in this section under following headings

5.1 Selection of the most acceptable three treatments

5.2 Quality evaluation of the selected products

5.3 Benefit cost analysis of the products

5.1 Selection of the most acceptable three treatments

Among the nine treatments and the control proposed, the most acceptable three treatments (including control) with maximum organoleptic qualities were selected. According to Kramer and Twigg (1970) the food quality detectable by our senses can be broken down into the main categories viz. appearance, colour, flavour, texture and taste. Quality is the degree of excellence and is a composite character determining the acceptability of a product (Neelofer, 1992).

The mean rank scores of the organoleptic parameters obtained by Kendall's W test (Table 2) did not give a clear picture as to which were the most acceptable three treatments. Hence weight age was given to different quality attributes in the selection of the most acceptable three treatments. More weight age was given to those sensory characteristics for which meat and meat products are valued by the consumers.

Among the organoleptic criteria, texture was given the maximum weight age followed by taste and flavour, while colour and appearance were given the same weight age.

Texture in meat is the ease of separation of lean tissue which determines the tenderness. Tenderness and juiciness are the two quality attributes which are closely associated with meat and tenderness is the most desired characteristic of meat (Srilakshmi, 2003). Texture was given the maximum weight age since Rareunrom *et. al.* (2007) points out that, to be accepted as a simulated meat analogue the product must have a certain texture associated with meat.

The next important criteria was taste followed by flavour, since one values food for its taste, Admin (2007) suggested that a meat analogue should be tasty and similar in structure to meat. Wrick (2007) also accepts this and suggests that meat analogue manufactured should closely approximate meat in sensory qualities primarily texture, flavour and appearance.

The weights assigned here for quality attributes were in such a manner that, in no way it affected the sequential ordering of the combinations. Thus in the present study, the selected treatments with maximum index were T_4 (107.75), T_6 (88.93) and the control (93.73).

5.2 Quality evaluation of the selected products

Quality is the main criterion on which the acceptability of any product depends (Anon, 1995). Hence, the quality evaluation of the product is important which was done on the basis of

5.2.1 Chemical constituents

5.2.2 Microbial quality

5.2.3 Organoleptic qualities

5.2.1 Chemical constituents

The initial moisture content of the meat analogues were found to be 8.8 per cent for control, 8.3 per cent for T_4 and 8.4 per cent for T_6 which was in accordance with the moisture content of sorghum soya based dehydrated foods, which was reported to have a moisture content of 8.2 per cent (Prasad *et. al.*, 2007). The moisture content of all the three treatments increased significantly during the storage period. Maximum moisture pick up after storage period was for control (12.28 %). This might be due to the uptake of moisture from the atmosphere and high relative humidity. The increase in the moisture content in dehydrated food products (skim milk powder) during storage was also been reported by Anon. (2001). The product picked up moisture even though it was packed in metallised polyester polyethylene pouches. Polyethylene pouches have been reported to be good barrier against moisture (Vijayalakshmi *et. al.,* 2003) and metallised polyester has been reported to have a low water vapour transmission rate (Fellow, 2003). The control sample had showed significantly high moisture content (8.8 %) even in the initial period.

The protein content of the control (23.22 %) was significantly low when compared to T_4 (24.84 %) and T_6 (26.00 %) during the initial period, which reduced significantly during the third month of storage. The rate of reduction in the protein content in T_4 (21.06 %) and T_6 (21.35 %) was very high, that after three months there was no significant difference in the protein content between treatments even with the control value (21.88 %). Protein content further reduced during the sixth month of storage which varied from 18.20 to 18.50 per cent, but between treatments there was no significant difference. This was found to closely relate to the protein content (20.69 $\pm$ 0.80 per cent) of meat analogue proposed by Filho *et. al.* (2005). To be accepted as a meat analogue, Hegarty and Ahn (1976) suggested that the products must have a protein content higher than or comparable to the product it is replacing. The protein content of non vegetarian foods ranged from 18.5 per cent (mutton muscle) to 25.9 per cent (fowl) (Srilakshmi, 2003). In the present study protein content of the products during the third month of storage was between 21.06 to 21.88 per cent.

