STANDARDISATION OF JACKFRUIT AND BREADFRUIT INCORPORATED MEAT ANALOGUES

By

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(2020-16-009)

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Standardisation of jackfruit and breadfruit incorporated meat analogues"** is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed during the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Date: 16/02/2023

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CERTIFICATE

Certified that the thesis entitled **"Standardisation of jackfruit and breadfruit incorporated meat analogues"** is a bonafide record of research work done independently by Ms. NOVA HENNA JEMIMAH KAILA under my guidance and that it has not been previously formed during the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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NOVA HENNA JEMIMAH KAILA

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Introduction

1. INTRODUCTION

Meat holds an irreplaceable place in terms of nutritional significance, organoleptic characteristics and the variety it brings to the plate. It's two folded role in culinary around the globe is obvious as it serves the part of being the source for all essential amino acids that are quintessential for tissue growth, maintenance and its repair whilst the other part of it laid a strong foundation for the growth and development of food industry and the industry continues to apply technologies to impart specific functionalities to the product of interest.

Consumer trends and behavior towards meat is under constant change due to which meat consumption patterns are unpredictable. Factors such as price, appearance, convenience, quality, safety, social, individual, economic and cultural aspects have the potential to influence consumer decision.

Gelation and other properties like meat particle binding and adhesion, emulsification and water holding capacity are the functionalities which are principal to processed meats. During the forecast period of 2020-25, the global meat market is estimated to grow at a compound annual growth rate of 7.35 per cent (Boukid, 2021).

Meat analogue is a food product that approximates the aesthetic qualities and chemical characteristics of meat. These are made from non-animal proteins and are simulated meat like products with similar texture, flavour, colour, and nutritive value which can be substituted directly for meat to all sections of the society. Meat analogues are the products in which plant proteins and non conventional proteins can be made into one unit within the three dimensional network of the compound (Kumar *et al.*, 2017).

Recent years witnessed a shift in interest and preferences among consumers towards plant based foods ever since health consciousness made human population give priorities to safe and healthy products. A potential reason to this transition is a known link between increased likelihood of diseases like obesity, maturity onset

diabetes, cardiovascular diseases and consumption of processed meat products. Additionally, the trend in production of healthy and delicious meat free food for satisfaction of vegetarian and personal well being resulted in the increased use of low cost vegetable proteins such as textured soy protein, mushroom, wheat gluten, pulses, *etc.* as a substitute for animal proteins (Kolodziejczak *et al* ., 2021).

Meat analogues are manufactured to have high biological value, better organoleptic properties and acceptance among people. The main source of meat analogues are vegetable proteins such as pulses, nuts, cereal proteins, vegetables and mycoproteins which influence flavour and textural aspects of the final product when mixed in adequate proportions (Kumar *et al.,* 2017).

Chickpea is a pulse crop with significant nutritional quality in terms of proteins and composition of amino acids. The excellent emulsifying and water binding capacity, along with its meaty flavour, texture and aroma makes it a promising ingredient in meat analogue. Cowpea is rich in potassium with good amount of calcium, magnesium and phosphorus. The nutritional quality of cowpea comes from its protein and carbohydrate content. Cowpea's protein level represents its major advantage for use in nutritional products (Jayathilake *et al*., 2018).

There certainly is a need for new interventions which increases the nutritional and physicochemical value of meat analogues and utilization of indigenous food sources is a promising way to achieve this. Tender jack fruit and breadfruit which are alike to meat in its characteristics, flavour and often used by vegans as meat replacer is a good choice for the incorporation into meat analogues.

Jackfruit, considered as poor man's food, is abundant in carbohydrates, proteins, fats, fibre and also in micronutrients like calcium, phosphorous, iron and vitamins. Major sugars are fructose, glucose and sucrose and predominant fatty acids are lauric acid, palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid whilst jacalin is the predominant protein.

Tender jackfruit is known for its antioxidant, anti-inflammatory, antibacterial, anti carcinogenic, anti-fungal, antineoplastic, hypoglycaemic, wound healing properties. *Artocarpus heterophyllus* holds a prominent place in dietary use as it is an important source of carbohydrates, protein, fat and micronutrients such as vitamins and minerals. It is shown in phytochemical studies that they possess useful compounds like sterols, flavonoids, prenyl flavones which give them distinct pharmacological properties (Baliga *et al.,* 2011).

Another remarkable species of the genus *Artocarpus, A. altilis* called breadfruit, is abundant in carbohydrates, protein, fatty acids, pro-vitamins, potassium, calcium, iron and dietary fibre along with many phytochemicals like triterpenes and flavonoids which give it many therapeutic benefits (Panghal *et al.,* 2001). Breadfruit flour which is also has significant physicochemical properties such as higher water absorption index, better oil absorption capacity, pliability and plasticity due to which it has been used as an ingredient in a wide range of processed foods, including meat, instant baby food, quick bread, instant cake, and instant noodles (Huang *et al*., 2020). Breadfruit also has a considerable untapped potential as a nutritious food particularly among the low-income groups of the society in developing countries, and has an advantage over cereals and tuber as it yields two or three times as much minerals and vitamins when compared to cereals and tubers (Sun *et al.,* 2021).

Cereals are composed of protein fractions namely prolamins, glutenins and gliadins. Gluten present inwheat has a cohesive, viscoelastic property which gives the needed textural property to the meat analogues accompanied by functional properties like solubility, viscosity, swelling (Panghal *et al.,* 2001).

Addition of mushrooms and soy protein can increase the quality of meat analogues with respect to its nutritional aspects and sensory appeal. Mushrooms can serve as a good source of protein, dietary fibre and mineral matter. Its natural protein makes it fibrous and imparts chewability simultaneously enhancing the protein and

lipid profile along with ample fibre content (Kumar *et al.*, 2017). Soy proteins from soy bean are incorporated in various physical forms of flour, soya bits, soya protein isolates, spray dried soya milk, texturized soya proteins, defatted soy flour, *etc.* each having respective distinctive properties. Its protein quality with score of 1.0 on PDCAAS (Protein Digestibility Corrected Amino Acid Score) scale, equivalent to animal meat, makes it even more acceptable among the consumers. Soya also contributes to the fibrous nature of meat analogues due to disulphide bonding which simulates meat texture. Defatted soy flour is also potential source of food protein, amino acids, ash and isoflavones (Kang *et al*., 2017).

Meat analogues contribute to the nutritional well being as they act as a dietary part of safeguarding the body against heart disease due to the presence of PUFA (poly unsaturated fatty acids) specifically the alpha linolenic acid (18:3 $\Delta^{9,12,15}$) which is widely known for its ability to suppress the pathogenesis of maladies like cardiovascular diseases, auto immune diseases, cancer, *etc.*. Being abundant in proteins, vitamins and minerals they're further nutritionally refined by adding fibre through other plant sources like vegetables due to which they are suitable for physiological complications like diabetes and obesity. They possess folate, phytochemicals, antioxidants which maintain nutritional self sufficiency. Introduction of plant based meat analogues is an efficient way to improve human health, conserve natural resources and secure welfare of animals (Sebastian *et al.*, 2021).

Meat analogues hold a tremendous scope in both food industry and its market due to which there is a need to refine its nutritional quality further with indigenous plant sources whilst still maintaining its organoleptic characteristics. So, the present study entitled "Standardisation of jackfruit and breadfruit incorporated meat analogues" was undertaken with the following objectives.

1. Standardisation of tender jackfruit incorporated meat analogues
2. Standardisation breadfruit incorporated meat analogues
3. Quality evaluation of selected meat analogues

Review of literature

2. REVIEW OF LITERATURE

The relevant literature on the study entitled "Standardisation of jackfruit and breadfruit incorporated meat analogues" has been briefly reviewed here under the following subtitles.

2.1. Future demand for protein
2.2. Significance of plant proteins
2.3. Major vegetal sources of protein
2.4. History of meat analogues
2.5. Components of meat analogues
2.6. Major constraints and future challenges

2.1. Future demand for protein

Protein is of paramount significance in human nutrition as this macro bio – molecule builds, repairs and maintains the tissues of living organism. Proteins perform physiological activities such as enhancement of nutrient absorption and growth stimulation in immune system modulation thereby defending against pathogens (Walther and Sieber, 2011).

Attaining adequate protein is fundamental to maintenance of muscle mass, healthy ageing, supports body's metabolism, regulates appetite and overall health (Churchward - Venne *et al.,* 2017). Certain proteins like immunoglobulins, casein, whey proteins *etc*. are considered to be bioactive peptides which are composed of peptide sequences that equip them with the potential to exert beneficial effect on human health (Daliri *et al.,* 2017). They regulate physiological pathways in the body including antihypertensive, antimicrobial, antioxidant and immunomodulatory functions (Sanchez and Vazquez, 2017).

Delgado (2003) reported that increased awareness regarding the role of protein in human body among consumers is one of the major driving factors in the

rise of demand for proteins. Recent studies on proteins make its role evident beyond muscle maintenance and development. Protein has an irreplaceable impact on satiety and weight management (Veldhorst *et al.*, 2008). Dietary sources of protein include plants, meat, dairy, fish, shellfish and other animal products sharing 57, 18, 10, 6 and 9 per cent respectively (FAO, 2010).

According to Klunder *et al.* (2012), hunger suppression in geriatrics can be addressed with hunger stimulating characteristics of proteins all of which contribute to continuous rise in demand for proteins.

Protein quality which includes essential amino acid composition, digestibility, energy deficit and infections are closely associated with prevalence of stunting (Boland *et al.*, 2013). Studies in the last two decades reveal that insufficient availability of amino acids have adverse effect upon cell and organismal growth (Cavazos and Gonzalez, 2013).

In our country, an overwhelming 83.4 per cent of men and 70.6 per cent of women in the age group of 15 to 49 years eat non vegetarian food daily, weekly or occasionally. The rest being vegetarian who never consumed chicken, meat or fish (IIPS and ICF, 2022).

Protein ingredient market globally accounts to USD 38 billion in 2019 and is expected to increase with a growth rate of 9.1% from 2020 to 2027. Demand for protein correlates with the increase in population. The human population in the year 2017 was 7.5 billion but it is predicted that population would keep rising to 8.5 billion in 2030. This being least, the year 2050 holds a projection of 10 billion population (Kołodziejczak *et al.*, 2021).

Cunsolo *et al.* (2012) reported that the consumption of protein increased by 31 per cent over the period of 1961 to 2011. This increase accounts to 61 grams per person per day in 1961 to 80 grams per person per day in 2011.

Projected consumption growth rates of meat and milk are higher with 164 and 172 per cent respectively in developing countries (Boland *et al*, 2013). Animal sources have higher biological value and essential amino acids that human body needs. In addition, meat is also a source of vitamins like vitamin A, thiamine, cobalamine and niacin and minerals like iron, zinc and other micronutrients. Meat, chicken, pork, fish, *etc*. not only have higher nutritive value but their other characteristics result in organoleptic experience which is favoured by many people around the globe. This gave rise to huge demand for food from animal sources. Henchion *et al.* (2017) reported a doubled demand for animal-derived protein in the world by 2050 giving rise sustainability and food security concerns.

Socioeconomic changes such as rising incomes and increased urbanization led to major transitions in population level dietary patterns in low and middle income countries and therefore, increase in demand for animal foods is seen in these countries (Popkin *et al.,* 2012). Additionally, both at production and manufacturing stages, existence of the global challenge to address food security together with conservation of non renewable resources has become another challenge in dynamics of protein market (Ismail *et al.,* 2020).

Protein also has tremendous role to play in combating protein energy malnutrition (PEM). In 2019, it was concluded that there were 14, 767,275 prevalence cases of protein energy malnutrition and the age standardized prevalent rate (ASPR) four per 1, 00,000 population with a significant increasing trend of 0.19 per cent in ASPR estimated annual percentage change (EAPC) globally (Zhang *et al.,* 2022). The study also reported that with regard to protein energy malnutrition, there were 212, 242 death cases and the age standardized death rate (ASDR) approximated to three per one lakh population due to PEM.

Study by FAO (2010) in Asia revealed that consumption of animal protein per capita increased by 225 per cent between 1961 and 2007 where the latter accounts

for 40 per cent of total protein consumption compared to 15 per cent in 1961 whilst crop derived protein for human consumption increased only by 22 per cent. Globally FAO (2010) reported that animal derived protein accounts for 40 per cent of total consumption and predicted an increase by 2050 substantially.

According to FAO, total consumption of meat and dairy products would increase by 102 and 82 per cent respectively from 2000 to 2050. This percentage translated into metric tonnes gives a numerical value of additional meat and milk which are 233 MT and 466 MT respectively (FAO, 2010).

Consumption of meat and dairy products would increase by 102 and 82 per cent respectively from 2000 to 2050. This percentage translated into metric tonnes gives a numerical value of additional meat and milk which are 233 MT and 466 MT respectively (FAO, 2010). Boland *et al.* (2013) reported that consumption growth rates of meat and milk are even faster for developing countries accounting to 164 and 172 per cent and total production of meat and dairy products by developing countries is projected to grow by 206 MT and 410 MT respectively. At present the demand for protein of 7.3 billion inhabitants of the world amounts to 202 million tones globally (Henchion *et al.*, 2017).

A staggering rise is expected in the demand for poultry in South East Asia with 25 per cent rise in 2000 to 2030. The primary reason to this tremendous rise is increasing per capita consumption rates rather than population levels (FAO, 2010). For countries under Organization for Economic Co - Operation and Development (OECD), consumption is estimated to increase through the decade of 2009 to 2019 by 38 per cent for poultry, 33 per cent for pork, 23 per cent for beef and 31 per cent for sheep meat (Boland *et al.*, 2013).

Milk and animal source protein show a positive association with linear growth in children (Hoppe *et al.*, 2006). Meat is one of the richest sources of essential amino acids and it has played a key role in food security in terms of providing

energy, high quality protein and essential micronutrients. It has high net protein utilization and digestibility (Bax *et al.,* 2013). Production and consumption of meat has its own challenges, one being the adverse effect on health it has due to higher content of saturated fatty acids.

Livestock is another major domain with huge input of protein required for its production. It comprises 40 per cent of agricultural GDP globally (Steinfeld *et al.,* 2006). Livestock production demands for protein input like forages, grains, legumes, animal meals and other by products. Poultry consumed 43 per cent of total compound feed produced globally followed by 25 per cent by pigs, 15 per cent by sheep, 5 per cent by dairy cattle and 13 per cent by other species including fish (IFIF, 2009).

Steinfeld *et al.* (2006) reported a consumption of 77 million tones of protein by the livestock annually from the feed that potentially suits human consumption while only 58 million tones of protein are being supplied by livestock products. World compound feed production was estimated to be 680 million tones equal to the use of approximately 150 to 170 million tones of protein (IFIF, 2009).

Industry of food processing and manufacturing now initiated to stream strategies to maximize revenue and value of the final food commodity along with adding value and maximizing the utility of by products as well. Proteins in many ways are used as food additives like gelling agents, emulsifiers with oil and water holding capacities (Day *et al.,* 2021).

Novel protein sources such as algae, *in vitro* meat, single celled proteins, meat analogues from plant proteins are extensively emerging both at production and consumption level (Henchion *et al.,* 2017). According to Maurya and Kushwaha (2019) insects and leaf protein extract could also be an excellent option in terms of protein quality, abundance, convenience and versatility.

2.2. Significance of plant proteins

Plant based foods are considered an important part of our diet as they add variety and colour to the plate. Vegetarian source is a predominant source of energy and other nutrients such as proteins, phytochemicals and antioxidants in our diet. They are abundant in vitamins such as B complex vitamins, C and other provitamins which make them a healthier option in diet (Ferreira *et al.*, 2021).

Dietary sources of protein are mainly from plant sources which make up to 57 per cent the rest being animal sources. Among the animal sources, 18 per cent, 10 per cent, 6 per cent and 9 per cent are occupied by meat, dairy, fish and shell fish and other animal products respectively (FAO, 2010).

Langelaan *et al.* (2010) reported major concerns with regard to meat production which include issues regarding the environment, public health and animal welfare. Meta-analyses and large prospective studies in western countries reveal that total mortality rates are considerably higher in subjects who prefer higher intakes of both red and processed meat (Rohrmann *et al.,* 2013).

Boland *et al.* (2013) reported that protein derived from animal source for human consumption is associated with loss of biodiversity, freshwater depletion, unfavourable climate changes and adverse effects on human health. Approximately 6 kg plant material is needed to feed livestock to produce one kilogram of high quality animal protein and this presents strain on natural resources. Crisis associated with non renewable resources lead to one of its contributing factors namely meat production (Ismail *et al.,* 2020).

According to the IARC (2015) processed meat is carcinogenic to humans as every 50 g portion of processed meat would raise the risk of colorectal cancer by 18 per cent and consumption of 100g red meat increases risk by 17 per cent.

According to Hurrell and Egli (2006) meat production is responsible for climate change and biodiversity issues with water, energy and chemical inputs leaving a negative impact on the environmental foot print.

Bax *et al.* (2013) also reported that animal protein is associated with negative impact on the environment as 12 per cent of green house gases emissions are derived from livestock production accompanied by 30 per cent human induced loss of terrestrial biodiversity. Ferreira *et al.* (2021) also reported that global production of an increased volume of food protein especially from animal sources could present sustainability crisis.

Higher consumption of processed meat is directly correlated with increased risk of cardiovascular diseases, obesity, colorectal cancer along with chronic diseases such as diabetes, rapid weight gain and hypertension (Pan *et al.*, 2011). The International Agency for Research on Cancer (IARC) revealed that diets high in processed meat are responsible to cause 34,000 deaths from cancer per year worldwide. IARC also put forth its conclusion that processed meat increases the risk of colorectal cancer by 9 per cent (IARC, 2015).

Bourvard *et al.* (2015) reported the components responsible for the carcinogenicity of processed meat to be N - nitroso compounds, heterocyclic aromatic amines and polycyclic aromatic hydrocarbons which are usually formed at higher cooking temperatures. Higher concentration of saturated fats in meat raise the level of low density lipoprotein cholesterol contributing to chronic morbidities such as hypertension, obesity and diabetes (Wolk, 2017).

Milk and dairy products dominate the protein market due to their functional and health benefits. Increase in biotechnology, food microbiology jointly with food processing technology raised both nutritive and economic value of dairy products. However, it is subject to numerous negative environmental impacts due to requirement of resources such as land, water, energy with huge production of green

house gases (Van Kernebeek *et al.,* 2016). At processing level, there is growing need to adopt sustainable manufacturing processes especially the conservation and management of energy and waste water (Teagasc Agriculture and Food Development Authority, 2016).

In the light of greenhouse gas emissions, the 100 g of beef, pork and poultry protein production releases 49.9 kg, 7.6 kg, 5.7 kg of CO_2 equivalents respectively whereas 100 g of grain protein production releases only 2.7 kg of CO_2 to equivalents (Mogensen *et al.,* 2020).Grain production requires approximately 4.6 m^2 of land per 100g of protein compared to utilization of 163.6m^2 of land to produce 100 g of beef protein (Ritchie and Roser, 2020).

According to Bernstein *et al.* (2010), a significantly lower risk of CVD's, stroke and type II diabetes are reported when diets with animal protein are replaced with plant sources of proteins namely pulses, whole grains and nuts. Springmann *et al.* (2016) also reported that adaptation from animal meat to more plant based diets can potentially reduce global mortality rates by 6 to 10 per cent.

Hurrell and Egli (2006) reported that an increased flux towards consumption of plant proteins may be due to their cost effectiveness and versatility in replacing animal proteins. Components of plant proteins also make them significant functional ingredients for product formulation in food industry (Lopez *et al.,* 2018). Increase in vegan, vegetarian and flexitarian populations gave rise to increased demand of plant proteins among consumers (Ismail *et al.,* 2020). Day *et al.* (2021) reported that consumers of today's market prefer plant based food options either for sustainability, health or ethical reasons.

Cereal and pulse proteins account for major portion of plant based dietary protein intake. According to Indian statistics, the cereal production amounts to 20.5 million MT whilst pulse production has an increased growth of 48 per cent which equals to 26.96 million MT in the FY 2022. Glutenins and gliadins which make up

the protein fractions of wheat are known to control the dough properties and quality of processed food products. The higher molecular weight (HMW) subunits of wheat glutenins are major determinants of the gluten elasticity which are of paramount significance in the food industry (Panghal *et al.,* 2001).

According to Henchion *et al.* (2017), the average proportion of nitrogen content between total alcohol soluble proteins (TASP) and total glutelins (TGlu) in cereals to be 47.8:33.2 in maize and 44.7:12.3 in wheat respectively.

Vegetal sources of protein dominate protein supply globally with sharing 57 per cent in the market (FAO, 2010). Plant based protein sources often lack the desired quantity of one or more amino acids to meet human nutritional requirements which are addressed by combining different proteins like cereal - pulse combinations and supplementation (Van Kernebeek *et al.,* 2016).

Plant sources have significant bioactive peptides along with other phytochemicals such as catechins, antioxidants, pigments, flavnoids and dietary fibre on which there is an increased attention in the recent years as they are safe and suitable remedy for chronic illnesses like diabetes, obesity and hypertension (Hernandez-Ledesma *et al.,* 2011). These compounds give bioactive potential to plant sources in terms of antioxidant, anti inflammatory, cholesterol lowering, satiety and anti diabetic activities (Malaguti *et al.*, 2014).

In a study conducted among adolescents revealed that higher levels of plant protein is associated with lower body fat percentage and body mass index (BMI) and therefore it could be healthier and safe option to address obesity (Lin *et al.,* 2015). According to Daliri *et al.* (2017), reduction in blood lipids including low - density lipoprotein cholesterol, non- high- density lipoprotein cholesterol and apolipoprotein B and an overall decrease in cardio-metabolic risk factors is observed with substitution of meat protein with plant based protein.

Plant proteins also improve the antioxidant status and systemic inflammation in patients with chronic kidney disease (CKD). A soy protein rich diet was found to decrease glomerular hyperfiltration in people with type 1 diabetes and early stage nephropathy and raised glomerular filtration rate (GFR) which is associated with kidney injury (D'Alessandro *et al.*, 2015). According to Cornet *et al.* (2020), animal protein diet showed decrease in whole body insulin sensitivity, but improvements in HbA1c, fasting glucose and fasting insulin levels were observed when animal protein sources were substituted with plant based proteins by at least 35 per cent in the diet for people with diabetes.

Polyphenols and other bioactive compounds in plant based foods are shown to have potential health benefits like reducing risk of cancer, osteoporosis and degenerative diseases (Pandey and Rizvi, 2009). Phytosterols, dietary fibre, biogenic amines, carotenoids, biologically active proteins, *etc.*posses antioxidative, anti inflammatory and immunomodulatory functions and therefore, are an excellent option for people with gut, immune, cardiovascular and neurological concerns (Samtiya *et al.,* 2020).

Mycoprotein of fungi is an excellent protein source but its use is limited due to slow digestibility. Li *et al.* (2017) reported that single cell proteins have high nucleic acid content and slow digestibility rate due to which their use is limited. Fungi, algae and yeast are dried to obtain single cell proteins which emulsify, add aroma and enhance nutritional quality of food products (Perez-Santaescolastica *et al.*, 2021).

According to Malav *et al.* (2015), textured proteins fulfill mouth feel, appearance and binding capacity along with enhancing nutrient quality by fortification. Plant proteins like vicillin and legumin give them distinctive physicochemical properties. These properties give them functional attributes like solubility, gelation, emulsifying ability, oil and water absorption capacity and

foaming. Hence, they are capable enough to impact the sensory, physical and chemical properties of foods (Singhal *et al.,* 2016).

Foaming and thickening capacity of plant proteins enhance functional characteristics such as mouth feel sensation, sensory experiences and contribute to satiety through complex integrated mechanisms of oral processing, dynamic texture sensation, transformation of food structure along with psychological, physiological and physical factors (Campbell *et al.,* 2017). Utility of plant proteins in food system is rapidly expanding with its adoption in food products such as meat analogues, beverages, bakery and pasta products.

Plant proteins especially soy and pea exhibit excellent functional properties such as water holding, gelling, fat absorbing and emulsifying the food products that they are added to. Others like gluten, a cereal protein have distinct cohesive and viscoelastic properties that could form fibrous proteinaceous networks and therefore used in meat analogues (Ismail *et al.,* 2020). Nutritional and functional properties of plant proteins can be enhanced through variety of methods such as thermal, enzymatic, chemical processing at the range of pH, temperature, pressure and ionic conditions (Singh *et al.,* 2021). Protein rich biosources such as vegetables and algae along with cereals and pulses have been extensively explored to mimic meat in flavor, texture, sensory and aromatic properties (Tan *et al.,* 2021).

2.3. Major vegetal sources of protein

Protein is of paramount importance for growth, reproduction and optimal performance of humans. It's not the protein alone but amino acids within it, that make it the type of important nutrient that it is to living organisms (Boland *et al*. 2013).

A typical protein contains 20 different amino acids in varied amounts, linked via peptide chains. The sources of dietary protein are plant sources (57%), meat (18%), dairy (10%) fish and shellfish (6%) and nine percent from other animal products (FAO,

2010). The determinants of nutritional value of proteins are content, digestibility coefficients and relative proportion of amino acids in dietary proteins. Amino acids provide nitrogen, hydrocarbon skeletons and sulphur which cannot be replaced by any other nutrients like carbohydrates and lipids. They are the precursors of substances that drive life such as RNA and DNA and enzymes, neurotransmitters like dopamine (Boland *et al*. 2013).

Based on the source, plant proteins may be deficient in some essential amino acids. Cereals contain low levels of lysine and tryptophan and pulses are usually low in sulfur containing amino acids as methionine and cysteine (Lazou *et al.*, 2007). Plants of the same species may vary in composition including macronutrients like proteins, lipids and micronutrients like minerals based on the differences in climate, soil diversity, geographic altitude and latitude, precipitation levels, agricultural practices and cultivars (Sun *et al.,* 2021). The following content discusses different sources of vegetal proteins.

Cereal proteins: Globally, cereal proteins account for the major portion of dietary protein intake. India is fond of cereals like wheat, paddy, sorghum, millets like bajra, barley, maize, *etc.* and wheat constitutes a large part of cookery in the world. Cereals are abundant source of polyphenols, antioxidants, calcium, dietary fibre, lipids and starch (Panghal *et al.,* 2001).

Proteins in cereals are made up of 5 to 15 per cent albumins, 3 to10 per cent globulins, 25 to 45 per cent prolamins and 30 to 40 per cent glutenins depending on the crops and sources of their origins (Pandey and Rizvi, 2009). As a food source, corn accounts for 25 per cent and 15 per cent of total maize consumption in developing countries and globally respectively which makes it significantly important for global food security (FAO, 2010). In Eastern and Southern Africa (ESA), Mesoamerican, Andean region, West and Central Africa (WCA) maize as a source of protein is similar in terms of its contribution to calories intake globally by 45, 61, 29 and 21 per cent. In South Asia, the percentage is around four. Millet is consumed extensively in West

Africa whilst staples like corn (maize), rice and wheat are consumed globally (Zhang *et al.,* 2016).

Wheat has protein content ranging from 5.6 per cent to 21 per cent , and this content depends upon agronomic practices, environmental and genetic factors (Panghal *et al.,* 2001). In storage proteins, largest content of protein is seen. Prolamins, globulins, germins are the storage proteins The molecular and functional relationships of cereal storage proteins namely the gluten, composed of glutenins and gliadins give unique properties which are responsible for elasticity, gas retention, strengthening of mixture, controlled expansion, improved water absorption and extending shelf life. Gliadins improve extensibility and product quality whilst glutenin imparts high elasticity and rubbery nature making the food product resistant to shear. Additionally, it helps strengthen frozen and refrigerated products (Cusolo *et al.,* 2012).

Oats is of highest protein quality among cereals with its amino acid content and quality comparable to soy protein. Higher content of essential amino acid lysine is seen in oats but is a poor source of proline and glutamic acid content which is advantageous to people with gluten intolerance and allergies (Cavazos and de Mejia, 2013). A significant reduction in greenhouse gases production was observed when oat protein substituted the animal based proteins proportionally with marked reduction of greenhouse gases production by 8 per cent and land use by 14 per cent respectively (Mogensen *et al.,* 2020).

Rice, however, does not contain large quantities of protein still they have better utilization values and hence considered best quality proteins (Panghal *et al.,* 2001). According to Revilla *et al.* (2009), rice protein possesses potential hypocholesterolemic and hypoallergenic characteristics which makes it highly valuable.

Millets: Pseudocereals like amaranth and quinoa have high bioavailability and a good profile of essential amino acids. Unlike cereals they contain adequate amount of the essential amino acid lysine (Aguilar *et al.*, 2015). Additionally they provide high

quality protein, fibres, unsaturated fatty acids and adequate levels of vitamins and minerals (Lopez *et al.*, 2018).

Millets are one of the richest sources of protein with the range of 5.6 to 12.70 per cent of protein content. Millets such as foxtail millet are rich in setarins which belong to the class of alcohol soluble proteins (prolamins) which constitute 60 per cent of its total protein with less content of disulfide cross linked proteins. These are the richest sources of mineral such as calcium and iron with content of 162 to 487 mg 100^{-1} and 3.61 to 5.42 mg 100^{-1} respectively which is a cost effective and suitable option for addressing a wide spectrum of calcium and iron deficiency disorders (Pragya and Rita, 2012).

Boland *et al.* (2013) also reported that the lysine content present in quinoa and amaranth is higher than the standard recommendation which makes them a potential and useful source of dietary supplements. The low *in vitro* protein digestibility (IVPD) of millets is improved by pretreatments such as dehulling, soaking and cooking which increased the IVPD from 72.3, 77.1 and 71.3 per cent to 85.5, 91.6 and 88.6 per cent in finger millet, foxtail millet and proso millet respectively (Annor *et al*., 2017). Their superior nutritional quality contributes to unique bioactive properties through which chronic diseases could be effectively managed (Sanchez and Vazquez, 2017).

Pulse proteins: These are one of the main plant derived protein sources and are preferred among people to animal based protein from an environmental perspective as it is associated with decreased need of land use and lower production of green house gases. Legumes offer unique benefits as they have an ability to fix nitrogen. Due to high cost and limited availability of animal proteins, plant sources, exclusively pulses are chosen as a part of daily menu which certainly makes them ‘poor - man's meat’. Proteins in pulses are seen in the cotyledons that constitute a major portion of pulse grain and embryonic axis of the seed with small amounts present in the seed coat. As cotyledons

occupy the major portion of the pulse grain they make the highest contribution to protein content (Ghanghas *et al*., 2022).

Pulses nutritionally are composed of 10 per cent moisture, 21 to 25 per cent crude protein, 1 to 1.5 per cent lipids, 60 to 65 per cent carbohydrates, and 2.5 to 4 per cent ash with chickpea as exception which has about 4 to 5 per cent lipids. Pulses such as soy bean and lupin have been reported as having up to 45 to 50 per cent protein (Jayathilake *et al.,* 2018). Pulses like cowpea are of novice interest among consumers and researchers worldwide due to its health beneficial properties which include anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory and anti-hypertensive. Recent publications reported that consumption of 54 to 360 grams of pulse a day showed improvements in blood lipid profile, blood pressure, inflammation biomarkers and body composition in terms of body weight and waist circumference (Ferreira *et al.,* 2021).

Unlike animal protein sources, plant proteins do not have all amino acids which are essential for the human body. Of nine essential acids body needs, pulses are deficient in sulphur containing amino acid – namely methionine. Also, pulses have anti-nutritional factors such as hydrolase inhibitors and lecithins which usually are the defense mechanism of seeds that inhibit biological functions. This can be minimized with the right cooking and preliminary methods like soaking, roasting, *etc.* (Henchion *et al.,* 2017). Combinations of different protein sources can help in improving the nutritional quality. Cereal and pulse combinations are the best example for this as they supplement each other filling the gaps of limiting amino acids of each group respectively. This is quintessential in a strict vegetarian or vegan diet to meet the daily protein needs (Onewezen *et al*., 2021).

Apart from that, proteins in pulses like vicillin and legumin give them distinctive physicochemical properties. These properties give them functional attributes like solubility, gelation, emulsifying ability, oil and water absorption capacity and

foaming. Hence, they are capable enough to impact the sensory, physical and chemical properties of food (Singhal *et al.*, 2016).

According to Bohrer (2019) textured vegetable protein is extracted from legumes such as soybeans, peas, faba beans to create and simulate meat texture in meat analogues. Novel textured products like defatted soy flour, its concentrate and isolate in combination with more stable wheat gluten are used in meat analogue formulation (Samard *et al.*, 2019).

The intrinsic molecular properties of pulse proteins could create a unique protein based fabric in foods in which the interactions at molecular level such as amino acid sequence and their disposition, molecular size, shape, confrontation and flexibility along with forces such as polarity, charge and hydrophilicity influence their functional behavior in that particular matrix of food (Chereau *et al*., 2016). Deciphering the technological and functional aspects of pulses and their proteins could increase the scope of formulating innovative food products that could address the detrimental consequences of protein deficiency (Bessada *et al*., 2019).

Nuts and oil seeds: Common nuts like almond, peanut, walnut, cashew, pecan, hazelnut, pine nut, sesame seed, groundnut, pumpkin seeds *etc.* represent important source of plant proteins as they provide other nutrients such as lipids, fibre, minerals and vitamins (Tan *et al.*, 2021). Acevedo - Juarez *et al.* (2022) reported that nuts are abundant sources of bioactive peptides like phenolic compounds, tannins and phytosterols due to which they have became key functional ingredients in industries of food, cosmetics and pharmaceuticals.

Nuts and oil seeds contain a wide variety of proteins namely glutelin, prolamin, globulin, albumin, amandine, *etc.* which account for 65 to 70 per cent of their total protein fraction. These are also nutritionally superior in terms of lipid profile containing sphingolipids, phospholipids, oleosins *etc.* accompanied by

equilibrated ratio of polyunsaturated and monounsaturated fatty acids make them suitable for people with cardiovascular issues (Manfredi *et al*., 2017).

Recent studies report rapid progress in research of functional properties of nuts and oil seed proteins in which their inter molecular matrix is shown to give them their unique properties such as emulsification, foaming capacity and gelling property when kept at isoelectric point (pH of 4 to 5) while subjecting them to heat treatment (Malik and Saini, 2018).

Behavoiur of proteins present in nuts and oil seeds act as binding, gelling, emulsifying, texturing, structuring agent while maintaining the three dimensional network that gluten forms in a food system which makes them a suitable ingredient to create meat like texture in meat analogues (Coelho *et al*., 2018).

Nuts and oil seeds are rich sources of phosphorous, potassium and calcium due to which they are better, safer and healthier option for celiac, pregnant women and lacto intolerant individuals (Bernat *et al*., 2015). They impart nutty taste, creamy texture and function as emulsifiers due to which they are added to enhance a food product in terms of its nutritive and organoleptic profile and therefore used to develop nut based beverages (Qamar *et al.*, 2020). According to Day *et al.* (2021), functional characteristics of nuts and oil seeds are used to develop food products such as soups, cakes, pastries, cookies, bars and pasta sauces like pesto.

Algae: Marine plants such as seaweeds and microalgae, collectively called algae are both multi and unicellular living organisms respectively. Seaweed is farmed globally whose marketable quantity equals to 24 million tones respectively (WRAP Food Futures, 2017).

Protein content of seaweeds range from 15 per cent ±3 to 47 per cent ±10 of dry weight in Phyaeophyceae (brown alage) and (Rhodophyta) red seaweed species respectively (Sampels, 2014). Seaweeds are reported to exhibit anti - inflammatory

and antioxidant properties due to higher nutritional profile as they contain polyphenols and other bioactive compounds (Pal *et al.*, 2014).

Organisms that belong to the genus *Chlorella (C. pyrenoidosa and C. vulgaris)* contain crude protein content of 51 to 58 per cent with the highest observed in the organism *Arthospira maxima* with a staggering range of 60 to 71 per cent of crude protein which also is an estimate of biologically active forms of protein like enzymes (Geada *et al.,* 2021). Algae proteins have distinctive amino acid composition in which they contain higher content of essential amino acids such as lysine and leucine at a known range of 3.50 to 8.40 and 8.80 to 11.0 g per 100 g protein, which is relatively higher than their respective contents in reference protein with only 5.30 and 8.80 g of the essential amino acids lysine and leucine per 100 g of protein respectively (Terriente-Palacios and Castellari, 2022).

Commonly used microalgae are *Scenedesmus obliquus, Spirulina* and *Dunaliella*which have a wide spectrum of nutritious compounds such as carbolydrates, lipids, vitamins, pigments, minerals and valuable trace elements besides higher content of protein. The estimated annual production of all microalagal species approximates to 10,000 tones per year (Adhikari *et al*., 2006). Peng *et al.* (2021) reported that mass production of protein rich microalgae to be a potential solution for the protein gap.

Fucoidans from brown algae exhibit antibacterial, antiviral, anticoagulant and antithrombic effects (Cumashi *et al.,* 2007). Ulvan, a sulphate polysaccharide from green algae is used as emulsifier, stabilizer and thickener in food product (Bajpai *et al.,* 2014). Carrageenans and agar from red algae are widely used as geling agents, thickening agents, emulsifiers in the food industry and as anticoagulant agents in biomedical and pharmaceutical industries (Henchion *et al.,* 2017). Microalgae are abundant sources of eicosapentaenoic acid (EPA) and dococsapentaenoic acid (DHA)

and therefore marketed as health foods, cosmetics and animal feed (Peters *et al.*, 2017).

2.4. History of meat analogues

An analogue is a term that refers to the meatless food that has taste, appearance and texture, approximately similar to that of a related food made from meat, poultry, fish or shellfish. Also, it is a compound that resembles another compound in structure but is different in composition and a meat analogue is similar to meat in structure but not in its composition (Joshi and Kumar, 2015).

It is also called as meat substitute, meat alternatives, fake or mock meat and imitation meat. *Tofu* (fried or dried frozen), wheat gluten, *temph*, *yuba* and nuts like peanuts are traditionally used as a main ingredient to make meat analogues. Until recently, soy protein isolates, modern textured soy protein products like spun soy protein fibres, TVP, textured soy protein concentrate, *etc.* are added (Ismail *et al*., 2020).

The term 'vegetable substitute for meat' was first used in U.S. patent 670,283 on June 3rd of the year 1899, titled 'vegetable food compound' by Dr. J.H Kellogg. The patent was issued on 19th March, 1901. Li-Yu-ying, the owner of tofu - company near Paris, France applies for a French Patent No. 428,718 titled *'Charcuterie de soja'* which means cold cuts and meat like products from soya. Jethro Kloss Health Food Co. in Brooke, Virginia first made an announcement regarding commercial alternative of meat in September, 1923. Description of textured meatless foods from spun vegetable protein was mentioned as a part of the U.S patent no. 2,682,66 applied by Boyer to whom the patent was issued on June 29, 1954. Spinning the soy protein filaments to create an entirely different and novel type of meat alternative increased with this significant discovery (Shurtleff and Aoyagi, 2014).

In 1953-54, the term ‘imitation meat’ and ‘simulated meat’ products were first used under different patent numbers by Robert Boyer and Harold Saewert as a team and Mortimer L. Anson and Morton Pader as another, both teams receiving patents in 1956-57 respectively. Some of the plant based meat alternatives throughout history are *tofu, tempeh, seitan, yuba, kinema, remisalgen, risofu, etc.* which are usually referred to as traditional fermented foods (Riaz, 2015).

With the increase in vegetarian population in the 1960’s and 70’s, more scope led to the research especially on soy proteins and its products. During the same period, increased production of meat analogues was seen along with the establishment of companies like Miles Laboratories, Worthington Foods, Morning Star Farms, Yves Tofu Wieners which began producing diversified meat alternatives like Tofurky (meat alternative for turkey), frozen alternatives, *etc.* (Bohrer, 2019).

Consumption of 6.25 g soy protein per serving per as a part of a healthy diet decreases the risk of heart diseases due to its low saturated fats and cholesterol (Juturu, 2014). This statement from thence was seen in the front of a number of meat like products made from soybean and proteins (Lee *et al*., 2020).

The early 1990’s till 2014 mark a significant period in the history of meat analogues as they were extensively commercialized from diverse plant products based on all previous research and technologies to develop them. Fast food chains like Burger King and other food processing industries like Kraft Foods Inc, and Gardein, began incorporating meat analogues in burgers, as sausages, patties, *etc.* which further increased their popularity and acceptability. The food industry extends its scope in exploring and exploiting different microbiological ways to produce nutritionally and organoleptically better meat analogues (Singh *et al*., 2021).

2.5. Components of meat analogues

Vegetable sources are the main source for meat analogues at present, these include wheat glutens, globulins of ground nut, cottonseed, peanut, sesame, yeast and soyabean. The main ingredients used in making the analogues of meat are soya protein, pulses, nuts, cereal proteins, vegetables, mycoproteins but wider consumer choices resulted in inclusion of newer ingredients in meat analogues which are manufactured to have high biological value of protein and to enrich a plant based monotonous diet. Meat analogues exist in three forms including coarse ground meat analogues used in burgers, sausages, batter or bread nuggets, meat balls, *etc.* or as emulsified meat analogues used in frankfurters, spreads and Deli' meats or as Loose fill used in taco fillings, chilli mixes and sloppy Joe (Joshi and Kumar, 2015).