The initial protein content was in close accordance with the protein content of the food products based on rice and soya blend developed by Andrews (1997) which had a protein content of 23.32 per cent. The protein content was found to decrease significantly during the storage period which might be due to the proteolytic action.

The initial fat content was significantly low in control (1.23 %) compared to T_4 (2.67 %) and T_6 (2.20 %). A significant reduction in the fat content was observed during storage and finally the fat content significantly varied between treatments with the highest value in T_6 (1.57 %) followed by T_4 (1.33 %). This was found to closely relate with the finding of Filho *et. al.* (2005) who found a fat content of 1.92 $\pm$ 0.37 per cent in the developed meat analogue. A similar fat content was reported by Prasad *et. al.* (2007) in sorghum soya based dehydrated foods. A decrease in the fat content was noticed which may be due to lypolytic action. When compared to meat, the fat content in the developed products is low and is of unsaturated in nature and has no cholesterol since the ingredients are of plant origin. Thus the quality of fats in meat analogues are superior when compared to meat and hence can be recommended in protein rich diets for patients who have to restrict animal foods due to high cholesterol content.

The initial fibre content was found to be significantly low in control (1.33 %) and a significant reduction in the fibre content was observed during storage, such that finally there was significant variation in the fibre content with T_6 (1.035 %) having the maximum fibre content. The reduction in the fibre content during storage may be due to the action of the microorganisms on unavailable carbohydrates. A significant increase in bacterial load was also observed in the products during storage.

The calcium content was found to be 276mg $100g^{-1}$ for all the three treatments and was observed to be constant during the storage period with only a slight decrease in the control (253mg /100g) but the difference was not significant. The calcium content was slightly higher than the calcium content of texturized vegetable protein (250 mg / 100g) to be used as a meat analogue (Osho, 2003).

During the initial period, T_6 (190.85mg /100g) and T_4 (190.82mg /100g) had significantly high values for phosphorous than control (177.94mg /100g). Phosphorus value significantly got reduced during storage to a range of 81.19 to 89.71mg $100g^{-1}$ with no significant difference between the treatments. The phosphorus value of the product in the present study during the initial and third month of storage was quite high compared to that reported by Banman (2008) i.e. 110mg $100g^{-1}$ in meat analogue. The phosphorus value in the sixth month of storage was also quite high as compared to the phosphorus content reported by Filho *et. al.* (2005) in meat analogue i.e 63.30 per cent. The initial phosphorus values of the products were closely related to the phosphorus content of flesh foods i.e. 150mg / 100g in mutton and 189 mg / 100g in buffalo meat (Gopalan *et. al.,* 1994).

In the initial period there was no significant difference in the iron content of T_6 (1.89mg /100g) and T_4 (1.87mg /100g) and control (1.77mg /100g) but during the third and sixth month of storage iron content was significantly low in control. A significant reduction in iron content was observed in each treatment during the storage. The result are in accordance with the iron content (1.65 $\pm$ 0.12mg 100g^{-1}) as reported by Filho *et. al.* (2005) in meat analogue. A decrease in the iron content during storage was also reported by Misfa *et. al.* (2000) in cereal grain products.

The initial potassium content was observed to range from 72.00mg 100g^{-1} to 73.00mg 100g^{-1} and by the end of the storage period it was found to be in a range between 56.33mg 100g^{-1} to 58.66mg 100g^{-1}. A significant reduction in the potassium content was observed in each treatment during storage, but the difference observed in potassium content between treatments in each storage period was not significant. The potassium content in the developed meat analogues were quite higher than that reported by Banman (2008) in a meat analogue (60mg 100g^{-1}) developed by him.

Nutritionally the products were found to be in close comparison with meat in nutrients like protein, calcium and iron while it also contained fibre and unsaturated fat which are not found in meat. Besides, the products are also free from cholesterol. So the products can be considered as nutritionally acceptable as suggested by Kuntz (1995) that, it is important that meat analogue should maintain the nutritional equivalence to the product it is replacing.