The key ingredients used in the preparation of meat analogues are discussed below:

Soy protein: It is known for its high quality nutritional and functional benefits. Soya is usually used as a partial or complete replacement of meat. Soya bean is the cheapest and most common source of protein from which meat like products could be obtained. Soya protein, on PDCAAS (Protein Digestibility Corrected Amino Acid Score) scale was reported to be similar to animal protein with a score of 1.0 which is the highest possible rating (Campbell *et al*., 2012).

Specific protein functionalities such as good gelling properties and water holding capacity along with being cost effective makes soy an apt ingredient for meat analogues. Soy protein is used in different physical forms namely soya flours, soyabits, isolated soy protein, spray dried soy milk and texturized soy proteins (Geerts *et al*., 2018).

Protein digestibility and nitrogen balance (g N/day) for textured vegetable protein from defatted soy flour was 66.1% and 1.16. For TVP made out of soy protein

concentrate the values are 63.4% and 1.31 N/day (Kumar *et al.,* 2017). Components intrinsic to soya bean such as soya saponin affect the hydrophobic features of the proteins which indirectly influence foamability and surface elastic behaviour (Zhu *et al*., 2020).

Defatted soy flour which contains 50 per cent protein content through the process of fractionation, is used to make concentrates and isolates which enrich the nutritional and sensory features of meat analogues (Xing *et al*., 2018). Along with wheat gluten and other vegetable proteins, soy protein isolates are used to prepare soya based meat alternatives which are mostly prepared by extrusion process in which the texture of the product remains fibrous due to disulfide bonding and resemble the texture of meat (Harvey and Philips, 2020).

Mushroom: Incorporation of mushroom in food products provides chewability as its natural protein is fibrous in nature. Dietary fibres present in the cell walls of hyphae are rich in whereas cell membranes contain polyunsaturated fatty acids whilst the cytoplasm has high quality proteins (Amit *et al*., 2017).

First commercial meat analogues like burger patties and sausages with mycelia were formulated using edible filamentous fungi. Fungi are composed of amino acids such as methionine and cysteine whose interactions with other amino acids such as glutamic acid produce meaty flavour due to which they are extensively used in European countries (Adhikari *et al*., 2008).

Protein content of mushrooms of the genus *Pleurotus* contain protein content of 4.00g $100g^{-1}$ with high biological value due to the presence of pronounced amounts of essential amino acids, which depends on environmental factors and its stage of maturity. The mycoprotein can reduce the harmful LDLs (low density lipoproteins) and enhances the beneficial HDLs (high density lipoproteins) and could contribute to fulfill our daily requirements of protein, minerals, and vitamins (Wang *et al*., 2014).

Unique interaction between protein and phenolic compounds in mushrooms exhibit protective effects in the body with remarkable reduction of free radical compounds and their intermediaries (Mishra *et al*., 2013).Healthy composition of lipids like sterols, sterol esters and phospholipids make mushroom one of the suitable ingredients for meat analogues (Kumar, *et al.,* 2017).

Wheat gluten:It is prepared as a byproduct during isolation of starch from wheat flour. It is an insoluble protein which is a cohesive, visco-elastic complex of enzymatic complex proteins which is widely used as it induces functional properties like binding, dough forming and leavening ability. It is because gluten is capable of imparting functional properties like solubility, viscosity, swelling, nutritional quality; it finds its way to inclusion in meat analogues (De Angelis *et al.*, 2020).

The molecular structure of wheat gluten gives it its characteristic property due to which it acts as both binder and a structuring agent at the same time. Gluten forms thin films of protein molecules upon simple deformation and elongation transforming the dough matrix of the meat analogue into a fibrous material (Krintiras *et al*., 2015).

The three dimensional fibrous material formed by gluten protein is the result of protein linking with sulphides as a disulphide protein linkage. This creates a definite matrix in food system that imparts better moisture retention, foaming and emulsifying properties (Wouters *et al*., 2016). Functionality of gluten depends on variables such as hydrostatic pressure and temperature and its interaction with other extrinsic compounds such as polyphenols which determine the matrix they form in the food system (Li *et al*., 2018).

Egg albumen:The clear liquid within the egg is called albumen whose incorporation into the analogues of meat contributes to binding along with bite during eating experience. Ovalbumin is a globular monomeric phosphoglycoprotein with free sulfhydryl groups. It has the ability to form heat induced gels. Globular protein

molecules of egg unfold to a molten globule structure partially, which increases the viscosity relative to the compact folded polymers of the same molecular weight, thereby adding to the binding effect of globular proteins (Kumar *et al.,* 2017). The incorporation of egg albumen showed significant effect on the physico-chemical properties of meat analogues, however, fat levels were least influenced by variation in incorporation of egg albumen in the products (Cornet *et al*., 2020).

Carbohydrates and gums: Gums of carbohydrate are used by the food industry as texture modifying agents in many products and starch is used extensively as food hydrocolloid. On hydration, starches, maltodextrins form gel and imitate fat like texture (Bajpai *et al*., 2014). Proteins have technological limitations like heat treatment resistance, compatibility with the other constituents as flavour components, *etc*.., restricting their scope of use in meat analogues to which gums of carbohydrates are a good choice. In addition to their functional properties, starches are cost effective. On addition of sucrose, the harsh hardening effect of salt was counteracted by preventing moisture removal and enhanced the product shelf life by bacterial growth inhibition (Amit *et al*., 2017).

Carrageenan are high molecular weight sulphated polysaccharides derived from red seaweeds or Rhodophyta. These are used as binders and extenders in the food industry and their use is approved by the U.S. Iota, kappa and lambda are the three types under this class where iota and kappa act as gelling agents whilst lambda carrageenan acts like a thickener (Kumar *et al.,* 2017).

A study conducted by Arora *et al.* 2017, mushroom based sausage analogues containing five per cent fat with carrageenan and xanthan gum exhibited improved textural properties like stability of emulsion and purge loss which was observed to be better than those produced with soy protein concentrate and casein. These properties give these polysaccharides their characteristic thickening and gel forming

functionality which increased their range of inclusion from binders to extenders (Warnakulasuriya and Nickerson, 2018).

The most commonly used ingredient in meat analogues like methylcellulose has distinctive thermal gelation with definite reversibility along with the ability to control formation of ice crystals. Optimal combinations of methylcellulose and other substances like alginate solutions provide adhesive properties forming a cold set gel due to which these are added in comminuted and emulsion type products (Howse *et al*., 2015).

Water: It acts as a medium of hydration for the dry ingredients in the mixture. It acts as a plasticizer and reaction agent during processing. In the extrusion process of developing meat analogues, determination of viscosity of the melt is done using water which also participates in the chemical reactions, influences friction and acts as a medium of energy transfer (Singh *et al*., 2021).

In food systems with low moisture pronounced difference is seen in the expansion and porosity was observed which is least acceptable with respect to meat analogues (Emin *et al*., 2017). Increased efficiency of disulphide bonds, hydrogen bonds and hydrophobic interactions is achieved in the presence of higher moisture due to which defined formation of fibrous structure was reported (Zhang *et al*., 2019).

Optimal moisture in meat analogues might increase its acceptability as water could impact sensory properties such as juiciness and mouth feel retaining them for a longer time (Cornet *et al*., 2020). Additionally, the molecular behaviour of water with biomolecules such as proteins give rheological characteristics to any food system like emulsification, foaming and thickening and gelling (Kyriakopoulou *et al.*, 2021).

Other ingredients: Meaty flavour is desired for meat analogues for the development of which extensive research has been done. Vegetable protein products and chemical flavouring compounds like glutamic acid and 5- ribonucleotides are

used for flavour potentiation (Emin *et al*., 2017). Development of synthetic meat flavour was done by Chambers *et al.* (2018) using surface response methodology. It was composed of simple sugars, amino acids, 5- nucleotides, glycoprotein, monosodium glutamate and salt along with fat as an optional component. He reported that the sulphur containing amino acids and simple sugars are crucial for flavour development whilst the other components masked the harsh 'sulphury' taste or enhanced the meaty flavour.

Caseinate is another component used widely as a ingredient in meat analogues. It is naturally present in milk in the form of a1, a2, b and k-casein. For application in the food industry, it is commercially produced in numerous compounds like sodium, calcium, potassium and magnesium caseinate. Peters *et al.* (2017) reported better solubility and functional properties in sodium and potassium caseinate compared to calcium caseinate.

2.6. Major constraints and future challenges:

Achieving the organoleptic characteristics of meat is a challenge in commercializing meat analogues as the sensory and organoleptic aspects of meat analogues are lower than the meat (Sasimaporn and Gi-Hyung, 2019).

The major challenge for food engineers is the development of the fibrous three dimensional structure from the plant proteins without any loss of their nutritive properties (Howse *et al*., 2015). Texturized wheat gluten in different forms varied in size, shape, density, colour and texture (Amit *et al*., 2017). Extensive production of wheat increases more interest towards texturized gluten. Researchers are now trying to develop varieties of wheat which are unique in terms of having minimum amount of gluten whilst maintaining its technological properties (Kumar *et al.*, 2017). Silencing of genes is another method of genetic engineering to enhance the quality of plant based food products (Kyriakopoulou *et al.*, 2021).

Plant sources and their proteins act as texturing agents in which shearing process is done to give 'fibrousness'. However, it is reported that this texture gives an impression of hardness and rubbery feeling (Langelaan *et al.,* 2010).

Consumers' preferences of meat analogues are that they should taste, feel and smell better or atleast similar to animal meat. It is obvious that unami flavour which is associated with meat and texture in terms of tenderness, fibrousness and juiciness are important determinants of the success of meat analogues and at the same time the biggest challenges for researchers and the food industry (Joshi and Kumar, 2015).

Recent most research and development made it possible to produce meat like texture by using plant based proteins and technologies like extrusion, shearing and mixing. Studies of consumer preference reveal that key motivations for people to move towards plant based diet which also includes meat analogues are part of their diet; are a plethora of health benefits and cost effectiveness. Other main factors are neophobia and meat attachment. So, these must be taken into note in innovation and development on meat analogue formation and production. Additionally, increased demand for sustainable ingredient sourcing, natural, clean label and nutritious products from the side consumers is present like never before (Sun *et al.,* 2021).

Soy based meat analogues have higher purine content than meat itself. These globular proteins make the meat analogues more proteinaceous with increased conversion efficiency, yet may pose complications for people with higher serum urates (hyperuraemia) hence, they must be limited in use (Havlik *et al*., 2010). It could be addressed by introducing be materials specifically those islolated proteins that relatively possess lower purine content in which however, research has not been fully extended (Chiang *et al.*, 2019).

Another major constraint lies at the consumer's end according to Adhikari *et al.* (2006) as it is consumer who has to recognize the meat analogues as a product that

could be eaten instead of meat and that the success depends upon their not only recognition but also incorporation in their meal. Meat analogues, being made out of vegetal sources may possess off flavours and cause allergic reactions. Allergens in plant foods such as proteins including vicilins, albumins, lectins, legumins, profilins, heveins and lipid transfer proteins trigger severe allergic reactions binding to IgE on the surface of mast cells and basophils (Couch *et al*., 2017). Seed storage proteins of nuts and oil seeds like 2S albumins are more resistant to digestion by proteolytic trypsin and pepsin enzymes causing the adverse allergic reaction to sustain for a long time (Stiefel *et al*., 2017).

Gliadins and glutenins in wheat gluten are known reason for different disorders such as celiac disease, IgE mediated allergies and non celiac gluten sensitivity in which immunological responses lead to intestinal inflammation and tissue damage due to which meat analogues may not be suitable meat replacers in the diet of people with this genetic disposition (Cook *et al*., 2017). However, protein sources for meat analogues are not limited to vegetable and plant proteins. Though, vegetable proteins are at large but algae, yeast, mushroom, and bacteria would probably supplement them in near future as they rapidly multiply without land and fit to consumer taste (Kyriakopoulou *et al*., 2021).

Materials and methods

3. MATERIALS AND METHODS

The materials used and methods followed for the standardisation of meat analogues followed by its quality evaluation such as nutrient profiling, *in vitro* studies, shelf life studies are given under following headings.

3.1. Collection of raw materials

3.2. Preparation of raw materials

3.2.1. Preparation of jackfruit flour

3.2.2. Preparation of breadfruit flour

3.2.3. Preparation of oyster mushroom flour

3.3. Spice broth formulation

3.4. Standardisation of tender jackfruit incorporated meat analogues

3.5. Standardisation of breadfruit incorporated meat analogues

3.6. Acceptability of the meat analogues

3.6.1. Selection of judges

3.6.2. Preparation of score card

3.6.3. Organoleptic evaluation

3.7. Selection of the meat analogues

3.8. Quality evaluation of the meat analogues

3.8.1. Nutritional profiling of selected meat analogues

3.8.2. *In vitro* studies

3.8.3**.** Shelf life studies

3.9. Cost of production

3.10. Statistical analysis

3.1. Collection of raw materials

Cowpea, chickpea, defatted soy flour, wheat gluten, spices and other ingredients were procured from the market. Tender jackfruit (*koozha*) and breadfruit were procured from homesteads. Oyster mushroom (*Pleurotus florida*) was procured from the Department of Plant pathology, College of Agriculture, Vellanikkara from which the flour was made as per standard procedures.

3.2. Preparation of raw materials

Whole pulses like cowpea and chickpea were subjected to pretreatment. They were washed and roasted for 15 minutes under low to medium flame. They were then soaked in water containing one percent sodium bicarbonate overnight. Pretreatments of raw materials like tender jackfruit, breadfruit and oyster mushroom are mentioned in 3.2.1, 3.2.2 and 3.2.3.

3.2.1. Preparation of tender jackfruit flour

Raw jackfruit flour was prepared by the standard procedure (Pandey and Ukkuru, 2004). The raw tender jackfruit were cut and separated from the rind. The flesh within was cut into uniform sized slices. They were blanched in boiling water for one minute to inactivate the micro-organisms and prevent discoloration. The blanched pieces were then immersed in lukewarm with 0.2 per cent KMS solution for ten minutes to preserve the colour. They were then dried in cabinet drier at 55 to 60^0C for 12 hours. The dried chips were milled into flour after retrieval from the drier (Plate 1).

3.2.2. Preparation of breadfruit flour

Standardisation of breadfruit flour preparation was given by Pillai (2001) in which the freshly collected produce was washed thoroughly, peeled and chipped and blanched in water at 90^0 C with 0.3 per cent citric acid and 1500 ppm of SO_2 for five

minutes followed by cabinet drying at 55 to 60^0 C for 8 to12 hours. These dried chips were milled, sieved to get uniform flour (Plate 2).

3.2.3. Preparation of oyster mushroom flour

The mushroom flour preparation was given by Piskov *et al*. (2020). Fresh mushrooms were cleaned thoroughly, sliced into thin slim strips and blanched in boiling water for two minutes and immersed in cold water for two minutes. The mushrooms were immersed in water containing 0.2 per cent potassium metabisulphite and one per cent citric acid. These were dried in cabinet drier at 60^0 C for six to eight hours till it reached $1/10^{th}$ weight of fresh product and were powdered (Plate 3).

3.3. Spice broth formulation

A pilot study was conducted wherein basic culinary spices like turmeric, pepper and salt were added to 500 ml water in different proportions from which the best composition was followed throughout for the development of meat analogues. The standardised ratio of water to salt, turmeric and pepper was as follows: 1: 0.4: 0.2: 0.3.

3.4. Standardisation of tender jackfruit incorporated meat analogues

Meat analogues were developed using pretreated cowpea, chickpea, wheat gluten, tender jackfruit flour, defatted soy flour and mushroom flour in various combinations. The treatments and their compositions are given in Table 1 and 2.

Table 1. Proportion of ingredients in tender jackfruit incorporated cowpea meat analogues

Treatments	Cowpea (%)	Tender jackfruit (%)	Wheat gluten (%)	Defatted soy flour (%)	Mushroom flour (%)
$T_{0\ (Control)}$	100	-	-	-	-
T_1	80	5	5	5	5
T_2	70	10	10	5	5
T_3	60	15	15	5	5
T_4	50	20	20	5	5
T_5	40	25	25	5	5

Table 2. Proportion of ingredients in tender jackfruit incorporated chickpea meat analogues

Treatments	Cowpea (%)	Tender jackfruit (%)	Wheat gluten (%)	Defatted soy flour (%)	Mushroom flour (%)
$T_{6\ (Control)}$	100	-	-	-	-
T_7	80	5	5	5	5
T_8	70	10	10	5	5
T_9	60	15	15	5	5
T_{10}	50	20	20	5	5
T_{11}	40	25	25	5	5

Fresh tender jackfruit

Separated from rind and cut into slice

Blanched in boiling water for one minute

Immersed in lukewarm with 0.2% KMS solution for ten minutes

Dried in cabinet drier at 55 – 60^{o} Celsius for 12 hours

Milled into flour

Plate 1. Flow chart of the preparation of tender jackfruit flour

Fresh breadfruit

Washed, peeled and chipped

Blanched in water at 90 ^{0}C with 0.3% citric acid and 1500ppm of SO_2 for five minutes

Dried in cabinet drier at 55 – 60^{0}C for 8 -12 hours

Milled into flour

Plate 2. Flow chart of the preparation of breadfruit flour

Fresh mushrooms

Cleaned and sliced into thin slim strips

Blanched in boiling water for two minutes

Immersed in water with 0.2 per cent KMS + 1 per cent citric acid

Dried in cabinet drier at 60^0 C for 6 to 8 hours

Milled into flour

Plate 3. Flow chart of the preparation of oyster mushroom flour

A pilot study was conducted wherein meat analogues were prepared with the above proposed compositions of pulse as base to which tender jackfruit flour, wheat gluten, defatted soy flour and mushroom flour were added.

During the study, to all the above treatments except control, ingredients such as tender jackfruit flour, wheat gluten, defatted soy flour and mushroom flour were added with one of the pulses as the base in different proportions. The pretreated pulse was washed gently under slow stream of tap water and then blended in a mechanical blender with necessary proportions of other ingredients.Later the mixture was pressure cooked at 121^0 C and 15 psi pressure for a time of 20 minutes. After the steam settled, the pressure cooked mass was transferred to a clean cutting board and cut into square shaped, even cubes. These fresh cubes of meat analogues were immersed in the spice broth for 10 minutes and dried in cabinet drier at 65^0 C for 8 to 12 hours (Plate 4).

3.5. Standardisation of breadfruit incorporated meat analogues

Meat analogues were developed using pretreated cowpea, chickpea, wheat gluten, breadfruit flour, defatted soy flour and mushroom flour in various combinations. The treatments and their composition are given in Table 3 and 4.

Table 3. Proportion of ingredients in breadfruit incorporated cowpea meat analogues

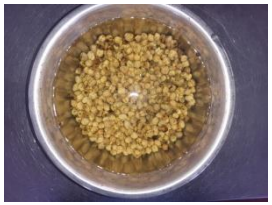
Treatments	Cowpea (%)	Breadfruit (%)	Wheat gluten (%)	Defatted soy flour (%)	Mushroom flour (%)
$T_{0\ (Control)}$	100	-	-	-	-
T_1	80	5	5	5	5
T_2	70	10	10	5	5
T_3	60	15	15	5	5
T_4	50	20	20	5	5
T_5	40	25	25	5	5

Table 4. Proportion of ingredients in breadfruit incorporated cowpea meat analogues

Treatments	Chickpea %	Breadfruit %	Wheat gluten %	Defatted soy flour %	Mushroom flour %
$T_{6\ (Control)}$	100	-	-	-	-
T_7	80	5	5	5	5
T_8	70	10	10	5	5
T_9	60	15	15	5	5
T_{10}	50	20	20	5	5
T_{11}	40	25	25	5	5


(or)


Pulse was washed and roasted for 15 minutes


(or)


Soaked in water containing one percent sodium bicarbonate overnight


or

+

+

+

+ Pretreated pulse

TJ BF WG OMF DSF

Blended

Pressure cooked for 20 minutes at 121^0 C + pressure of 15 psi and cut into cubes

Immersed in the spice broth for 10 minutes

Plate 4 continued….

Cabinet dried at 65^0 C for 8 to 12 hours

Plate 4. Flow chart of the preparation of meat analogues

During the study, to all the above treatments except control, ingredients such as breadfruit flour, wheat gluten, defatted soy flour and mushroom flour were added with one of the pulses as the base in different proportions.

The pretreated pulse was washed gently under slow stream of tap water and then blended in a mechanical blender with necessary proportions of other ingredients. Later the mixture was evenly spread on the platters and pressure cooked for 20 minutes at 121^0 C and pressure of 15 psi. After the steam settled, the pressure cooked mass was transferred to a clean cutting board and cut into square shaped, even cubes. These fresh cubes of meat analogues were immersed in the spice broth for 10 minutes and dried in cabinet drier at 65^0 C for 8 to 12 hours (Plate 4).

3.6. Acceptability of meat analogues

Developed meat analogues were evaluated for their organoleptic qualities.

3.6.1. Selection of panel members for the organoleptic evaluation

A panel of 20 judges (between 18-35 years) was selected by using a triangle test suggested by Jellinek (1985) carried out in the laboratory. The acceptability trials of the meat analogues were done by this panel.

3.6.2. Preparation of score card

The nine-point hedonic scale, originally developed by the US Army was used for the organoleptic evaluation of the food mixtures by the panel members. The score card is given in Appendix I.

3.6.3. Organoleptic evaluation

The meat analogues underwent a series of sensory evaluation by a panel of 20 selected judges using the nine-point hedonic scale. Meat analogues were reconstituted in lukewarm water for 20 minutes (Plate 11). A standard procedure suggested by Philip (1993) for recipe was followed in which meat was replaced

with the reconstituted meat analogues as shown in Plate 12 (Appendix II). The sensory evaluation was carried out and quality attributes like appearance, colour, flavour, texture, taste and overall acceptability was evaluated.

3.7. Selection of meat analogues

On the basis of organolpetic scores using nine point hedonic scale, the best treatment from each set of two experiments with highest organoleptic score along with the control treatments were selected for further studies.

3.8. Quality evaluation of selected meat analogues

The selected meat analogues were evaluated for their nutritional and organolpetic qualities. They were also evaluated for their shelf life attributes.

3.8.1. Nutrient profiling of the selected meat analogues

Selected meat analogues along with the controls were stored for three months and were evaluated for the following nutritional aspects initially and also at the end of storage period.

3.8.1.1. Moisture

Moisture content of the meat analogues was estimated using the method of AOAC (1980). For the determination of moisture content, five gram of the powdered sample was taken in a petri dish and dried in hot air oven at 60^0C to 70^0C, 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.

3.8.1.2. Total carbohydrate

The total carbohydrate content of the meat analogues was estimated by method suggested by Sadasivam and Manickam (1992). The 50 mg of sample was hydrolysed with five ml of 2.5N HCl for three hours and cooled to a room

temperature. The residue was neutralized with solid sodium carbonate until effervescence ceases. The volume was made up to 100 ml and centrifuged. The supernatant (0.2 ml) was made up to one ml and then four ml of Anthrone reagent was added and heated for eight minutes, cooled rapidly and intensity of green to dark colour was read at 630 nm. A standard graph was prepared using standard glucose at serial dilution. From the standard graph, the amount of total carbohydrate present in sample was estimated and expressed in g per 100 g of sample.

3.8.1.3. Protein

AOAC (1980) method was followed to estimate the protein content of the sample. Powdered meat analogues were digested with six ml conc. H_2SO_4 after adding 0.4 g of $CuSO_4$ and 3.5 g of K_2SO_4 in a digestion flask until the sample became colourless. After digestion, it was diluted with water and made up to 1000 ml, out of which ten ml of the sample was pipette out. 25 ml of 40 per cent NaOH was pumped. This distillate was collected in 20 per cent boric acid with 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 expressed in percentage.

3.8.1.4. Total fat

AOAC (1980) method was followed to estimate the total fat content of the sample. Five gram of powdered meat analogue was taken in a thimble and plugged with cotton. The material was extracted with petroleum ether for six hours without interruption by gentle heating in a soxhlet apparatus. Extraction flask was then cooled, and ether was removed by heating and weight was taken. The fat content was expressed in g $100g^{-1}$ of the sample.

3.8.1.5. Total ash

The AOAC (1980) method was followed for the estimation of total ash. First, a clean and dry crucible was properly weighed and recorded. To get the

exact weight of the sample, about two gram of it was put in the crucible and weighed again. The sample was put in a partly open crucible in an electric burner for the sample to be burned with initial smoky expulsion. The crucible was then put in a muffle furnace and heated to 600^0 C for two hours. The crucible was carefully removed from the furnace and allowed to cool to room temperature before being weighed again.

3.8.1.6. 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 H_2SO_4 for thirty minutes. It was filtered through a muslin cloth and washed with 1.25 per cent 25 ml H_2SO_4, three times with 50 ml water and finally with 25 ml 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 expressed as percentage calculated from the loss in weight on ignition.

3.8.1.7. Calcium

Titration method with EDTA method by Page (1982) was followed to estimate the calcium content in which two gram of dried and powdered sample was pre-digested with 10 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 NaOH 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 $100g^{-1}$ of the sample.

3.8.1.8. Iron

Colourimetric analysis using ferric ion, which gives a blood red colour with potassium thiocyanate was followed to determine iron content in the meat analogues (Raghuramulu *et al.*, 2003). To an aliquot of 6.5 ml diacid solution, one ml of 30% H_2SO_4, one ml of 7 per cent potassium persulphate solution and 1.5 ml of 40% potassium thiocyanate were added. The intensity of the red colour was measured within 20 minutes at 540 nm. Using serial dilution of standard iron solution, a standard graph was prepared from which the sample's iron content as estimated and expressed in mg $100g^{-1}$.

3.8.1.9. Magnesium

The amount of magnesium in the meat analogues was calculated using the standard procedure suggested by Perkin-Elmer (1982). The 0.2 gram of dried and powdered meat analogues sample was pre-digested in 10 ml of nitric acid and perchloric acid in 9:4 ratio. In the Atomic Absorption Spectrometer, the diacid extract of the meat analogues was used to estimate magnesium. The proportion of minerals in the assay was measured in milligram per 100 gram.

3.8.1.10. Phosphorous

Phosphorous content in meat analogues was analyzed colourimetrically by the procedure suggested by Jackson (1973), which gives yellow colour with nitric acid and vandatemolybdate reagent. The sample of 0.2g was pre-digested with ten milliliters of 9:4 diacid and the volume was made up to 100 ml. Five milliliters each of nitric acid and vandatemolybdate reagent was added to five milliliters predigested aliquot and the volume was made up to 50 ml with distilled water and OD reading was taken at 420 nm after ten minutes. Using serial dilution of standard phosphorous solution, a standard graph was prepared from which the phosphorous content of the sample were estimated and expressed in mg $100g^{-1}$.

8.8.1.11. Sodium

Flame photometer was used as suggested by Jackson (1973) to estimate the content of sodium in the meat analogues. In the flame photometer, the digested diacid solution of meat analogues was directly read and the value was expressed in mg $100g^{1}$ of the sample.

8.8.1.12. Potassium

Jackson (1973) suggested the use of flame photometer in estimation of potassium content in a sample. One ml of the digested solution was made upto 25 ml and read directly in flame photometer. The potassium content was expressed in $100g^{-1}$ of the sample..In the atomic absorption spectrophotometer, the diacid solution was read and the value is expressed in mg $100g^{-1}$ of the sample.

3.8.1.13. Zinc

The zinc content of the meat analogues was estimated by atomic absorption spectrometric method using diacid extract taken from the sample (Perkin - Elmer, 1982). In the atomic absorption spectrophotometer, the diacid solution was directly read and the value was expressed in mg $100g^{-1}$ of the sample.

3.8.2. *In vitro* studies

In this study, *in vitro* digestibility of protein and mineral bioavailability of meat analogues were estimated. *In vitro* protein digestibility is evaluated by the amount of nitrogen consumed and absorbed by the animal. The *in vitro* bioavailability of a mineral is commonly defined as the fraction of total minerals in food or a meal that is used for normal biological functioning. *In vitro* studies are accurate, quick, inexpensive, simple, robust, adaptive, and relevant to digestion, absorption, and metabolic processes.

3.8.2.1. *In vitro* digestibility of protein

Protein *in vitro* digestibilityof meat analogues was estimated using method suggested by Satterlee (1979).

To the powdered and sieved sample, ten milliliters of glass distilled water was added. The sample as allowed to be hydrated for at least one hour at 5^0 C. The sample was then equilibrated at pH 8.0 at 37^0 C. Three enzyme solution composed of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase per ml in glass distilled water was added to the sample suspension in the amount of one ml and stirred while being held at 37^0 C. After exactly ten minutes from the time of addition of three enzyme solution, one ml of bacterial protease solution was added which was immediately transferred to 55^0 C water bath. After nine minutes precisely, the solution was transferred back to 37^0 C water bath. The pH of the hydroslyate was measured exactly after ten minutes of bacterial enzyme addition. This is called the 20 minute pH. The *in vitro* protein digestibility was calculated using the following equation: % digestibility = 234.84 – 22.56 X (where X is the measured pH after 20 minutes incubation).

3.8.2.2. *In vitro* availability of minerals

The method of Duhan *et al.* (2001) was used to determine the *in vitro* availability of calcium, iron, potassium, phosphorus, zinc and magnesium. The HCl extractability of minerals was computed for *in vitro* availability. The selected meat analogue samples were collected in a shaker for three hours at 37^0 C with 0.03 N H Cl. Whatman No. 40 filter paper was used to filter the sample. The clear extract was dried in an oven at 100^0 C before being digested with moist acid. The amount of HCl extractable calcium, iron, potassium, phosphorus, zinc and magnesium in the digested sample was then calculated using the above-mentioned

methods for mineral estimation. To calculate the HCl extractability, the following formula was recommended.

$$\text{Mineral availability (\%)} = \frac{\text{Mineral extractability in 0.03 N HCl} \times 100}{\text{Total mineral}}$$

3.8.3. Shelf life studies

The meat analogues selected on the basis of their acceptability along with the controls were stored for a period of three months in food grade HDPE covers (250 gauge) at both ambient and refrigerated temperature conditions for a period of three months (Plate 13). Samples were analyzed for their organoleptic qualities, microbial quality and insect infestation at monthly intervals. Nutrient profiling and *in vitro* studies of the elected meat analogues were done initially and at the end of storage period.

3.8.3.1.Organoleptic qualities

Organoleptic evaluation of the selected meat analogues was conducted using score card by a panel of 15 judges as described in 3.5.3. Quality attributes like appearance, colour, flavour, texture, taste and overall acceptability were evaluated at monthly intervals of the storage period.

3.8.3.2. Nutritional and *in vitro* studies at the end of storage period

Studies with respect to nutrient and *in vitro* studies of meat analogues were conducted initially and at the end of storage period as mentioned in 3.8.1. and 3.8.2.

3.8.3.3. Enumeration of total microflora

The selected meat analogues were evaluated for the presence of bacteria, fungi and yeast at monthly intervals following the plate count method as described by Agarwal and Hasija (1986). Ten ml of the sample was added to 90 ml of sterile water

and shaken for 20 minutes. One ml of this solution was transferred to a test tube containing 9 ml sterile water which is 10^{-2} dilution and simultaneously dilutions till 10^{-6} were prepared. Enumeration of total microflora was carried out using media such as Nutrient Agar for bacteria, Sabouraud's Dextrose Agar for yeast and Potato Dextrose Agar for fungi.

3.8.3.3.1. Enumeration of bacterial colony

In the nutrient agar medium, the total number of bacterial colonies was counted in a 10^{-5}dilution. Using a micropipette, pour one ml of 10^{5} dilution into a clean petri dish. Pour about 20ml of the nutrient agar medium into the petri dish, which is equally distributed in the petri dish by spinning clockwise and anticlockwise. The enumerated petri dishes were incubated for 48 hours at room temperature for bacterial colonies. The total number of bacterial colonies were counted and expressed in colony forming units per gram (cfu/g).

3.8.3.3.2. Enumeration of fungal colony

In Potato Dextrose Agar, the total number of fungal colonies was counted in a 10^{-3}dilution.Using a micropipette, pour one ml of 10^{3} dilution into a clean petri dish. Pour about 20 ml of Potato Dextrose Agar medium into a petri dish and spread evenly. The petri dishes were incubated at room temperature for 4 to 5 days to count the fungal colonies. The number of fungal colonies counted in total. The total number of fungal colonies were counted and expressed in colony forming units per gram (cfu/g).

3.8.3.3.3. Enumeration of yeast colony

In Sabouraud's Dextrose Agar medium, the total number of yeast colonies was counted ina10^{3}dilution. Using a micropipette, pour one ml of 10^{-3} dilution in to a clean petridish. Pour about 20 ml of Sabouraud's Dextrose Agar medium into the petridish, rotating it to evenly distribute the medium. The petridishes were incubated in room temperature for 4 to 5 days to count the yeast

population. The total number of yeast colonies were counted and expressed in colony forming units per gram (cfu/g).

3.8.3.4. Insect infestation

By visual observation and by examining microscopically, the presence of storage insects in meat analogues were assessed at monthly intervals.

3.9. Cost of production

To determine the cost of production of the selected meat analogues along with the controls were calculated by considering the material cost (market value), labour charges, fuel and electricity charges and packing cost. The price was determined based on 100 gram.

3.10. Statistical analysis

The observations of three independent recorded determinations were tabulated and the data were analyzed statistically using completely randomized design (CRD) and Duncan's multiple range test (DMRT) for nutrient and *in vitro* studies. The scores of organoleptic evaluation were assessed using Kendall's coefficient (W) from mean values of observations. The effect of storage on sensory characteristics of the meat analogues were studied using scores of organoleptic evaluation. Nutrient and *in vitro* studies under different storage conditions were analyzed from the recorded three independent determinations to which paired t - test was applied.

Results

4. RESULTS

The results of the study entitled "Standardisation of jackfruit and breadfruit incorporated meat analogues" are presented under the following headings.

4.1. Standardisation of tender jackfruit incorporated meat analogues

4.1.1. Organoleptic evaluation of tender jackfruit incorporated cowpea meat analogues -Set I

4.1.2. Organoleptic evaluation of tender jackfruit incorporated chickpea meat analogues -Set II

4.1.3. Selection of the best treatment

4.2. Standardisation of breadfruit incorporated meat analogues

4.2.1. Organoleptic evaluation of breadfruit incorporated cowpea meat analogues - Set I

4.2.2. Organoleptic evaluation of breadfruit incorporated chickpea meat analogues - Set II

4.2.3. Selection of the best treatment.

4.3. Quality evaluation of selected meat analogues

4.3.1. Nutrient studies of the selected meat analogues

4.3.1.1. Moisture, total carbohydrates, protein, total fat, total ash and fibre content of selected meat analogues of selected meat analogues

4.3.1.2. Mineral composition of selected meat analogues

4.3.2. *In vitro* studies of selected meat analogues

4.3.2.1. *In vitro* protein digestibility of selected meat analogues

4.3.2.2. *In vitro* mineral availability of selected meat analogues

4.4. Shelf life studies

4.4.1. Organoleptic evaluation of selected meat analogues on storage

4.4.2. Effect of storage conditions on the nutrient composition of the selected meat analogues on storage

4.4.2.1. *In vitro* protein digestibility of selected meat analogue on storage

4.4.2.2. *In vitro* mineral availability of selected meat analogue on storage

4.4.3. Enumeration of total microflora in selected meat analogues on storage

4.4.4. Insect infestation in selected meat analogues on storage

4.5.Cost benefit analysis

4.1.Standardisation of tender jackfruit incorporated meat analogues

Meat analogues were standardised using ingredients such as tender jackfruit (TJ), cowpea (CWP), chickpea (CP), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) with varied proportions in different treatments. In this study the eleven treatments were divided into two sets with T_0 to T_5 using cowpea (CWP), as the first and T_6 to T_{11} using chickpea (CP), as the second set with tender jackfruit (TJ), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) as common ingredients with varied proportions corresponding to their respective treatments. The treatments standardised were [**T_0**- 100 % CWP (Control), **T_1** – 80 % CWP + 5 % TJ + 5 % WG + 5 % DSF + 5 % OMF, **T_2** - 70 % CWP + 10 % TJ + 10 % WG + 5 % DSF + 5 % OMF, **T_3** - 60 % CWP + 5 % TJ + 5 % WG + 5 % DSF + 5 % OMF, **T_4** - 50 % CWP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP (Control), **T_7** - 80 % CP + 5 % TJ + 5 % WG + 5 % DSF + 5 % OMF, **T_8** - 70 % CP + 10 % TJ + 10 % WG + 5 % DSF + 5 % OMF, **T_9** - 60 % CP + 5 % TJ + 5 % WG + 5 % DSF + 5 % OMF, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF and **T_{11}** - 40 % CP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF]. The developed fresh meat analogue cubes were soft, springy with distinctive 'pulse' flavour and the cut surface showed defined interconnected inner framework of its ingredients. These fresh cubes of meat analogues were immersed in the spice broth for 10 minutes and dried in cabinet drier at 65^0 C for 8 to 12 hours.

These dried meat analogues had similar appearance to dried beef (Eg: Plate 7) with notable rigidity which when reconstituted showed regained springiness.

4.1.1. Organoleptic evaluation of tender jackfruit incorporated cowpea meat analogues – Set I

The eleven treatments were divided into two sets with T_0 to T_5 using cowpea, as the first and T_6 to T_{11} using chickpea, as the second set. The meat analogues were organoleptically evaluated by replacing meat with reconstituted meat analogues in standard meat recipe. Meat analogues from each treatment was evaluated for its organolpetic qualities such as appearance, colour, flavour, texture, taste and overall acceptability using nine point hedonic scale by 20 judges. The values from score cards were used for statistical analysis to select the best treatment. Mean scores obtained for the organoleptic attributes of meat analogues of set I (T_0 to T_5) are presented in Table 5.

As revealed in Table 5, highest total score for set I of meat analogues was seen in the treatment (T_5) with a highest total score of 51.62 followed by T_4, T_3, T_2, T_1 and T_0 with total score of 48.93, 47.10, 46.24, 43.82 and 43.26 respectively. Control T_0 (100 per cent CWP) had lowest score (7.62) in the attribute appearance and the highest score for appearance was seen in T_5 (8.50) of set I. With regards to the attribute colour, treatments T_1 and T_2 ranked the lowest (7.81) with the highest (8.45) score for colour seen in T_5 of set I. Treatment T_1 had the lowest score in both flavour (7.21) and texture (6.24) while the treatment T_5 had the highest scores of flavour (8.53) and texture (8.78) of set I respectively. The control T_0 had lowest (7.50) score for taste and the highest (8.55) was observed in T_5 of set I correspondingly. In overall acceptability of different treatments of meat analogues, T_0 ranked the lowest (6.54) while T_5 of set I ranked the highest (8.81) in overall acceptability. Treatment T_5 was observed to have highest scores for all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability.

Among the treatments of set I, T_0 had the highest mean rank score in all attributes except appearance while T_2 has the highest mean score for appearance and T_1 scoring the lowest in mean rank scores in all the five attributes.

4.1.2. Organoleptic evaluation of tender jackfruit incorporated chickpea meat analogues – Set II

Meat analogues from each treatment was evaluated for its organolpetic qualities such as appearance, colour, flavour, texture, taste and overall acceptability using nine point hedonic scale by 20 judges. The meat analogues were organoleptically evaluated by replacing meat with reconstituted meat analogues in standard meat recipe. The values from score cards were used for statistical analysis to select the best treatment. Mean scores obtained for the organoleptic attributes of tender jackfruit incorporated chickpea meat analogues of set II (T_6 to T_{11}) are presented in Table 6.

As revealed in Table 6, treatment T_{10} had the highest total score for set II of tender jackfruit incorporated chickpea meat analogues with highest total score of 51.01 followed by T_{11}, T_8, T_9, T_6 and T_7 with total score of 48.25, 45.88, 45.43, 44.35, and 42.43 respectively. The treatment T_7 had lowest score (7.36) in the attribute appearance and the highest score (8.64) for appearance was seen in T_{10} of set II.With regards to the attribute colour, treatment T_9 ranked the lowest (7.57) with the highest score (7.85) for colour seen in T_{10} of set II. Treatment T_7 had the lowest score in flavour (6.24) texture (6.40) and taste (7.45) while the treatment T_{10} of set II had the highest scores of flavour (8.48) texture (8.76) and taste (8.64) respectively. In overall acceptability of different treatments of meat analogues, T_6 ranked the lowest (7.16) while T_{10} of set II ranked the highest (8.64) in overall acceptability. Treatment T_{10} was observed to have highest scores for all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability.