5.2.2 Microbial quality

Microbial quality of the product is the result of several factors such as quality of raw materials, storage and processing temperature, processing technique and environment, storage container etc. (Bryan, 1974, Nanu *et. al.*, 1992). There has been a strong and growing demand for foods that is natural, convenient and containing few additives. Although there has been a marked increase in the meat analogue foods, the safety of these foods should be fully evaluated (Messina and Messina 1997).

The bacterial load of treatments during the initial period was found to range from 0.167 to 0.333 x 10^{6} cfu g^{-1}. During the third month, the range increased to 1.167 to 1.333 x 10^{6} cfu g^{-1} and further increased to 2.167 to 2.5 x 10^{6} cfu g^{-1}. The difference observed between the treatments in each storage period was not significant. Hence the bacterial load of all the three treatments in the initial period can be expressed as a mean value of 0.278 x 10^{6} cfu g^{-}1,and as a mean value of 1.278 x 10^{6} cfu g^{-1} during the third month, and a mean value of 2.278×10^6 cfu g^{-1} during the sixth month of storage. But there was a significant increase in the microbial load in each treatment with storage period. During third month of storage, T_4 and T_6 showed higher bacterial load (1.333×10^6 cfu g^{-1}) than control (1.167×10^6 cfu g^{-1}) but after sixth month, microbial load in control was significantly high (2.5×10^6 cfu g^{-1}). This comparatively high bacterial load in the control can be due to the high moisture content of control (12.28 %) after sixth month of storage. It can also be due to the comparatively high initial microbial load of the control (0.167×10^6 cfu g^{-1}).The bacterial count so found for the products in the present study was less than that reported by Fihlo *et. al.* (2005), who studied the microbiological quality of canned meat analogue for a period of twelve months and reported a minimum bacterial count of 3.15 ± 0.22 and a maximum of 3.28 ± 0.15. Anon., (2001) also indicated that, an increase in the moisture content allowed bacterial growth in dried skim milk powder stored at a temperature of 37° C with a relative humidity of 90 per cent for a period of three months.

Initially no fungal or yeast count was noted in the products. Since there was no significant difference with fungal load between treatments during each storage period, the fungal load for all the treatments can be expressed as a mean value of 0.722×10^3 cfu g^{-1} during the third month, and as a mean value of 1.556×10^3 cfu g^{-1} during the sixth month of storage. But a significant increase in the fungal load was observed in each treatments with storage period. After sixth month, fungal load was significantly high in T_6 (1.667×10^3 cfu g^{-1}). With yeast count also, there was no significant difference between treatments in each storage period which can be expressed as a mean value of 0.553×10^3 cfu g^{-1} for all treatments during third month, and as a mean value of 0.718×10^3 cfu g^{-1} during the sixth month of storage. The difference observed in yeast count in each treatment with regard to storage period was also not significant. This was again found to be lower than the fungal and yeast count reported by Filho *et.al.* (2005) in the canned meat analogue which showed a minimum count of 2.95 ± 0.34 and a maximum of $3.13 \pm 0.28 \times 10^3$ cfu g^{-1}.

The low microbial burden found in the products of the present study may be due to the use of spices like cinnamon, in its preparation, which contains cinnamic aldehyde that is reported to have antimicrobial activity (Bullerman *et.al.,* 1977). Fung and Gailani (1986) reported that the dried products in general is the safest mode of food storage since dry state does not promote microbial survival or growth and may maintain the population at undetectable levels.

There was also no insect infestation in the products during storage. The low microbial load and lack of infestation may be due to the packaging of the products in metallised polyester polyethelyne laminate pouches. Potter and Hotchkiss (1996) has reported that, laminate pouches are excellent barrier against microorganism and dirt. Aluminium foil laminate pouches have been reported to preserve the food products with minimum microbial load in mango bar (Singh *et. al.*, 2003).