Plate 5. Control meat analogues (T_0)

T_0 (100% CWP)

Plate 6. Control meat analogues (T_6)

T_6 (100% CP)

Plate 7. Selected treatment of tender jackfruit incorporated cowpea meat analogues (T_5)

T_5 (40 % CWP + 25 % TJ+ 25 % WG + 5 % DSF + 5 % OMF)

Table 5. Mean scores for the organoleptic qualities of tender jackfruit incorporated cowpea meat analogues – Set I ($T_0 – T_5$)

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_0(100% CWP)	7.62 (3.89)	7.93 (5.82)	7.27 (5.29)	6.40 (5.64)	7.50 (5.14)	6.54 (4.79)	43.26
T_1 (80%CWP+5%TJ +5%WG+5%DSF +5%OMF)	7.71 (2.89)	7.81 (1.75)	7.21 (1.43)	6.24 (1.93)	7.74 (1.50)	7.11 (2.29)	43.82
T_2(70%CWP+10%TJ +10%WG +5%DSF +5%OMF)	7.71 (3.93)	7.81 (3.11)	7.74 (3.29)	7.62 (2.64)	7.81 (3.18)	7.55 (3.29)	46.24
T_3(60%CWP+5%TJ +5%WG +5%DSF +5%OMF)	7.81 (3.72)	7.86 (4.61)	8.00 (4.36)	7.81 (4.64)	7.76 (4.39)	7.86 (4.21)	47.10
T_4(50%CWP+20%TJ +20%WG +5%DSF +5%OMF)	8.02 (3.29)	7.88 (2.46)	8.11 (2.86)	8.45 (2.79)	8.02 (2.96)	8.45 (2.93)	48.93
T_5(40%CWP+25%TJ +25%WG +5%DSF +5%OMF)	8.50 (3.79)	8.45 (3.25)	8.53 (3.79)	8.78 (3.36)	8.55 (3.82)	8.81 (3.50)	51.62
Kendall's value (W)	0.11*	0.67*	0.58*	0.64*	0.49*	0.26*	

CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour, DSF - Defatted soy flour

Figures in parenthesis indicate mean rank scores

* - Significant at 5% level

Table 6. Mean scores for the organoleptic qualities of tender jackfruit incorporated chickpea meat analogues – Set II (T_6 – T_{11})

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall Acceptability	Total score
T_6(100% CP)	7.50 (3.89)	7.78 (5.82)	7.64 (5.29)	6.69 (5.64)	7.57 (3.82)	7.16 (2.93)	44.35
T_7 (80%CP+5%TJ +5%WG +5%DSF +5%OMF)	7.36 (3.93)	7.62 (1.75)	6.24 (1.43)	6.40 (1.93)	7.45 (1.50)	7.36 (2.29)	42.43
T_8(70%CP+10%TJ +10%WG +5%DSF +5%OMF)	7.45 (2.39)	7.64 (4.36)	7.59 (3.11)	7.78 (2.64)	7.64 (3.18)	7.78 (3.39)	45.88
T_9 (60%CP+5%TJ +5%WG +5%DSF+5%OMF)	7.69 (3.71)	7.57 (2.29)	7.40 (3.29)	7.40 (3.36)	7.59 (5.14)	7.78 (4.79)	45.43
T_{10}(50%CP+20%TJ +20%WG +5%DSF +5%OMF)	8.64 (3.79)	7.85 (4.61)	8.48 (3.79)	8.76 (4.64)	8.64 (4.39)	8.64 (3.50)	51.01
T_{11}(40%CP+25%TJ +25%WG +5%DSF +5%OMF)	7.93 (3.29)	7.64 (2.46)	7.78 (2.86)	8.45 (2.79)	7.90 (2.96)	8.55 (3.25)	48.25
Kendall's value (W)	0.17*	0.67*	0.58*	0.64*	0.49*	0.27*	

CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour

Figures in parenthesis indicate mean rank scores

* - Significant at 5% level

Plate 8. Selected treatment of tender jackfruit incorporated chickpea meat analogues (T_{10})

T_{10} (50 % CP + 20 % TJ+ 20 % WG + 5 % DSF + 5 % OMF)

Plate 9. Selected treatment of breadfruit incorporated cowpea meat analogues (T_4)

T_4 (50 % CWP + 20 % BF+ 20 % WG + 5 % DSF + 5 % OMF)

Plate 10. Selected treatment of breadfruit incorporated chickpea meat analogues (T_{11})

T_{11} (40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF)

Among the treatments of set II, control T_6 had the highest mean rank scores in colour, flavour and texture while highest mean rank score for appearance was observed in T_7 and T_9 had the highest mean rank scores for taste and overall acceptability. The treatment T_7 was observed to have lowest scores of mean rank in all sensory attributes except for appearance for which T_8 has the lowest mean rank score.

4.1.3. Selection of the best treatment

The one best treatment from the two sets of meat analogues set I - T_0 to T_5 and set II - T_6 to T_{11} along with their controls were selected based on the overall acceptability and total score. The treatments T_5 and T_{10} were best selected treatments from tender jackfruit incorporated meat analogues in experiment I. Statistical analysis by applying Kendall's (W) test showed that Kendall's (W) value was highly significant with regards to all quality attributes. Hence, the treatments selected for further studies from experiment I were $T_5$and T_{10} along with controls (T_0 and T_6).

4.2. Standardisation of breadfruit incorporated meat analogues

Meat analogues were standardised using ingredients such as breadfruit flour (BF), cowpea (CWP), chickpea (CP), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) with varied proportions in different treatments. The eleven treatments were divided into two sets with T_0 to T_5 using cowpea (CWP), as the first and T_6 to T_{11} using chickpea (CP), as the second set breadfruit flour (BF), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) as common ingredients with varied proportions corresponding to the respective treatments. The treatments standardized were [**T_0** – 100 % CWP, **T_1** – 80 % CWP + 5 % BF + 5 % WG + 5 % DSF + 5 % OMF, **T_2** – 70 % CWP + 10 % BF + 10 % WG + 5 % DSF + 5 % OMF, **T_3** – 60 % CWP + 5 % BF + 5 % WG + 5 % DSF + 5 % OMF, **T_4** – 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** – 40 % CWP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_7** – 80 % CP + 5 % BF + 5 % WG + 5 % DSF + 5

% OMF, **T_8** – 70 % CP + 10 % BF + 10 % WG + 5 % DSF + 5 % OMF, **T_9** – 60 % CP + 5 % BF + 5 % WG + 5 % DSF + 5 % OMF, **T_{10}** – 50 % CP + 20 % BF + 20 % WG +5 % DSF + 5 % OMF and **T_{11}** – 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF]. The developed fresh meat analogue cubes were soft, springy with distinctive 'grainy' texture which in cut surface showed defined interconnected inner framework of its ingredients. These fresh cubes of meat analogues were immersed in the spice broth for 10 minutes and were dried in cabinet drier at 65^0 C for 8 to 12 hours. These dried meat analogues had similar appearance to dried beef with notable rigidity (Eg: Plate 9) which when reconstituted showed regained springiness.

4.2.1. Organoleptic evaluation of breadfruit incorporated cowpea meat analogues – Set I

The eleven treatments of breadfruit incorporated meat analogues were divided into two sets with T_0 to T_5 using cowpea, as the first and T_6 to T_{11} using chickpea, as the second set. The meat analogues were organoleptically evaluated by replacing meat with meat analogues in standard meat recipe. Meat analogues from each treatment was evaluated for its organolpetic qualities such as appearance, colour, flavour, texture, taste and overall acceptability using nine point hedonic scale by 20 judges. The values from score cards were used for statistical analysis to select the best treatment. Mean scores obtained for the organoleptic attributes of meat analogues of set I (T_0 to T_5) are presented in Table 7.

Plate 11. Reconstituted meat analogues

Plate 12. Meat analogue curry

Plate 13. Storage of meat analogues in food grade HDPE covers

Table 7. Mean scores for the organoleptic qualities of breadfruit incorporated cowpea meat analogues – Set I

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_0 (100 % CWP)	7.55 (1.75)	7.59 (3.07)	7.09 (2.11)	6.43 (1.64)	7.43 (2.18)	6.67 (1.50)	42.76
T_1(80 % CWP + 5 % BF + 5 % WG + 5 % DSF + 5 % OMF)	8.07 (3.64)	7.62 (2.89)	7.12 (2.07)	6.38 (1.57)	7.62 (2.79)	7.07 (2.18)	43.88
T_2 (70 % CWP + 10 % BF + 10 % WG + 5 % DSF + 5 % OMF)	8.26 (4.46)	7.57 (2.82)	7.62 (3.11)	7.67 (3.61)	7.74 (3.32)	7.55 (3.36)	46.41
T_3 (60 % CWP + 5 % BF + 5 %WG + 5 % DSF + 5 % OMF)	8.05 (3.64)	7.83 (3.89)	8.07 (4.39)	7.67 (3.54)	7.74 (3.36)	7.86 (3.86)	47.22
T_4 (50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 %OMF)	8.17 (3.93)	8.07 (4.39)	8.30 (4.82)	8.74 (5.64)	8.36 (4.82)	8.52 (5.25)	50.16
T_5 (40 % CWP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF)	8.02 (3.57)	7.93 (3.93)	8.14 (4.50)	8.57 (5.00)	8.19 (4.54)	8.38 (4.86)	49.23
Kendall's value (W)	0.26*	0.14*	0.48*	0.82*	0.31*	0.65*	

CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour

Figures in parenthesis indicate mean rank scores

* - Significant at 5% level

As shown in Table 7, the treatment (T_4) had the highest total score of 50.16 followed by T_5, T_3, T_2, T_1 and T_0 with total score of 49.23, 47.22, 46.41, 43.88 and 42.76 respectively. Control T_0 (100% CWP) had lowest score (7.55) in the attribute appearance and the highest (8.26) score for appearance was seen in T_2 of set I. With regards to the attribute colour, treatment T_2 ranked the lowest (7.57) with the highest score (8.07) for colour seen in T_4 of set I. The treatment T_1 had lowest score (6.38) for texture and the highest score (8.74) for texture was observed in T_4 of set I correspondingly. Pertaining to the sensory quality flavour of different treatments of meat analogues, T_0 ranked the lowest (7.09) while T_4 of set I ranked the highest (8.30). The control T_0 had the lowest score in both taste (7.43) and overall acceptability (6.67) while the treatment T_4 had the highest scores of taste (8.36) and overall acceptability (8.52) of set I respectively. The best treatment from set I therefore was T_4 with highest (8.52) overall acceptability. Treatment T_4 was observed to have highest scores for the sensory attributes including colour, flavour, texture, taste and overall acceptability. Among the treatments of set I, T_2 had the lowest mean rank scores for sensory qualities of colour, flavour and texture and T_0had lowest mean rank score in appearance, taste and overall acceptability. Highest mean rank scores in all attributes except for appearance were observed in T_4 while T_2 had highest mean rank score for appearance.

4.2.2. Organoleptic evaluation of breadfruit incorporated chickpea meat analogues – Set II

Meat analogues from each treatment was evaluated for its organolpetic qualities such as appearance, colour, flavour, texture, taste and overall acceptability using nine point hedonic scale by 20 judges. Standard meat recipe was prepared in which meat was replaced with the reconstituted meat analogues. The values from score cards were used for statistical analysis to select the best treatment. Mean score obtained for the organoleptic attributes of breadfruit incorporated chickpea meat analogues of set II (T_6 to T_{11}) are presented in Table 8.

Table 8. Mean scores for the organoleptic qualities of breadfruit incorporated chickpea meat analogues – Set II

Treatment	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_6 (100 % CP)	7.55 (1.96)	7.59 (2.64)	7.09 (2.14)	6.43 (1.64)	7.43 (2.29)	6.67 (1.46)	42.76
T_7(80 % CP + 5 % BF + 5 % WG + 5 % DSF + 5 % OMF)	8.07 (3.82)	7.76 (3.29)	7.04 (1.96)	6.38 (1.57)	7.62 (2.79)	7.07 (2.07)	43.94
T_8 (70 % CP + 10 % BF + 10 % WG + 5 % DSF + 5 % OMF)	7.93 (3.50)	7.92 (3.68)	7.61 (2.93)	7.67 (3.71)	7.74 (3.18)	7.55 (3.25)	46.42
T_9 (60 % CP + 5 % BF + 5 % WG + 5 % DSF + 5 % OMF)	8.04 (3.89)	7.90 (3.54)	8.07 (4.46)	7.67 (3.61)	7.74 (3.46)	7.86 (3.64)	47.28
T_{10}(50 % CP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF)	8.02 (3.50)	7.78 (2.79)	8.09 (4.32)	8.21 (4.71)	7.97 (3.75)	8.45 (4.86)	48.52
T_{11} (40 % CP + 25% BF + 25 % WG + 5 % DSF + 5 % OMF)	8.26 (4.32)	8.45 (5.07)	8.33 (5.18)	8.78 (5.75)	8.55 (5.54)	8.81 (5.71)	51.18
Kendall's value (W)	0.21*	0.24*	0.55*	0.82*	0.38*	0.78*	

CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour

Figures in parenthesis indicate mean rank scores

* - Significant at 5% level

The control T_6 (100% CP) ranked the lowest in the sensory attributes such as appearance (7.55), colour (7.59) and taste (7.43). The highest score for appearance (8.26), colour (8.45), flavour (8.33) and texture (8.78) taste (8.55) observed in treatment T_{11}. Lowest scores for the sensory attributes flavour (7.04) and texture (6.38) was seen in treatment T_7 Among the treatments of breadfruit incorporated chickpea meat analogues, overall acceptability was seen to be lowest and highest in T_6 (6.67) and T_{11} (8.81) respectively. The total score was seen highest in T_{11} (51.18) and lowest (42.76) in control T_6 which is 100 per cent chickpea. Treatment T_{11} was observed to have highest scores for all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability. In set II, T_6 had the lowest mean rank scores for attributes such as appearance, colour, taste and overall acceptability while lowest mean rank score for flavour and texture was observed in T_7. The treatment T_{11} had the highest mean rank scores in all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability.

4.2.3. Selection of the best treatment

The best treatment along with controls from the two sets of breadfruit incorporated cowpea and chickpea meat analogues, set I - (T_0 to T_5) and set II - (T_6 to T_{11}) were selected based on the overall acceptability and total score. From set I and II, T_4 and T_{11} were best selected treatments respectively from breadfruit incorporated cowpea and chickpea meat analogues in experiment II. Statistical analysis by applying Kendall's (W) test showed that Kendall's (W) value was highly significant with regards to all quality attributes. Hence, the treatments selected for further studies from experiment II were T_4 and T_{11} along with controls (T_0 and T_6).

4.3. Quality evaluation of the selected meat analogues

Table 9 shows the best treatments and the control of both tender jackfruit and breadfruit meat analogues that were selected with respect to their organoleptic qualities for further studies. From experiment I, best treatments were T_5 with

tender jackfruit and cowpea along with T_{10} whose main ingredients were tender jackfruit and chickpea. Best treatments from experiment II were T_4 with breadfruit and cowpea as main ingredients and T_{11} with breadfruit and chickpea as main ingredients. The controls were composed of 100 per cent cowpea as in T_0 while T_6 contained 100 per cent chickpea. The controls were the same for both experiments I and II.The treatments along with their controls were evaluated for their quality in terms of nutrient studies, *in vitro* studies and shelf life studies.

Table 9. Combinations of best selected treatments and controls

Treatments	Combination	
T_0	100 % CWP	Control
T_6	100 % CP	
T_4	50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF	Best treatments
T_5	40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF	
T_{10}	50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF	
T_{11}	40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF	

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

4.3.1. Nutrient studies of the selected meat analogues

Nutrient studies of the selected meat analogues and their controls included estimation of moisture, total carbohydrate, protein, total fat, total ash, fibre and minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc. The results of the nutrient studies of best selected treatments and the control (T_0 and T_6) are furnished in Table 10 and Table 11.

4.3.1.1. Moisture, total carbohydrates, protein, total fat, total ash and fibre content of selected meat analogues

Moisture, total carbohydrate, protein, total fat, total ash and fibre in the control (T_0 and T_6) and the selected treatments (T_4, T_5, T_{10} and T_{11}) of meat analogues are furnished in Table 10.

The highest moisture content was observed in treatment T_{11} (10.62%)and lowest moisture content of 9.25 per cent was observed in T_0 (100% CWP). Among the six treatments, T_{10} was on par with rest of the treatments when analysed using DMRT.

The highest content of total carbohydrates (53.29 g $100g^{-1}$) was seen in T_6 (100 % CP) and the lowest content of total carbohydrates was observed in T_5 (32.46 g $100g^{-1}$) among selected meat analogues. Significant difference in total carbohydrate content was observed in some treatments except for T_5 and T_{11} which were seem to be on par with treatment T_4.

Protein content of meat analogues ranged between 20.79 to 38.03 g $100g^{1}$ (Table 10). The controls T_0 and T_6 differed significantly with the rest of the treatments with regards to the content of protein on the basis of DMRT.

Total fat content was observed to be highest in T_0 (1.92 g $100g^{-1}$) and the lowest content of 1.06 g $100g^{-1}$ was observed in T_4 treatment . Treatment T_0 differed significantly with all the treatments T_4, T_5 and T_6 in the content of total fat on the basis of DMRT.

Highest total ash content was observed in T_5 (5.66 g $100g^{-1}$) and the lowest was observed in T_0 (2.92 g $100g^{-1}$). Treatments T_0, T_4 and T_6 showed significant difference in their total ash content against T_5, T_{10}, T_{11} on the basis of DMRT.

Fibre content was found to be between the range of 2.23 to 7.30 g $100g^{-1}$ in the meat analogues. The treatments T_5 and T_{10} differed significantly with the rest of the treatments pertaining to fibre content on the basis of DMRT analysis.

4.3.1.2. Mineral composition of selected meat analogues

The results of the mineral profiling of best selected treatments and the controls are furnished in Table 11 with content of minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc.

Calcium content was observed to be the highest in T_{10} (94.67 mg 100g $^{-1}$) and the lowest calcium content (80.25 mg 100g $^{-1}$) was observed in T_0 (100% CWP). On the basis of DMRT, no significant difference was observed between the treatments T_0, T_5 and T_6.

Phosphorous content was found to be highest (325.46 mg 100g $^{-1}$) in T_0 and lowest phosphorous content was observed in T_{11} (255.62 mg 100g $^{-1}$). On the basis of DMRT, all the treatments differed with each other significantly in phosphorus content except for T_6 and T_{11} which were on par with each other.

Sodium content ranged between 23.52 to 74.43 mg 100g^{-1} in the meat analogues with significant difference observed amongst all the treatments except for T_0 and T_6 which were on par with each other.

The treatment T_4 had the highest potassium content (631.50 mg 100g ^{1}) and lowest potassium content was seen in T_6 (510.49 mg 100g $^{-1}$). Significant difference was not observed between the treatments T_4, T_5 and T_{10}, T_{11} in terms of their potassium content.

The range of magnesium content in the selected meat analogues ranged between 103.64 to 181.69 mg 100g^{-1} and the lowest magnesium content was observed in T_{11} and highest content observed in treatment T_4. The treatments (T_0, T_4) and (T_6, T_{11}) showed no significance difference with each other with regards to magnesium content.

The content of the iron was observed to be highest (5.73 mg 100g $^{-1}$) in T_0 and the lowest iron content was observed (4.17 mg 100g $^{-1}$) in T_5 and the treatments T_0 and T_5 differed significantly with each other and also rest of the

treatments. Significant difference was not observed in the iron content of the treatments T_4, T_6, T_{10} and T_{11}.

Zinc content ascended from the lowest content of 3.17 mg $100g^{-1}$ in T_5 to 3.96 mg $100g^{-1}$ in T_{10}. Th treatments T_4, T_5 and T_6 showed no significant difference with respect to their zinc content. Similarly, the treatment T_6, T_{10} and T_{11} also were not significantly different with each other.

Table 10. Estimation of moisture, total carbohydrates, protein, total fat, total ash and fibre of selected meat analogues (per 100g)

Treatments	Moisture (%)	Total carbohydrate (g)	Protein (g)	Total fat (g)	Total ash (g)	Fibre (g)
T_0	9.25^{c}	48.91^{b}	24.96^{c}	1.92^{a}	2.92^{b}	3.10^{cd}
T_4	10.54^{ab}	35.95^{de}	34.55^{b}	1.06^{b}	3.23^{b}	3.85^{bc}
T_5	9.40^{c}	32.46^{e}	38.03^{a}	1.43^{b}	5.66^{a}	7.30^{a}
T_6	9.60^{bc}	53.29^{a}	20.79^{d}	1.20^{b}	2.96^{b}	2.23^{d}
T_{10}	9.70^{abc}	42.10^{c}	37.13^{a}	1.60^{ab}	5.33^{a}	6.86^{a}
T_{11}	10.62^{a}	36.94^{d}	36.61^{ab}	1.63^{ab}	5.00^{a}	4.69^{b}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

DMRT column wise comparison

Values with same superscript do not have significant difference

Values are mean of three independent determinations

Table 11. Mineral profiling of the selected meat analogues (per 100g)

Treatments	Calcium (mg)	Phosphorous (mg)	Sodium (mg)	Potassium (mg)	Magnesium (mg)	Iron (mg)	Zinc (mg)
T_0	80.25^{c}	325.46^{a}	23.88^{e}	603.03^{b}	178.02^{ab}	5.73^{a}	3.62^{a}
T_4	92.50^{b}	316.04^{b}	52.17^{b}	631.50^{a}	181.69^{a}	4.33^{b}	3.22^{b}
T_5	85.10^{c}	308.95^{c}	74.43^{a}	618.89^{a}	162.01^{b}	4.17^{c}	3.17^{b}
T_6	85.16^{c}	255.67^{e}	23.52^{e}	510.49^{d}	110.58c^{d}	4.54^{b}	3.26^{b}
T_{10}	94.67^{a}	269.58^{d}	34.85^{d}	540.20^{c}	120.93^{c}	4.67^{b}	3.96^{a}
T_{11}	93.28^{a}	255.62^{e}	38.720^{c}	522.42^{c}	103.64^{d}	4.21^{b}	3.89^{a}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

DMRT column wise comparison

Values with same superscript do not have significant difference

Values are mean of three independent determinations

4.3.2. *In vitro* studies of selected meat analogues

In vitro studies in the selected meat analogues and their controls were analysed. Estimation of *in vitro* protein digestibility and *in vitro* availability of minerals including calcium, phosphorous, sodium, potassium, magnesium, iron and zinc were evaluated.

4.3.2.1. *In vitro* protein digestibility of selected meat analogues

In vitro protein digestibility of the selected treatments and the controls are furnished in Table 12. The treatment T_6 had the lowest protein *in vitro* digestibility of 62.12 per cent while T_4 was observed to have the highest (80.30 %) of protein *in vitro* digestibility. The statistical analysis showed that the treatment T_5 was on par with treatments T_4, T_{10} and T_{11} with respect to *in vitro* protein digestibility. Significant difference was not observed in the *in vitro* protein digestibility between both the controls (T_0 and T_6).

Table 12. *In vitro* protein digestibility of selected meat analogues

Treatment	Protein digestibility
T_0	63.76 ^{c}
T_4	80.30^{a}
T_5	73.74 ^{ab}
T_6	62.12 ^{c}
T_{10}	65.43 ^{b}
T_{11}	76.17^{a}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF
(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)
DMRT column wise comparison
Values with same superscript do not have significant difference
Values are mean of three independent determinations

4.3.2.2. *In vitro* mineral availability of selected meat analogues

Table 13 shows the *in vitro* availability of minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc of the selected treatments (T_4, T_5, T_{10} and T_{11}) and the controls (T_0 and T_6).

Calcium *in vitro* availability was observed to be the highest (87.62 %) in T_4 and lowest *in vitro* calcium availability was observed in T_6 (34.43 %). On the basis of DMRT, all the treatments differed significantly except for T_{10} which was on par with T_4, T_5 and T_{11}.

Phosphorous *in vitro* availability was observed to be the lowest (47.62%) in T_6 (100% CP) with the highest (71.43 %) observed in T_5. The treatments T_{10} and T_{11} were on par with all the other treatments with respect to *in vitro* phosphorous availability.

Sodium *in vitro* availability ranged between 57.66 to 77.20 per cent in the selected meat analogues. The treatment T_{11} was found to be on par with the treatments T_4, T_5 and T_{10} with respect to their *in vitro* sodium availability.

The treatment T_5 (82.85 %) had the highest potassium *in vitro* availability and lowest (62.54 %) was seen in T_{11}. Significant difference was not observed between treatments (T_4, T_5), (T_0, T_{10}) and (T_6. T_{11}).

The range of *in vitro* availability of magnesium in the selected meat analogues was between 54.40 to 63.73 per cent with the lowest *in vitro* magnesium availability observed in T_5 and highest observed in T_4. Significant difference was not observed between the treatments T_0 and T_4, similarly, the treatments T_{10} and T_{11} also showed no significant difference with regards to their *in vitro* magnesium availability.

The *in vitro* availability of iron was observed to be highest (73.32 %) in T_{11} and the lowest (52.40 %) in T_0 (100 % CWP). Significant difference was not observed between the treatments T_4 and T_5 (which were on par with each other) and T_6 and T_{10} (which were on par with each other).

Zinc *in vitro* availability was found to be highest (64.28%) in T_{11} and the lowest (55.89%) was reported in T_0 (100% CWP). Both the control treatments

(T_0 and T_6) were on par with each other with respect to their zinc *in vitro* availability. Among the treatments, the treatments T_4, T_{10} and T_{11} showed no significant difference.

Table 13. *In vitro* mineral availability of the selected meat analogues (%)

Treatment	Calcium	Phosphorous	Sodium	Potassium	Magnesium	Iron	Zinc
T_0	45.58^{c}	61.18^{b}	58.52^{c}	68.34^{b}	62.84^{a}	52.40^{d}	55.89^{c}
T_4	87.62^{a}	57.13^{b}	66.33^{b}	80.62^{a}	63.73^{a}	58.22^{c}	62.42^{a}
T_5	78.25^{b}	71.43^{a}	77.20^{a}	82.85^{a}	54.40^{d}	57.51^{c}	60.18^{b}
T_6	34.43^{d}	47.62^{c}	57.66^{c}	62.75^{c}	60.84^{b}	68.73^{b}	56.46^{c}
T_{10}	81.47^{ab}	55.50^{bc}	68.49^{b}	68.53^{b}	59.74^{c}	69.49^{b}	63.14^{a}
T_{11}	85.76^{a}	64.22^{ab}	71.40^{ab}	62.54^{c}	58.42^{c}	73.32^{a}	64.28^{a}

T_0- 100 % CWP, **T_4 -** 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5 -** 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10} -** 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11} -** 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

DMRT column wise comparison

Values with same superscript do not have significant difference

Values are mean of three independent determinations.

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4.4. Shelf life studies

The selected meat analogues were packed in food grade HDPE covers (250 gauge) and kept at both ambient temperature and refrigerated conditions for a period of three months. At monthly intervals, aspects such as organoleptic qualities, enumeration of total microflora, insect infestation were studied. Nutrient and *in vitro* studies were conducted initially and at the end of storage period.

4.4.1. Organoleptic evaluation of selected meat analogues on storage

The meat analogues, both the best selected treatments and their controls were stored in ambient and refrigeration storage conditions for a storage period of three months. At monthly intervals, they were evaluated for their organoleptic qualities such as appearance, colour, flavour, texture, taste and overall acceptability. The respective results are furnished in the Tables 14 (a), 14 (b) and 15 (a), 15 (b).

Table 14 (a) and 14 (b) shows how organoleptic qualities of the meat analogues in both controls and their treatments are influenced by storage in both aspects of time - three months and of condition - ambient temperature.

The sensory quality appearance of the selected treatments throughout the storage was observed to be highest (8.64) initially in T_{10} and the lowest (6.45) was seen in T_0 at its third month of ambient storage.

Organoleptic evaluation for colour in the selected treatments throughout the storage was highest (8.45) in both T_5 and T_{11} initially while the lowest (6.48) was observed in T_6 after third month of ambient storage.

Lowest score (6.30) for the sensory attribute flavour among the selected treatments throughout the storage was seen in T_6 at its third month of storage while highest score (8.53) for flavour was seen in T_5 initially.

Table 14 (a). Organoleptic qualities of selected meat analogues stored at ambient temperature

Sensory parameters	T_0				T_4				T_5			
	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)
Appearance	7.62	7.21	7.03	6.45	8.17	8.14	8.11	8.10	8.50	8.47	8.38	7.34
Colour	7.93	7.80	7.55	6.91	8.07	8.05	8.02	7.00	8.45	8.22	8.21	7.19
Flavour	7.27	7.21	7.10	6.98	8.30	8.26	8.24	8.23	8.53	8.23	8.22	8.11
Texture	6.40	6.20	6.50	5.45	8.74	8.73	8.72	8.69	8.78	8.18	8.12	8.09
Taste	7.50	7.20	7.11	7.10	8.36	8.08	8.03	7.00	8.55	8.44	8.41	8.11
Overall acceptability	6.54	6.25	6.13	6.11	8.52	8.47	8.33	8.23	8.81	8.80	8.49	7.21
Total score	43.26	41.87	41.42	39.00	50.16	49.73	49.45	47.25	51.62	50.34	49.83	46.05

MAS – Month after storage

T_0- 100 % CWP, T_4 - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, T_5 - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF

Values are mean of three independent determinations

Table 14 (b). Organoleptic qualities of selected meat analogues stored at ambient temperature

Sensory parameters	T_6				T_{10}				T_{11}			
	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)
Appearance	7.50	7.45	7.33	6.56	8.64	8.49	8.31	8.20	8.26	8.11	8.09	8.02
Colour	7.78	7.43	7.23	6.43	7.85	7.71	7.09	7.02	8.45	8.44	8.12	8.09
Flavour	7.64	7.47	7.44	6.30	8.48	8.47	8.20	8.12	8.33	8.21	8.19	8.17
Texture	6.69	6.10	6.02	6.00	8.76	8.67	7.33	6.98	8.78	8.68	8.21	8.14
Taste	7.57	7.47	7.45	6.94	8.64	8.44	8.43	7.87	8.55	8.21	8.11	7.89
Overall acceptability	7.16	7.11	7.09	6.04	8.64	8.08	8.05	7.23	8.81	8.75	8.53	8.12
Total score	44.34	43.03	42.56	38.27	51.01	49.86	47.41	45.42	51.18	50.40	49.25	48.43

MAS – Month after storage

T_6- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

Values are mean of three independent determinations

The treatment T_0 had lowest score (5.45) for the sensory attribute texture among the selected treatments throughout the storage while T_5 had highest score (8.78) at the start of ambient storage.

The taste of the selected treatments throughout the storage was observed to be highest (8.64) initially in T_{10} and the lowest score (6.94) was seen in T_6 after third month of ambient storage.

The overall acceptability of the selected treatments throughout the storage was highest (8.81) in both T_5 and T_{11} initially and the lowest (6.04) was observed in T_6 after third month of ambient storage.

At the end of third month of ambient storage, highest total score (48.43) was observed in T_{11} while the lowest (38.27) was seen in control T_6. Initially the highest total score (51.62) was seen in T_5 and the lowest score (43.26) was observed inconrol T_0.

A decreasing trend was observed in the organoleptic qualities as the total score in all the treatments stored at ambient temperature decreased with increase in the time of storage.

Table 15 (a) and 15 (b) shows how organoleptic qualities of the meat analogues both controls and their treatments are influenced by storage in both aspects of time - three months and of condition - refrigerated temperature.

The sensory quality score for appearance of the selected treatments throughout the storage was observed to be highest (8.64) initially in T_{10} and the lowest (7.21) was seen in T_0 after third month of refrigerated storage.

The highest (8.85) score for the sensory attribute colour was seen in T_{10} initially while the lowest (7.33) was observed in T_6 after third month of refrigerated storage.

Lowest score (7.15) for the sensory attribute flavour of the selected treatments was seen in control T_6 after third month of storage while highest score

(8.53) at the start of refrigerated storage for flavour was seen in T_5 throughout the storage period.

The treatment T_6 had lowest score (6.09) for the sensory attribute texture among the selected treatments throughout the refrigerated storage while T_5 had highest score (8.78) initially.

The sensory quality taste of the selected treatments throughout the storage was observed to be highest (8.64) initially in treatment T_{10} and the lowest (7.19) was seen in treatment T_6 after third month of refrigerated storage.

The overall acceptability of the selected treatments throughout the storage was highest (8.81) in both T_5 and T_{11} initially and the lowest was observed in T_0 (6.23) after third month of refrigerated storage.

At the end of third month of refrigerated storage, highest total score (51.18) was observed in treatment T_5 while the lowest (41.98) was seen in treatment T_6. Initially, highest (51.62) total score was seen in T_5 and the lowest total score of 43.26 was observed in control T_0.

Table 14 (a), 14 (b) and 15 (a), 15 (b) provide a clear understanding about the influence of different storage conditions on the selected meat analogues for a period of three months. At the initial period all the six treatments had higher scores in all the sensory attributes such as appearance, colour, flavour, taste, texture and overall acceptability.

In both ambient and refrigerated conditions, there was reduction in organoleptic scores, for instance, T_0 (100 % CWP) had the total score of 43.26 which at the end of storage decreased to 39.00 (with difference of 5.26) in ambient condition. However, in refrigerated condition the same showed a difference of only 0.86 with final total score of 42.40. This implies that meat analogues stored under refrigerated condition showed better sensory qualities than their ambient stored counterparts throughout storage.

Table 15 (a). Organoleptic qualities of selected meat analogues stored at refrigerated temperature

Sensory parameters	T_0				T_4				T_5			
	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)
Appearance	7.62	7.54	7.52	7.50	8.17	8.11	8.09	8.05	**8.50**	8.49	8.47	8.44
Colour	7.93	7.92	7.87	7.85	8.07	8.05	8.03	8.00	8.45	8.43	8.42	8.40
Flavour	7.27	7.26	7.23	7.18	8.30	8.28	8.23	8.21	8.53	8.51	8.48	8.47
Texture	6.40	6.35	6.33	6.23	8.74	8.65	8.58	8.44	8.78	8.75	8.71	8.69
Taste	7.50	7.48	7.44	7.41	8.36	8.32	8.29	8.23	8.55	8.52	8.51	8.46
Overall acceptability	6.54	6.50	6.43	6.23	8.52	8.49	8.42	8.40	8.81	8.78	8.74	8.72
Total score	43.26	43.05	42.82	42.40	50.16	49.9	49.64	49.33	51.62	51.48	51.33	51.18

MAS – Month after storage

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF

Values are mean of three independent determinations

Table 15 (b). Organoleptic qualities of selected meat analogues stored at refrigerated temperature

Sensory parameters	T_6				T_{10}				T_{11}			
	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)	Initial	1st (MAS)	2nd (MAS)	3rd (MAS)
Appearance	7.50	7.42	7.32	7.21	8.64	8.42	8.24	8.28	8.26	8.23	8.13	8.12
Colour	7.78	7.44	7.42	7.33	7.85	7.82	7.63	7.61	8.45	8.44	8.43	8.45
Flavour	7.64	7.36	7.18	7.15	8.48	8.38	8.37	8.31	8.33	8.32	8.30	8.28
Texture	6.69	6.26	6.11	6.09	8.76	8.64	8.51	8.20	8.78	8.68	8.62	8.59
Taste	7.57	7.34	7.22	7.19	8.64	8.62	8.59	8.32	8.55	8.40	8.38	8.36
Overall acceptability	7.16	7.11	7.02	7.01	8.64	8.44	8.42	8.12	8.81	8.79	8.75	8.62
Total score	44.34	42.93	42.27	41.98	51.01	50.32	49.76	48.84	51.18	50.86	50.61	50.42

MAS – Month after storage

T_6- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

Values are mean of three independent determinations

Table 16 shows mean values of all sensory qualities and total score of each treatment from first initial to third month of storage, thereby providing an understanding of the effect of ambient and refrigerated conditions over the organoleptic qualities of the meat analogues. All treatments had better scores for appearance, colour, flavour, texture, taste and overall acceptability along with total score at refrigerated condition.

4.4.2. Effect of storage conditions on the nutrient composition of selected meat analogues

Meat analogues were stored in HDPE covers (250 gauge) for three months in ambient and refrigerated conditions. The nutrient composition of meat analogues was analysed and results are furnished in Tables 17 to 22.

Table 17 depicts the moisture content of selected meat analogues (T_4, T_5, T_{10} and T_{11}) and the controls (T_0 and T_6) at the initial and final months of storage at both refrigerated and ambient storage conditions. In both the storage conditions, an increase in the moisture content was observed on storage. At ambient condition, highest moisture content was observed in T_5 (12.41 % $100g^{-1}$) at the end of storage period. On the basis of paired t – test, all the treatments showed significance at one per cent level except T_4 and T_6 which showed five per cent level of significance in the increase in moisture content between the initial and final ambient storage condition. At refrigerated storage, the highest moisture content was 11.87 per cent per 100g at the end of storage period. Increase in moisture was not significant in any of the treatments after three months of refrigerated storage. The table also reveals that the moisture content of initial and final values at ambient condition showed more variation in contrast to refrigerated condition where all the treatments were not significantly different.

Table 16. Average organoleptic scores of selected meat analogues under ambient and refrigerated storage conditions (comparison)

Treatments	T_0		T_4		T_5		T_6		T_{10}		T_{11}	
Storage condition	**Amb**	**Ref**	**Amb**	**Ref**	**Amb**	**Ref**	**Amb**	**Ref**	**Amb**	**Ref**	**Amb**	**Ref**
Appearance	7.07	7.54	8.13	8.10	8.17	8.47	7.21	7.36	8.41	8.39	8.12	8.18
Colour	7.54	7.89	7.78	8.03	8.01	8.42	7.21	7.49	7.41	7.72	8.27	8.44
Flavour	7.14	7.23	8.25	8.25	8.27	8.49	7.21	7.33	8.31	8.38	8.22	8.30
Texture	6.13	6.32	8.72	8.60	8.29	8.73	6.20	6.28	7.93	8.52	8.45	8.66
Taste	7.22	7.45	7.86	8.30	8.37	8.51	7.35	7.333	8.34	8.54	8.19	8.42
Overall acceptability	6.25	6.42	8.38	8.45	8.32	8.76	6.85	7.07	8.00	8.40	8.55	8.74
Total score	41.38	42.88	49.14	49.75	49.46	51.40	42.05	42.88	48.42	49.98	49.81	50.76

T_0- 100 % CWP, **T_4 -** 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5 -** 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10} -** 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11} -** 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF)

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Amb - Ambient storage condition

Ref - Refrigerated storage condition

Table 18 shows the changes in the content of total carbohydrates among the treatments in ambient and refrigerated storage conditions at initial and final stage of storage. In both the storage conditions, a general decrease in the total carbohydrate content was observed on storage. Initially, total carbohydrate content ranged from 31.46 to 53.29 g $100g^{-1}$ while the range was 31.72 to 51.24 g $100g^{-1}$ at the end of three months of refrigerated condition. However, the range of total carbohydrate content was observed to be 29.05 to 49.92 g $100g^{-1}$ in ambient condition with one and five per cent level of significant difference after storage. All the treatments differed significantly with regards to change in total carbohydrate content at the end of storage in ambient condition. Change in total carbohydrate content in refrigerated condition showed five per cent level of significance only in treatments T_4 and T_6 while the rest showed no significant difference at the end of storage at refrigerated condition.

The changes in protein content of selected meat analogues on storage at ambient and refrigerated condition is shown in Table 19. Highest protein content at final storage was observed in T_{10} with 35.86 g $100g^{-1}$and 34.20 g $100g^{1}$ at refrigerated and ambient conditions respectively. The protein content in all the treatments showed a decreasing trend while on storage in both ambient and refrigerated conditions. Paired t – test revealed that all the treatments showed five per cent level of significance in the change in protein content except in T_4 which differed with one per cent level of significance. In refrigerated condition, all the treatments differed with five per cent level of significance while T_4 and T_5 showed no significant difference in the decrease in protein content at initial and final storage.

Table 20 shows the fat content of all the treatments at initial and final storage at both ambient and refrigerated conditions. There was a reduction in the total fat content in all the treatments under both storage conditions. The highest fat content was observed in treatment T_0 with 1.92 g $100g^{1}$ at initial stage of study. The fat content of meat analogues stored in ambient temeperature for three months showed a total fat content ranging from 0.90 to 1.54 g $100g^{-1}$. Significant difference was not

observed in the change in content of fat in the treatments T_4 and T_5 after three months of ambient storage. The final stage of refrigerated condition showed no significant change in the total fat content in treatments T_4, T_{10} and T_{11}. The total fat content in refrigerated stored meat analogues ranged from 0.57 to 1.60 g $100g^1$ after third month of storage.

The changes in total ash content in both ambient and refrigerated conditions after storage of three months is depicted in Table 21. There was a reduction in the total ash content in all the treatments under both storage conditions. Total ash content ranged from 2.92 to 5.66 g 100^{-1}g at initial stage while the content of total ash was between 1.80 to 4.62 g 100^{-1}g and 1.68 to 4.67 g $100g^{-1}$ at end of storage at ambient and refrigerated condition respectively. At ambient condition, all the treatments showed a decrease in total ash content at five per cent level of significance. All the treatments differed significantly except in treatment T_5 which showed no significant difference in the content of total ash at the end of refrigerated storage.

Table 22 reveals the changes in fibre content of all the treatments at initial and final storage at both ambient and refrigerated conditions. A decrease in fibre content was observed in all the treatments at the end of storage at both ambient and refrigerated conditions. The range of fibre was 2.32 to 5.25 g 100^{-1}g after three months of ambient condition. All the treatments showed a decrease in fibre content after three months of storage. The changes in fibre content in the treatments T_4, T_5 and T_{11} were not significant, while the other treatments differed significantly with an observed range (2.20 to 6.02 g 100^{-1}g) at final stage of refrigerated condition.

Changes in the composition of minerals of both the controls and best treatments of meat analogues stored in HDPE covers (250 gauge) for three months in ambient and refrigerated conditions are furnished in Table 23 to 29.

The changes in calcium content of selected meat analogues initially and after three months of storage at both ambient and refrigerated conditions are shown in

Table 23. A decrease in calcium content was observed in all the treatments stored under ambient condition. Calcium content ranged from 78.54 to 93.24 mg 100^{-1}g at final ambient storage while it was 80.11 to 94.11 mg $100g^{1}$at final refrigerated condition with values of most treatments under refrigerated condition showing no significant difference from initial values. All the treatments differed significantly in the calcium content after three months of storage under ambient condition. Meat analogues stored in refrigerated condition showed no significant change in their calcium content after storage.