5.2.3 Organoleptic qualities

According to Watts *et.al.* (1984) the information on specific sensory characteristics of a food must be obtained by product oriented tests. Organoleptic qualities can be defined as qualities affecting the body organs or senses particularly, of a combination of taste and aroma (Sindhu, 1995). Sensory evaluation of foods is assumed to be increasing significantly as this provides information which may be utilized for the development of the product and its improvement (Nair, 1999).

A gradual decrease in the total mean score for each quality parameter in all the three treatments was noticed during the storage period. There was no significant difference in the appearance of the products during initial and third month of storage, but during the sixth month, T_6 (6.60) had significantly high score for appearance. Regarding colour, even though there was no significant difference during the initial period, during the third month mean score for colour was significantly high for T_4 (6.60) followed by T_6 (6.30), but by sixth month the mean score became significantly high in T_6 (6.15) followed by T_4 (5.85). There was no significant difference in the flavour of the products initially but during the third month, T_6 (6.45) had the highest score for flavour and by sixth month both T_6 and T_4 had the same score (6.00).

Texture of the products showed no significant variation in the initial and third month but after sixth month, score for texture was significantly high in T_4 (6.00). Regarding taste there was significant difference in the score even from the initial period, and T_4 scored the highest in the initial (7.00), third month (6.30) and sixth month of storage (6.00). This difference in the quality attributes had also been reflected in the overall acceptability of the products. There was a significant reduction in the overall acceptability of the products during storage. Initially there was no significant difference in the overall acceptability scores for the treatments but during the third month and sixth month, overall acceptability was significantly high in T_4 (6.48 and 5.97 respectively) followed by T_6 (6.30 and 5.86 respectively). This high overall acceptability for T_4 is contributed by the high scores in attributes like colour, appearance, texture and taste in the third month and high score for flavour, texture and taste during the sixth month.

The gradual decrease in the overall acceptability was mainly due to the hardness in the products. The hardness may be due to the use of gluten in the products whose firming property has been reported by Kobs (1999), while it is this property of gluten that makes it a valuable ingredient in simulated meat. Wrick (2007) have reported that when gluten is cooked in a broth, it absorbs the surrounding liquid and becomes firm to bite thus used in vegetarian cuisine. In the present study, actually 80g of gluten was proposed per 100g of ingredients used for meat analogue preparation. But during the pilot study the products were found to be too hard so gluten was reduced to 40g per 100g of ingredient, with acceptable texture of the products. Even then, the texture became hard during storage. So further studies are needed to standardize the amount of gluten to be used with respect to the type of ingredients used for meat analogue preparation. Hsu and Sun (2006) also reported that there was disagreement among the consumers regarding the sensory qualities of meat analogue. Hsu and Chung (1998) reported that consumers prefer a harder texture while Hsu and Yu (2002) reported that the harder texture could have a negative effect on the sensory qualities.

5.3 Benefit cost analysis and acceptability of the product

The cost of preparation of the products were worked out and it was compared with a similar product in the market i.e. soya chunks, a texturized vegetable protein. The cost of one kg of prepared meat analogue varied from approximately Rs 152 – 155 whereas the market price of soya chunks is Rs 100 per kg.

The high cost of production of the developed products is due to several reasons that can be overcome by large scale production.

1. In the present study, for the preparation of meat analogues, all the raw materials (food ingredients) in limited quantities were purchased from the local super market whereas soya chunks produced in large scale in factories, where the main raw material i.e. soya bean is procured from the farmers by the manufacturer itself. This substantially reduces the cost of product. Besides they do not contain any spices and are bland in taste.

2. In the preparation of meat analogues at laboratory level, wheat gluten is used as a texturizer of the product. In this study gluten was extracted from whole wheat flour which itself is costly. This also added to the cost of the products. In large scale production of meat analogues, commercial gluten is used which is a by product of the wheat processing industries in the manufacture of gluten free wheat products.

The above two factors which increased the actual cost of production of the meat analogues can thus be overcome by large scale production. Further, soya chunks in the market, is less acceptable due to beany taste and bland flavour whereas the developed products are acceptable with added spices which gives it a flavour that resembles meat preparations.