Significant difference was observed in the phosphorous content of treatments T_0, T_4 and T_6 in ambient condition while the change in phosphorous content was not significant in all the treatments of meat analogues stored at refrigerated condition after three months of storage (Table 24). The range of phosphorous content at the initial stage of storage was 255.62 to 325.46 mg $100g^{1}$while after three months of ambient temperature it ranged from 254.97 to 324.16 mg $100g^{1}$. The meat analogues stored in refrigerated temperature reported a phosphorous content ranging from 255.17 to 325.22 mg $100g^{1}$.

Table 17. Effect of storage conditions on the moisture content of selected meat analogues

Treatments	Moisture (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	9.25	11.58	7.50**	9.25	10.91	1.89^{NS}
T_4	10.54	11.21	3.78*	10.54	10.07	2.00^{NS}
T_5	9.40	12.41	7.46**	9.40	10.41	1.23^{NS}
T_6	9.60	10.67	2.27*	9.60	11.34	1.8^{NS}
T_{10}	9.70	12.04	4.61**	9.70	9.71	0.60^{NS}
T_{11}	10.62	11.96	2.40**	10.62	10.96	1.51^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 18. Effect of storage conditions on the total carbohydrate content of selected meat analogues

Treatments	Total carbohydrates (g $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	48.91	46.25	19.00*	48.91	48.25	1.32^{NS}
T_4	35.95	31.62	8.00*	35.95	33.62	5.56*
T_5	32.46	29.05	1.90*	32.46	31.72	0.22^{NS}
T_6	53.29	49.92	1.84**	53.29	51.24	3.27*
T_{10}	42.10	40.21	1.94*	42.10	41.11	0.87^{NS}
T_{11}	36.94	34.97	1.96**	36.94	36.30	1.00^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 19. Effect of storage conditions on the protein content of selected meat analogues

Treatments	Protein (g $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	24.96	20.62	6.04*	24.96	23.12	4.91*
T_4	34.55	29.89	14.00**	34.55	33.22	1.51^{NS}
T_5	38.03	30.72	1.90*	38.03	33.33	0.90^{NS}
T_6	20.79	17.45	1.17*	20.79	19.39	1.89*
T_{10}	37.13	34.20	3.00*	37.13	35.86	2.00*
T_{11}	36.61	32.82	1.22*	36.61	35.61	1.00*

T_0- 100 % CWP, **T_4 -** 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5 -** 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10} -** 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11} -** 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 20. Effect of storage conditions on the total fat content of selected meat analogues

Treatments	Total fat (g $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	1.92	1.30	3.98*	1.92	1.60	1.00*
T_4	1.06	1.33	2.61^{NS}	1.06	1.54	0.82^{NS}
T_5	1.43	1.54	2.40^{NS}	1.43	1.20	2.19*
T_6	1.20	0.90	1.65*	1.20	0.57	1.77*
T_{10}	1.60	1.05	3.46*	1.60	1.32	1.33^{NS}
T_{11}	1.63	0.97	1.00*	1.63	1.40	0.23^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; NS - Not significant

Table 21. Effect of storage conditions on the total ash content of selected meat analogues

Treatments	Total ash (g $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	2.92	1.80	3.45*	2.92	2.12	1.73*
T_4	3.23	3.08	2.44^{NS}	3.23	4.64	1.00*
T_5	5.66	4.21	7.00**	5.66	4.70	1.73^{NS}
T_6	2.96	2.19	14.14**	2.96	1.68	1.00*
T_{10}	5.33	4.62	1.63*	5.33	2.02	4.34*
T_{11}	3.23	2.89	2.00*	3.23	2.56	0.48*

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 22. Effect of storage conditions on the fibre content of selected meat analogue

Treatments	Fibre (g $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	3.10	2.44	5.50**	3.10	2.11	2.47*
T_4	3.85	2.85	1.73*	3.85	3.18	1.00*
T_5	7.30	5.25	4.00**	7.30	6.02	0.92^{NS}
T_6	2.23	2.32	59.00**	2.23	2.02	1.00^{NS}
T_{10}	6.90	4.90	5.19**	6.90	4.90	2.00*
T_{11}	4.69	3.35	1.00*	4.69	4.69	0.96^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 23. Effect of storage conditions on the calcium content of selected meat analogues

Treatments	Calcium (mg 100g^{-1})					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	80.25	78.54	4.41**	80.25	80.11	0.85^{NS}
T_4	92.50	91.24	15.31**	92.50	93.08	1.21^{NS}
T_5	85.10	84.14	1.21^{NS}	85.10	85.02	1.00^{NS}
T_6	85.16	84.03	4.58*	85.16	86.09	1.31^{NS}
T_{10}	94.67	93.24	1.41^{NS}	94.67	94.11	3.67^{NS}
T_{11}	93.28	92.09	1.81^{NS}	93.28	93.16	4.43^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 24. Effect of storage conditions on the phosphorous content of selected meat analogues

Treatments	Phosphorous (mg $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	325.46	324.16	9.67**	325.46^{a}	325.22^{a}	0.75^{NS}
T_4	316.04	315.90	10.00**	316.04^{b}	316.00^{b}	3.32^{NS}
T_5	308.95	308.81	3.83*	308.95^{c}	308.02^{c}	0.87^{NS}
T_6	255.67	254.97	5.76**	255.67^{e}	255.17^{e}	0.85^{NS}
T_{10}	269.58	269.01	3.82*	269.58^{d}	269.18^{d}	2.31^{NS}
T_{11}	255.62	255.03	2.25*	255.62^{e}	255.22^{e}	1.65^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 25. Effect of storage conditions on the sodium content of selected meat analogues

Treatments	Sodium (mg $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	23.88	23.02	2.21^{NS}	23.88	23.78	0.22^{NS}
T_4	52.17	51.87	16.90**	52.17	52.03	1.86^{NS}
T_5	74.43	73.03	3.91*	74.43	74.15	0.65^{NS}
T_6	23.52	23.14	3.68^{NS}	23.52	23.19	0.23^{NS}
T_{10}	34.85	34.11	8.97**	34.85	34.23	0.32^{NS}
T_{11}	38.72	38.31	3.00^{NS}	38.72	38.25	1.42^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 26. Effect of storage conditions on the potassium content of selected meat analogues

Treatments	Potassium (mg $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	603.03	602.21	7.09**	603.03	602.55	2.39 ^{NS}
T_4	631.50	630.97	14.00**	631.50	631.35	0.85^{NS}
T_5	618.89	617.23	6.31**	618.89	618.73	1.26 ^{NS}
T_6	510.49	509.03	7.22**	510.49	520.89	3.41 *
T_{10}	540.20	539.26	14.00**	540.20	540.18	1.87 ^{NS}
T_{11}	522.42	521.94	10.00**	522.42	522.32	1.25 ^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 27. Effect of storage conditions on the magnesium content of selected meat analogues

Treatments	Magnesium (mg $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	178.02	177.36	3.21*	178.02	178.00	1.26^{NS}
T_4	181.69	180.94	12.54**	181.69	181.14	0.23^{NS}
T_5	162.01	161.41	3.22*	162.01	161.97	1.32^{NS}
T_6	110.58	103.68	3.40*	110.58	109.09	0.85^{NS}
T_{10}	120.93	119.52	4.56**	120.93	120.02	0.76^{NS}
T_{11}	103.64	100.34	13.89**	103.64	103.25	1.98^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 28. Effect of storage conditions on the iron content of selected meat analogues

Treatments	Iron (mg $100g^{-1}$)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	5.73	4.73	2.81^{NS}	5.73	5.63	2.44^{NS}
T_4	4.33	4.23	2.86^{NS}	4.33	4.73	0.43^{NS}
T_5	4.17	4.09	2.21^{NS}	4.17	4.89	3.41^{NS}
T_6	4.54	4.01	7.09**	4.54	4.71	2.21^{NS}
T_{10}	4.67	4.22	4.52**	4.67	4.98	1.94^{NS}
T_{11}	4.21	4.04	6.21**	4.21	4.18	2.31^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 29. Effect of storage conditions on the zinc content of selected meat analogues

Treatments	Zinc (mg 100g^{-1})					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	3.62	3.12	3.41 ^{NS}	3.62	3.40	2.45 ^{NS}
T_4	3.22	3.10	3.78 ^{NS}	3.22	3.14	0.87^{NS}
T_5	3.17	3.08	7.81**	3.17	3.13	1.21 ^{NS}
T_6	3.26	2.93	7.94**	3.26	3.22	1.74 ^{NS}
T_{10}	3.96	3.04	4.56**	3.96	3.94	2.37 ^{NS}
T_{11}	3.89	3.11	2.90 ^{NS}	3.89	3.77	2.45^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 25 represents the changes in sodium content of the selected meat analogues and the controls (T_0 and T_6) at the initial and final stages of storage at both refrigerated and ambient storage conditions. The meat analogues stored in ambient storage condition showed a decrease in their sodium content. The range of sodium content at storage was between 23.52 to 74.43 mg $100g^1$ initially while after three months of ambient temperature it ranged from 23.02 to 73.03 mg $100g^1$. The meat analogues stored in refrigerated temperature reported sodium content ranging from 23.19 to 74.15 mg $100g^1$ at the end of three months storage. The significant change in sodium content was observed in most of the treatments after three months of ambient storage except in treatments T_0, T_6 and T_{11}. In the case of refrigerated storage all the treatments showed no significance in the change of sodium level after three months.

The potassium content of treatments at both ambient and refrigerated conditions after three months of storage is depicted in Table 26. A change in potassium content was observed at both ambient and refrigerated condition after three months of storage. The range of potassium content at the initial stage of storage was 510.49 to 631.50 mg $100g^1$ while after three months of ambient temperature it declined to 509.03 to 630.97 mg $100g^1$. The meat analogues stored in refrigerated temperature reported potassium content ranging from 520.89 to 631.35 mg $100g^1$ respectively. At ambient condition, the change in potassium content of all the treatments showed significance in potassium content after three months storage. Similarly, the decrease in potassium content was not significant statistically in most of the treatments after three months of refrigerated storage.

Table 27 shows the statistical significance in the change in the content of magnesium among the treatments in both ambient and refrigerated storage condition at initial and final stage. Magnesium content of meat analogues at ambient storage ranged from 103.64 to 181.69 mg $100g^1$ initially and it ranged from 100.34 to 180.94 mg $100g^1$ at final month of storage respectively. At refrigerated condition, the range

was 103.25 to 181.14 mg $100g^{-1}$ in the meat analogues after three months of storage. Paired t – test analysis showed that all the treatments differed significantly in the magnesium content after three months of storage under ambient condition. The change in magnesium content in refrigerated condition was shown to have no significance in all the treatments after three months storage period.

The iron content of the selected meat analogues at initial and final storage of both ambient and refrigerated conditions is shown in Table 28. There was pronounced change in the iron content in all the treatments at the end of storage at ambient and refrigerated conditions. Iron content ranged from 4.01 to 4.73 mg $100g^{1}$ at final ambient storage while it was 4.18 to 5.63 mg $100g^{1}$ at final refrigerated condition. Data from Table 28 reveals that most of the treatments differed significantly with regards to change in iron content after three months storage at ambient condition except in T_0, T_4 and T_5 in which no significant difference was observed. In refrigerated condition, all the treatments showed no significant difference in the initial and final content of iron after three months of storage.

Table 29 shows the significance in the change of zinc content among the treatments in both ambient and refrigerated storage condition at initial and final stage. The range of zinc content at the initial stage of storage was observed to be from 3.17 to 3.96 mg 100^{-1} while at final stage of ambient temperature it was 2.93 to 3.12 mg 100^{-1}g. In refrigerated storage, the zinc content in meat analogues ranged from 3.13 to 3.94 mg 100^{-1}g at the end of storage period. Except in treatments T_0, T_4 and T_{11}, the decrease in zinc content in all the treatments was statistically significant after three months of ambient storage. All the treatments were significantly not different in the change of zinc content after three months of storage under refrigerated condition.

4.4.2.1. *In vitro* protein digestibility of selected meat analogues on storage

Protein *in vitro* digestibility was shown to significantly differ in the selected treatments of meat analogues along with the controls from the initial and final storage at both ambient and refrigerated conditions of storage (Table 30). The *in vitro* digestibility of protein of meat analogues stored in ambient condition showed a general trend of decrease in all the treatments. The initial *in vitro* protein digestibility ranged from 62.12 to 80.30 per cent and after storage for three months the *in vitro* protein availability ranged from 61.22 to 80.07 per cent after three months of ambient storage. The change in the *in vitro* protein availability was not significant in the case of treatment T_4 stored at ambient condition. The *in vitro* digestibility of meat analogues stored in refrigerated condition for three months ranged between 62.04 to 80.21 per cent. Difference in the change in *in vitro* digestibility of protein after refrigerated storage for three months was only significant in T_{11}.

4.4.2.2. *In vitro* mineral availability of selected meat analogues on storage

The level of significance of different treatments pertaining to the calcium *in vitro* availability with respect to initial and final storage of both ambient and refrigerated conditions is shown in Table 31. There was a change in calcium *in vitro* availability at the end of storage at both ambient and refrigerated conditions. Calcium *in vitro* availability ranged from 32.19 to 86.81 per cent at final ambient storage while it was 33.78 to 86.91 per cent at the third month of storage at refrigerated condition. Paired t - test revealed that all the treatments differed with one per cent level of significance except T_4 and T_6 which showed with five per cent level of significance in the change in calcium *in vitro* availability after storage period at ambient condition. In refrigerated condition, all the treatments showed no significant difference in the change in calcium *in vitro* availability while T_6 and T_{11} were significantly different at five per cent level of significance in the decrease in protein *in vitro* availability after three months of refrigerated storage.

Comparison of phosphorous *in vitro* availability at both ambient and refrigerated conditions comparing initial and final stage of storage is depicted in Table 32. There was a change in phosphorous *in vitro* availability at the end of storage at both ambient and refrigerated conditions. The range of phosphorous *in vitro* availability at the initial stage of storage was 47.62 to 71.43 per cent while after three months of storage at ambient temperature it was 46.17 to 69.53 per cent. The observed range of phosphorous *in vitro* availability of meat analogues stored at refrigerated condition was 46.17 to 70.12 per cent. At ambient condition, all the treatments showed significant difference with respect to the change in phosphorous *in vitro* availability after storage. In refrigerated condition, only the treatment T_6 showed significant difference with respect to change in phosphorous *in vitro* availability at the end of storage period. Similarly, the all treatments except T_5 and T_6 showed no significant difference in the *in vitro* availability of phosphorous after refrigerated storage.

Table 33 depicts the sodium *in vitro* availability of the selected meat analogues and the controls (T_0 and T_6) at the initial and final stages of storage at both refrigerated and ambient storage conditions. There was a general change in sodium *in vitro* availability at the end of storage at both ambient and refrigerated conditions. Range of sodium *in vitro* availability at ambient storage showed variance 57.66 to 77.20 per cent initially to 55.82 and 73.74 per cent after three months of ambient storage respectively. Range of sodium *in vitro* availability after three months of refrigerated storage ranged between 57.93 and 76.84 per cent. All the treatments were significantly different at the end of storage under ambient condition. Significant difference was not observed in the change in sodium *in vitro* availability in all the treatments stored at refrigerated condition.

The changes in potassium *in vitro* availability in different treatments at initial and final storage of both ambient and refrigerated conditions are shown in

Table 34. There was a slight decrease in potassium *in vitro* availability at the end of storage at both ambient and refrigerated conditions. Initial *in vitro* availability of potassium ranged from 62.54 to 82.85 per cent which reduced to 59.82 to 80.04 per cent at the end of storage at ambient condition. The *in vitro* availability of potassium ranged between 61.51 and 81.81 per cent at end of storage at refrigerated condition. All the treatments differed significantly with regards to the decrease in potassium *in vitro* availability after three months of storage under ambient condition. In refrigerated condition, no significant difference was observed in any of the ttreatments with respect to *in vitro* availability of potassium at the end of three months of storage.

Table 35 shows the changes in the *in vitro* availability in magnesium of the treatments stored in both ambient and refrigerated storage condition at initial and final stage of storage. There was a general change in magnesium *in vitro* availability at the end of storage at both ambient and refrigerated conditions. Range of magnesium *in vitro* availability at ambient storage showed variance from 54.40 to 63.73 per cent at initial stage and 53.02 to 65.63 per cent at final stage respectively. The magnesium *in vitro* availability of the treatments was observed to be in the range of 54.06 to 67.91 per cent at the end of storage at refrigerated condition. All the treatments differed significantly at five per cent except T_{10} which differed with one per cent level of significance in magnesium *in vitro* availability at the end of storage at ambient condition. All the treatments were not significantly different in the *in vitro* availability of magnesium after three months of storage at refrigerated condition.

The *in vitro* availability of iron in the selected treatments and controls at initial and final storage of both ambient and refrigerated conditions is shown in Table 36. Change in iron *in vitro* availability at the end of storage at both ambient and refrigerated conditions was observed. Iron *in vitro* availability ranged from 51.66 to 72.24 per cent at final ambient storage while it was 51.92 to 73.30 per cent at final

stage of refrigerated storage. Statistical analysis using paired t - test revealed that all the treatments showed significant difference observed with regards to change in iron *in vitro* availability after three months of storage at ambient condition. In refrigerated condition, all the treatments showed no significant difference in the *in vitro* availability of magnesium at the end of storage.

Table 37 shows the initial and final zinc *in vitro* availability in the treatments stored in both ambient and refrigerated storage condition at initial and final stage. There was an observed reduction in zinc *in vitro* availability at the end of storage at both ambient and refrigerated conditions. The range of zinc *in vitro* availability at the initial stage of storage was between 55.89 and 64.28 per cent while at final stage of ambient temperature it was between 54.34 and 63.41 per cent. The *in vitro* zinc availability after three months of refrigerated storage ranged from 55.44 to 64.79 per cent. All the treatments differed significantly with respect to change in zinc *in vitro* availability after three months of storage at ambient condition. Significant difference in the change in zinc *in vitro* availability was not observed in any of the treatments after three months of storage under refrigerated condition.

Table 30. Effect of storage conditions on the *in vitro* protein digestibility of selected meat analogues

Treatments	Protein (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	63.76	63.12	2.25*	63.76	63.57	0.25^{NS}
T_4	80.30	80.07	0.87^{NS}	80.30	80.21	0.22^{NS}
T_5	73.74	72.11	1.87*	73.74	73.19	0.87^{NS}
T_6	62.12	61.22	3.42*	62.12	62.04	0.28^{NS}
T_{10}	65.43	64.92	2.42*	65.43	65.22	0.33^{NS}
T_{11}	76.17	75.62	3.52*	76.17	75.97	2.37*

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 31. Effect of storage conditions on the *in vitro* calcium availability of selected meat analogues

Treatments	Calcium (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	45.58	43.43	4.80**	45.58	44.32	1.35 ^{NS}
T_4	87.62	86.81	2.95*	87.62	86.91	2.25 ^{NS}
T_5	78.25	76.01	3.25**	78.25	77.90	1.27 ^{NS}
T_6	34.43	32.19	2.89*	34.43	33.78	3.09*
T_{10}	81.47	79.52	14.00**	81.47	80.93	1.25^{NS}
T_{11}	85.76	84.48	4.35**	85.76	83.39	3.87*

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 32. Effect of storage conditions on the *in vitro* phosphorous availability of selected meat analogues

Treatments	Phosphorous (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	61.18	59.47	5.41**	61.18	60.03	1.87^{NS}
T_4	57.13	56.34	2.41*	57.13	57.00	0.41^{NS}
T_5	71.43	69.53	5.30**	71.43	70.12	2.10^{NS}
T_6	47.62	46.17	7.87**	47.62	46.17	5.41*
T_{10}	55.50	54.67	4.32**	55.50	55.21	0.43^{NS}
T_{11}	64.22	63.07	3.89*	64.22	63.91	1.42^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 33. Effect of storage conditions on the *in vitro* sodium availability of selected meat analogues

Treatments	Sodium (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	58.52	57.71	3.28*	58.52	58.10	1.25^{NS}
T_4	66.33	64.54	13.22**	66.33	66.97	2.25^{NS}
T_5	77.20	73.74	15.30**	77.20	76.84	2.81^{NS}
T_6	57.66	55.82	7.00**	57.66	57.93	1.85^{NS}
T_{10}	68.49	65.91	12.00**	68.49	68.02	1.90^{NS}
T_{11}	71.40	69.02	14.21**	71.40	71.19	0.27^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 34. Effect of storage conditions on the *in vitro* potassium availability of selected meat analogues

Treatments	Potassium (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	68.34	67.11	2.83*	68.34	67.93	1.85^{NS}
T_4	80.62	79.11	3.29*	80.62	80.16	0.35^{NS}
T_5	82.85	80.04	4.58**	82.85	81.81	1.90^{NS}
T_6	62.75	61.52	10.00**	62.75	62.45	1.22^{NS}
T_{10}	68.53	67.43	7.63**	68.53	67.92	2.91^{NS}
T_{11}	62.54	59.98	12.65**	62.54	61.51	3.81^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 35. Effect of storage conditions on the *in vitro* magnesium availability of selected meat analogues

Treatments	Magnesium (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	62.84	65.63	2.31*	62.84	67.91	0.23^{NS}
T_4	63.73	62.91	3.81*	63.73	63.51	1.83^{NS}
T_5	54.40	53.02	4.26*	54.40	54.06	2.85^{NS}
T_6	60.84	64.31	4.20*	60.84	67.31	6.13*
T_{10}	59.74	57.24	12.38**	59.74	59.24	0.32^{NS}
T_{11}	58.42	56.53	2.04*	58.42	58.53	0.21^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 36. Effect of storage conditions on the *in vitro* iron availability of selected meat analogues

Treatments	Iron (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	52.40	51.66	1.89*	52.40	51.92	1.45^{NS}
T_4	58.22	57.90	4.57*	58.22	58.01	0.75^{NS}
T_5	57.51	56.30	5.21**	57.51	57.59	3.21^{NS}
T_6	68.73	67.11	7.65**	68.73	68.95	2.89^{NS}
T_{10}	69.49	68.22	4.84*	69.49	69.21	0.47^{NS}
T_{11}	73.32	72.24	2.54*	73.32	73.30	0.29^{NS}

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

Table 37. Effect of storage conditions on the *in vitro* zinc availability of selected meat analogues

Treatments	Zinc (%)					
	Ambient storage			Refrigerated storage		
	Initial	Final	t - value	Initial	Final	t - value
T_0	55.89	54.45	4.83**	55.89	55.44	0.85 NS
T_4	62.42	62.02	3.91*	62.42	62.31	0.29 NS
T_5	60.18	59.96	5.32**	60.18	60.00	1.37 NS
T_6	56.46	54.34	10.00**	56.46	56.41	0.90 NS
T_{10}	63.14	62.91	7.65**	63.14	63.21	1.17 NS
T_{11}	64.28	63.41	3.21*	64.28	64.79	1.53 NS

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

* - Significant at 5 % level; ** - Significant at 1 % level; NS - Not significant

4.4.3. Enumeration of total microflora in selected meat analogues on storage

Under microbial studies, micro organisms such as bacteria, fungi and yeast were enumerated in meat analogues (control and best treatments) throughout the storage period of three months under two different conditions (ambient and refrigerated storage).