When compared to the market price of beef (Rs120 / kg) the products are costlier but beef consumption is limited in other parts of our country due to religious beliefs. So, when compared to the cost of mutton (Rs 170 / kg) which is popularly used in India, these products are economical.

When compared to red meat with respect to the nutritional quality and health aspects, the developed products had the benefit of good quality protein with unsaturated fat since it is a product of plant origin. It can be consumed by non vegetarians, who have to avoid meat for health reasons such as diabetes, CVD, hypertension etc. Besides good quality proteins, these products also contain unsaturated fat and fibre that is lacking in meat / mutton.

Because of the above advantages over meat, this product can be commercialized which will reduce the cost significantly and also it had the various benefits as mentioned above.

These products can also be used in therapeutic diets as a source of good quality acceptable protein comparable to animal protein without the adverse effects of meat and with the added benefit of fibre. Knox (2000) also reported that the meat analogue may appear some what pricy at the first glance but they compare quite well to meat. And meat substitute consumers, as suggested by Wong (2006), agrees that the price quality relation when buying foods was most important to them.

Summary

6. SUMMARY

To develop a product which could simulate nutritional and sensory qualities of meat and to evaluate the shelf life qualities of meat analogue a study was undertaken entitled "Standardization of green gram based meat analogues". Meat analogue was prepared by combining ingredients like green gram, wheat and soya in different proportions along with spices like ginger, garlic, cinnamon and pepper as flavouring and taste enhancing agents and wheat gluten as texturizing agent. The ingredients required for the preparation of meat analogues were collected from the local market.

The ingredients were subjected to pre treatments prior to the preparation of the meat analogues. Green gram was first roasted and then soaked for 12 hours. Wheat and soya were also soaked for 12 hours. Sodium bicarbonate was added to the soaking water of soya. After soaking, soya was pressure cooked for 15 minutes. The soaked grains were then ground with 40g of gluten and 40g of spices per 100g of the grain in appropriate proportions, to a coarse paste. This was then spread in trays and steamed for 35 – 40 minutes. After cooling, it was cut into half inch pieces and then dried in a cabinet dryer at 65° C for 18 hours.

A standardized procedure for preparing the meat recipe was followed to prepare the meat analogue recipe by replacing meat. This was organoleptically evaluated by a panel of ten judges rating for different quality parameters, like appearance, colour, flavour, texture, taste and overall acceptability, on a nine point hedonic scale. The scores obtained for the ten treatments were statistically analyzed by Kendall's (W) test. Then an index was worked out after assigning weights to different quality attributes giving more importance to the parameters valued for a meat substitute. The ranks obtained by different treatments after calculating the index was used for selecting the most acceptable three treatments. T_4, control and T_6 were selected since they obtained a final score of 107.75, 93.73 and 88.93 respectively.

The selected three treatments (100g/pack) were stored for a period of six months in metallised polyester polyethylene laminate pouches under ambient storage conditions. Quality evaluation of the meat analogues were carried out by assessing the chemical constituents, organoleptic characteristics and microbial load with respect to bacteria, fungi and yeast at an interval of three months up to a period of six months.

The moisture content of the meat analogues ranged from 8.3 per cent to 8.8 per cent during the initial month of storage and finally reached to a range of 11.13 to 12.28 per cent by the end of the storage period. There was a significant increase in the moisture content during the storage.

The protein content was found to be in a range of 23.22 per cent to 26.00 per cent initially, which reduced significantly to a range of 21.06 per cent to 21.88 per cent by the third month of storage. By the end of the storage period the protein content decreased even further below to a range of 18.20 per cent to 18.84 per cent.

A significant reduction in the fat content was observed in storage and finally the fat content significantly varied between treatments with the highest value in T_6 (1.57 %) followed by T_4 (1.33%).

Initially the fibre content was found in a range between 1.33 per cent to 1.82 per cent which also reduced to a range of 1.10 per cent to 1.36 per cent during the third month and then decreased to a range of 0.87 per cent to 1.03 per cent during the sixth month. Finally there was significant variation in the fibre content, with T_6 (1.04 %) having the maximum fibre content.

The calcium content was found to be similar for all the three treatments during the initial month i.e. 276 mg $100g^{-1}$ which then reduced to 253 mg $100g^{-1}$ for control during the third month and then remained constant for the rest of the storage period, while the calcium content for T_4 and T_6 remained same from the beginning to the end of storage period.

The phosphorus content was initially observed to be in a range of 177.94mg, to 190.85mg $100g^{-1}$ which reduced significantly on storage ranging from 81.19mg to 89.71mg $100g^{-1}$ during the last month of storage without any significant difference between treatments.

Initially the iron content ranged from 1.77mg to 1.89mg $100g^{-1}$. This reduced to a range of 1.18mg $100g^{-1}$ to 1.48mg $100g^{-1}$ in the third month and then reduced to a range of 0.93mg $100g^{-1}$ to 1.05mg $100g^{-1}$ at the end of the storage period. Iron content was significantly low in control after storage period.

The potassium content of the meat analogues were in a range of 72.00mg to 73.00mg $100g^{-1}$ initially. This then decreased to a range of 62.66mg $100g^{-1}$ to

66.00mg $100g^{-1}$ during the third month and finally ranged from 56.53mg to 58.66mg $100g^{-1}$ during the sixth month of storage.

The microbial load was also evaluated with respect to bacteria, fungi and yeast. The bacterial mean count ranged from 0.167×10^6 to 0.333×10^6 cfu g^{-1}. A gradual increase was noticed during the third month and finally a mean count ranging from 2.167×10^6 to 2.5×10^6 cfu g^{-1} was observed during the sixth month of storage.

The fungal and the yeast count for all the three treatments was observed to be nil during the initial month which gradually increased with a mean count of 1.5×10^3 to 1.667×10^3 cfu g^{-1} for fungi by the end of the storage period. The yeast load increased with a mean count ranging from 0.667×10^3 to 0.833×10^3 cfu g^{-1} during the sixth month of storage period.

From the organoleptic point of view, a reduction in the scores for various quality parameters was noticed which affected the overall acceptability of the products. High overall acceptability observed for T_4 (5.97) was mainly contributed by the high score for attributes like colour, appearance, texture and taste in the third month and high score for flavour, texture and taste during the sixth month.

When compared to soya chunks, these products were found to be costly, cost ranging from approximately Rs 152 – 155 per kg. The price of these products developed in the laboratory is comparatively higher than its available substitutes in the market, which can be reduced considerably through large scale production. Hence, cost factor alone cannot be considered as a constraint, in the development of meat analogues.

Even though there was a reduction in the chemical constituents during storage, nutrients like protein, calcium and iron of T_4 and T_6 were comparable to meat. More than meat, it had the added quality of unsaturated fats and fibre and is also cholesterol free.

The low microbial load of the products is also a point of significance. Regarding acceptability the treatment T_4 was found to be highly acceptable followed by T_6.

Since the products are of plant origin, this can be consumed by the vegetarians. This can also be consumed by the non vegetarians who have to avoid meat for health reasons. This product can also be used in therapeutic diets as a source of good quality protein of acceptable sensory qualities comparable to meat but without the adverse effects of meat.

Hence T_4 (60% green gram, 10% soya and 30% wheat) and T_6 (80% green gram,. 10% soya and 10% wheat) can be recommended as a meat analogue with acceptable qualities.

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* Originals not seen

Appendices

APPENDIX-I

Standard meat recipe

Ingredients

Meat – 1kg

Onion (sliced) – ¾ kg

Garlic (chopped) – 6-7 flakes

Coriander powder – 2 tbsp

Chilli powder – 1½ tsp

Turmeric powder – ¼ tsp

Pepper powder – ¼ tsp

Garam masala powder – ¼ tsp

Green chilies (slit) – 2-3nos

Curry leaves – few

Vinegar – 1 tsp

Coconut milk (dilute) – 2 cups

Coconut milk (thick) – 1cup

Oil – 2 tbsp

Salt – to taste

Procedure

1. Heat the oil in a pan and add the sliced onions, green chillies and sauté it till they become golden brown.
2. Then add the ground spice paste i.e. a paste of coriander powder, chilli powder, turmeric powder, pepper powder, garlic and garam masala powder, and sauté it for another 5 mins.
3. Then add the meat pieces, salt and vinegar and stir for 5-7 mins.
4. Add the dilute coconut milk and allow it to boil till the pieces are cooked.
5. Finally add the thick coconut milk and cook to get the desired consistency.
6. Add curry leaves.

APPENDIX-II

Score card for organoleptic evaluation of meat analogue recipe

Date: Name of the judge:

No	Character	1	2	3
1	Appearance			
11	Colour			
111	Flavour			
1V	Texture			
V	Taste			
V1	Over all acceptability			

Evaluate the product on the basis of the scores given below

Description	Score
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

Signature

STANDARDIZATION OF GREEN GRAM BASED MEAT ANALOGUES

By

NISHA

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Home Science

(FOOD SCIENCE AND NUTRITION)

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2008

// ABSTARCT

Meat analogue, a simulated product that resembles meat, is suggested as the food for future owing to its nutritional qualities, beneficial health effects and sensory characteristics. In the present study an attempt was made to develop a product possessing both the nutritional and sensory characteristic of meat. It is health beneficial and at the same time is at par with the shortcomings of meat.

Meat analogues were prepared using green gram as the base, blended with soya bean and wheat in different proportions. Gluten was used as a texturizing agent and spices like ginger, garlic, pepper and cinnamon were used for flavouring. Totally ten treatments were tried with different proportions including one control with 100 per cent green gram.

A standard meat recipe was prepared by replacing meat with the developed products and were evaluated organoleptically using a nine point hedonic rating scale. Three treatments were selected based on maximum index scores, T_4 (107.75), control (93.73) and T_6 (88.93). The selected three products were packed in metallised polyester polyethylene laminate pouches and kept for shelf life studies for a period of six months. Chemical constituents, sensory qualities and microbial load were analyzed during the initial period, third and sixth month of storage. The products were also subjected to benefit cost analysis by comparing with a similar product available in the market and also with fresh meat and mutton.

Meat analogue was observed to be a good source of protein; with a maximum protein content in T_6 (26.00g per 100g), invisible fat; which ranged from 1.23g to 2.67g $100g^{-1}$ with a maximum fat content in T_4, fibre; ranging from 1.33g to 1.82g $100g^{-1}$ with T_6 having the maximum fibre content, and minerals like calcium (276mg $100g^{-1}$), potassium; ranging from 72.00mg to 73.00mg $100g^{-1}$, phosphorus; ranging from 177.94mg to 190.85mg 100 g^{-1} and iron ranging from 1.77mg to 1.89mg 100 g^{-1}. However, on storage there was a significant reduction in the nutrients. A gradual increase in the microbial load was also noticed with the advancement of storage period. The bacterial and the fungal load increased significantly, from 0.167 to a maximum of 2.5×10^6 cfu g^{-1} and from zero to 1.667×10^3 cfu g^{-1} respectively, whereas the yeast count remained insignificant with a maximum count of 0.833×10^3

cfu g^{-1}, even at the end of the storage period. However no insect infestation was noticed till the end of storage.

Organoleptic evaluation was done by preparing a recipe as in the case of treatment selection. The evaluation revealed that there was decrease in the organoleptic attributes which affected the over all acceptability of the products. High over all acceptability was observed for T_4 (5.97). This was mainly contributed by the high scores for attributes like colour, appearance, taste and texture in the third month and high score for flavour, texture and taste during the sixth month.

The price of the developed products is comparatively higher than its available substitute in the market. The cost can be reduced considerably through large scale production. Hence, considering the health benefits compared to red meat, cost factor alone cannot be considered as a constraint in the development of the meat analogues. Therefore in this study, treatments T_4 (60% green gram, 10% soya and 30% wheat) and T_6 (80% green gram, 10% soya and 10% wheat) with comparable nutritional qualities and acceptability can be recommended as a meat analogue for popularization and large scale production.