Table 38. Total bacterial count of meat analogues during storage at ambient condition ($\times 10^5$cfu/g)

Treatment/ Storage period	T_0	T_4	T_5	T_6	T_{10}	T_{11}
Initial	3.33	3.00	3.33	3.33	3.00	3.22
1st (MAS)	5.00	3.33	4.33	4.00	4.66	3.66
2nd (MAS)	4.66	4.66	5.33	4.66	5.33	4.33
3rd (MAS)	5.33	5.00	6.66	5.22	6.33	7.00

MAS – Month after storage

T_0- 100 % CWP, **T_4 -** 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5 -** 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10} -** 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11} -** 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

Table 38 depicts the total bacterial count of meat analogues stored at ambient storage condition. Initially, all the treatments showed viable bacterial count in the range between 3.00 to 3.33×10^5 cfu/g.

The treatment T_0 showed highest (5×10^5cfu/g) viable bacterial count and the lowest (3.33×10^5cfu/g) was observed in T_4 after first month of storage under ambient

condition. The range of viable bacterial count after the second month of storage at ambient condition was observed to increase between 4.33 to 5.33 $\times 10^5$cfu/g. At three months of storage, the highest (7×10^5cfu/g) viable bacterial count was found in treatment $T_{11,}$ while the lowest (5.00×10^5cfu/g) count was observed in T_6.

Table 39. Total bacterial count of meat analogues during storage at refrigerated condition ($\times 10^5$cfu/g)

Treatment/ Storage period	T_0	T_4	T_5	T_6	T_{10}	T_{11}
Initial	3.33	3.00	3.33	3.33	3.00	3.22
1st (MAS)	3.66	3.33	4.00	3.66	4.33	4.00
2nd (MAS)	4.00	3.66	4.66	4.21	4.66	4.66
3rd (MAS)	4.66	4.00	5.11	5.11	5.00	5.00

MAS – Month after storage

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

Table 39 depicts the total bacterial count of meat analogues stored at refrigerated storage condition. Initially, all the treatments showed viable bacterial count in the range between 3.00 to 3.33×10^5cfu/g.

In refrigerated condition, after the first month of storage, the viable bacterial count was in the range of 3.33 to 4.00 $\times 10^5$cfu/g. After the second month of storage,

the range of viable bacterial count was between 3.66 and 4.66 $\times 10^5$cfu/g at refrigerated storage. After third month of storage, the range of bacterial viable count was between 4.00×10^5cfu/g (T_4) to 5.11×10^5cfu/g (T_5 and T_6) at refrigerated storage.

At both ambient and refrigeration storage conditions, bacterial colonies increased with increase in storage time. However, viable bacterial count was higher in treatments of meat analogues stored under ambient condition against refrigerated condition.

Table 40. Total fungal count of meat analogues during storage at ambient condition ($\times10^3$cfu/g)

Treatment/ Storage period	T_0	T_4	T_5	T_6	T_{10}	T_{11}
Initial	ND	ND	ND	ND	1.66	ND
1st (MAS)	0.33	0.33	0.33	1.66	2.00	ND
2nd (MAS)	1.66	1.88	1.33	2.33	3.22	2.33
3rd (MAS)	2.33	3.88	2.88	2.88	3.88	3.21

MAS – Month after storage

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

ND – Not detected

Table 40 depicts the total fungal count of meat analogues stored at ambient condition. Initially, viable fungal count was seen only in treatment T_{10} (1.66×10^3cfu/g) whereas no viable fungal colonies were detected in the rest of the treatments.

After first month of storage, treatment T_{10} showed an increase in viable fungal count (2×10^3cfu/g) while other treatments showed no viable fungal count at ambient condition. At the end of second month of storage, viable fungal count was highest(3.22×10^3cfu/g) in T_{10} and treatment T_5 had lowest count(1.33×10^3cfu/g) at ambient condition. The range of fungal viable count was 2.33 to 3.88 $\times10^3$cfu/g at the end of storage at ambient condition.

Table 41. Total fungal count of meat analogues during storage at refrigerated condition ($\times10^3$cfu/g)

Treatment/ Storage period	T_0	T_4	T_5	T_6	T_{10}	T_{11}
Initial	ND	ND	ND	ND	1.66	ND
1st (MAS)	ND	ND	ND	ND	1.88	ND
2nd (MAS)	0.22	0.23	0.43	0.88	2.00	0.21
3rd (MAS)	0.66	0.88	0.66	2.33	2.33	0.29

MAS – Month after storage

T_0- 100 % CWP, **T_4 -** 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5 -** 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10} -** 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11} -** 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

ND – Not detected

Table 41 depicts the total fungal count of meat analogues stored at refrigerated condition. Initially, viable fungal count was seen only in treatment T_{10} (1.66×10^3cfu/g) whereas no viable fungal colonies were detected in the rest of the treatments.

In refrigerated condition, no fungal viable count was detected in all the treatments except T_{10} with 1.88×10^3cfu/g count at the first month of storage. At the second month of storage, viable fungal count was highest(2.00×10^3cfu/g) in T_{10} and treatment T_{11} had lowest count (0.21×10^3cfu/g) at refrigerated condition. At the end of third month of storage, the range of fungal count was between 0.66 and 2.33×10^3cfu/ml under refrigerated condition with the highest fungal count observed in treatment T_6 and T_{10} and the lowest in the treatment T_{11}.

At both ambient and refrigeration storage conditions, fungal colonies increased with increase in storage time. However, viable fungal count was higher in treatments of meat analogues stored under ambient condition against refrigeration condition.

Table 42. Total yeast count of meat analogues during storage at ambient condition ($\times 10^3$cfu/g)

Treatment/ Storage period	T_0	T_4	T_5	T_6	T_{10}	T_{11}
Initial	ND	ND	ND	ND	ND	ND
1st (MAS)	ND	0.22	ND	0.11	ND	ND
2nd (MAS)	0.13	0.26	0.12	0.33	0.24	0.33
3rd (MAS)	0.23	0.88	0.22	0.66	0.45	0.56

MAS – Month after storage

T_0- 100 % CWP, **T_4 -** 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5 -** 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6-** 100 % CP, **T_{10} -** 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11} -** 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

ND – Not detected

Table 42 depicts the total yeast count of meat analogues stored at ambient condition. Initially, no viable yeast count was detected in any of the treatments of meat analogues. At the end of first month of ambient storage, treatments T_4 and T_6 showed viable yeast count of 0.22 and 0.11×10^3cfu/g respectively while the others showed no yeast growth. After the second month of storage, viable yeast count was observed to be in the range of 0.12 to 0.33 $\times10^3$cfu/g at ambient condition. The range of yeast viable count was 0.22 to 0.88 $\times10^3$cfu/g at the end of storage at ambient condition with highest yeast count observed in treatment T_4 and the lowest in treatment T_5.

Table 43. Total yeast count of meat analogues during storage at refrigerated condition (×10^3cfu/g)

Treatment/ Storage period	T_0	T_4	T_5	T_6	T_{10}	T_{11}
Initial	ND	ND	ND	ND	ND	ND
1st (MAS)	ND	ND	ND	ND	ND	ND
2nd (MAS)	0.11	ND	ND	0.34	ND	ND
3rd (MAS)	0.23	0.11	0.22	0.55	0.21	0.33

MAS – Month after storage

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour)

Values are mean of three independent determinations

ND – Not detected

Table 43 depicts the total yeast count of meat analogues stored at refrigerated condition. Initially, no viable yeast count was detected in any of the treatments of meat analogues.

In refrigerated condition, no yeast viable count was detected in all the treatments at the end of first month of storage. The treatments T_0 and T_6 showed viable yeast count of 0.11 and 0.34 ×10^3cfu/g at the end of second month of storage at refrigerated condition. At the end of storage, the range of viable yeast count was between 0.11 to 0.55 × 10^3cfu/g at refrigerated condition with the lowest yeast count reported in treatment T_4 and the highest in treatment T_6.

At both ambient and refrigeration storage conditions, yeast colonies increased with increase in storage time. However, viable yeast count was higher in treatments of meat analogues under ambient condition against refrigeration condition.

4.4.4. Insect infestation

Meat analogues were assessed at monthly intervals during the storage period of three months. Insect infestation was not observed in any of the different treatments of meat analogues.

4.5. Cost of production for selected meat analogues

The cost of production of the selected meat analogues and their controls were estimated per 100 g of the finished products and the details are furnished in Table 44. The cost for the controls ranged from Rs. 34.00 to Rs. 39.00/100g for 100 per cent cowpea and chickpea respectively. The cost incurred for the production of tender jackfruit incorporated cowpea meat analogues was Rs. 57.00/100g and for tender jackfruit incorporated chickpea meat analogues it was Rs. 60.00/100g. The cost of production of breadfruit incorporated cowpea meat analogues approximated to Rs. 58.00/100g and for breadfruit incorporated chickpea meat analogues it was Rs. 59.00/100g.

Table 44. Cost of production of selected meat analogues

Treatments	Cost (Rs./ 100g)
T_0	34.00
T_4	58.00
T_5	57.00
T_6	39.00
T_{10}	60.00
T_{11}	59.00

T_0- 100 % CWP, **T_4** - 50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF, **T_5** - 40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF, **T_6**- 100 % CP, **T_{10}** - 50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF, **T_{11}** - 40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF

(CWP – Cowpea, CP – Chickpea, TJ – Tender jackfruit, BF – Breadfruit, WG – Wheat gluten, OMF – Oyster mushroom flour , DSF - Defatted soy flour

Discussion

5. DISCUSSION

The discussion of the study entitled "Standardisation of jackfruit and breadfruit incorporated meat analogues" is presented under the following headings.

5.1. Standardisation of tender jackfruit incorporated meat analogues

5.1.1. Organoleptic evaluation of tender jackfruit incorporated meat analogues

5.1.2. Selection of the best treatment

5.2. Standardisation of breadfruit incorporated meat analogues

5.2.1. Organoleptic evaluation of breadfruit incorporated meat analogues

5.2.2. Selection of the best treatment.

5.3. Quality evaluation of selected meat analogues

5.3.1. Nutrient studies of the selected meat analogues

5.3.2. *In vitro* studies of selected meat analogues

5.4. Shelf life studies

5.4.1. Organoleptic evaluation of selected meat analogues on storage

5.4.2. Effect of storage conditions on the nutrient composition of the selected meat analogues on storage

5.4.2.1. *In vitro* protein digestibility of selected meat analogue on storage

5.4.2.2. *In vitro* mineral availability of selected meat analogue on storage

5.4.3. Enumeration of total microflora in selected meat analogues on storage

5.4.4. Insect infestation in selected meat analogues on storage

5.5. Cost benefit analysis

Plant-based foods are considered an essential portion of our cuisine as they offer complexity and vibrancy to the menu platter. The preponderance of the energy and other nutrients in our diet, including proteins, phytochemicals, and antioxidants, comes from vegetarian sources. They are a healthier option for consuming due to their richness in vitamins such as the B complex, C, and other provitamins (Walther and Sieber, 2011).The largest of the protein in the diet comes from plant sources, which constitute for 57 per cent of the sum total of dietary protein. Plant protein may

be incomplete but adequate adding up of ingredients makes a food product complete in terms of healthy composition of essential amino acids. Different plant sources of food possess different compounds whose inter molecular interactions induce functional characteristics like emulsifying capacity, elasticity, chewiness, texturization, gelation, foaming and so on which when constituted into one product could create a novel food product with desirable characteristics (Fardet, 2017). Plant food sources like wheat gluten, soy protein, soy beans, green gram, whey protein, mushroom, egg albumin, gums and carbohydrates have been in explored for their ability to simulate meat and its characteristics which were successfully utilized and standardized to optimum compositions. Utilizing plant-based proteins and techniques like extrusion, shearing, and mixing, recent research and development have made it possible to manufacture a texture that resembles that of meat (Coelho *et al*., 2018).

India is the country with bountiful indigenous and highly distinctive plant sources of foods like jackfruit and breadfruit which are nutritionally adequate and capable of refining the nutritional quality of the foods to which they are added. Utilizing these could not only increase the scope of food industry to formulate novel and nutritional products but also reduce post harvest losses which account for 40 per cent of the field produce. Hence, in the present study, several combinations of ingredients including tender jackfruit, breadfruit, wheat gluten, oyster mushrooms, defatted soy flour, cowpea, and chickpea were explored for the development of meat analogues. The nutritional value and shelf life studies of the best organoleptic treatments and their controls were examined, further advancing the "meat analogue" food sector.

5.1. Standardisation of tender jackfruit incorporated meat analogues

In this experiment, meat analogues were standardised using ingredients such as tender jackfruit (TJ) flour, cowpea (CWP), chickpea (CP), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) with varied in proportions of different treatments.

The eleven treatments were divided into two sets with T_0 to T_5 using cowpea (CWP), in the first and T_6 to T_{11}using chickpea (CP), in the second set with tender jackfruit (TJ), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) as common ingredients in varied proportions corresponding to their respective treatments.

The necessary ingredients were added and blended with the pretreated pulse. This blended mass was then cut into cubes and immediately immersed in a spice broth after being pressure boiled for 20 minutes at 121^0C and 15 psi. They were then dried for 8 to 12 hours at 65^0C in a cabinet dryer. As the number of treatments proceeded, the percentage of breadfruit flour and wheat gluten increased, with corresponding decline in the percentage of pulses (cowpea and chickpea).

The developed meat analogues had appearance and colour similar to dried meat with distinctive 'pulse' flavour. Nisha (2008) reported that green gram meat analogues had acceptable appearance and colour with no distinctive flavour.

5.1.1. Organoleptic evaluation of tender jackfruit incorporated meat analogues

Sensory evaluation is a multidisciplinary field that includes the description, measurement, and interpretation of product features that can be sensed by senses. Objective and subjective exams can be used to evaluate sensory abilities. The first analysis produces as much impartial information as possible, ideally comparable to data processed using chemical or physical instruments. Subjective testing, on the other hand, are based on personal impressions, such as the ultimate consumers' preference for the product under consideration. When used effectively, sensory evaluation enables understanding and control of the critical features for the market success of food goods and beverages (Sirangelo, 2019). Meat analogues from each treatment was evaluated for its organolpetic qualities such as appearance, colour, flavour, texture, taste and overall acceptability using nine point hedonic scale by 20 judges. The meat analogues were organoleptically evaluated by replacing meat with reconstituted meat analogues in standard meat recipe. The values from score cards were used for statistical analysis to select the best treatment.

As shown in Table 5, the treatment (T_5) had the highest overall score for the tender jackfruit incorporated cowpea meat analogues followed by the other treatments T_4, T_3, T_2, T_1, and T_0. The best-selected treatment hence selected was T_5, was found to have the highest scores for all sensory qualities, including appearance, colour, flavour, texture, and overall acceptability.

In tender jackfruit incorporated cowpea meat analogues, treatment T_{10} had the highest organoleptic total score followed by T_{11}, T_8, T_9, T_6 and T_7 as shown in Table 6. Treatment T_{10} was observed to have highest scores for all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability and hence was considered to be the best selected treatment.

Appearance and taste of the tender jackfruit incorporated meat analogues among all treatments (T_0 to T_{11}) was highest in T_{10} and the highest scores in colour, flavour, texture was highest in T_5. The overall acceptability of the best treatment (T_5) of set I (tender jackfruit incorporated cowpea meat analogues) which was T_5 had higher overall acceptability when compared to T_{10} which was the best treatment of set II (tender jackfruit incorporated chickpea meat analogues) which signifies that preference and acceptance for cowpea incorporated meat analogues was higher than chickpea incorporated tender jackfruit meat analogues.

5.1.2. Selection of the best treatment

The fresh meat analogue cubes developed had unique "pulse" flavour and were firm and springy. When cut, the cubes revealed a clearly delineated, interconnected interior network of ingredients. Chickpea meat analogues had more meaty flavour with pale yellow colour compared to cowpea meat analogues which had better textural properties and a distinctive dark brown colour. However, clear colour distinction was absent with the addition of new components, notably tender jackfruit flour which had clear, opaque white body.

Based on each treatment's overall acceptability and total score in the organoleptic scores, the best treatments from the two sets of set I (T_0 to T_5) and set II

(T_6 to T_{11}) were chosen. Applying Kendall's (W) test statistical analysis revealed that the Kendall's (W) value was very significant in relation to all quality metrics. The treatments showed no significant difference in sensory attributes such as appearance and colour. However, as the proportion of wheat gluten and tender jackfruit increased, the texture, flavour, taste and over all acceptability of meat analogues was improved. Wheat gluten of 20 to 25 per cent added better textural properties such as chewiness, elasticity, springiness and flavour retention. According to Cunsolo *et al.* (2012) the components of gluten namely glutenin which adds high elasticity, rubbery character that makes the food product resistant to shear along with gliadins that enhance extensibility and product quality. This is further supported by Samtiya *et al.* (2020) who reported that addition of optimal gluten could induce properties such as flexibility, gas retention, strengthening, controlled expansion, enhanced water absorption, and increased shelf life to the food product.

Pretreatments and heat processing of tender jackfruit were shown to increase its capacity for water and oil absorption (Odoemelam, 2005). Addition of tender jackfruit added to the gumminess and cohesiveness of the meat analogues which is in accordance with the observations of Rana *et al.* (2019) according to whom jackfruit at tender stage exhibits textural properties such as springiness, adhesiveness and chewiness which showed inverse proportional relation with maturity. Unlike wheat gluten or other ingredients, tender jackfruit shows greater affinity to fat due to its proximate composition due to which it improves the texture and increases the consumer acceptability of the food system to which they were added (Sultana *et al*., 2015).

The cut surface of dried meat analogues showed fine interconnected fabric of different ingredients with irregular spaces distributed all throughout the matrix. According to Krintiras *et al.* (2015), under simple strain and expansion gluten forms thin films of protein molecules, altering the dough matrix of the meat analogue into a fibrous substance. The gluten protein links with hydrogen sulfide to generate a three-dimensional fibrous structure known as a disulfide protein linkage. As a result, the

food system is given a visible framework with improved moisture retention, foaming, and emulsifying capabilities (Wouters *et al*., 2016).

The mean weight of the pressure cooked tender jackfruit meat analogues (314.09 g) was more than the homogenized batter (182.04g). This increase in the weight of tender jackfruit meat analogues could be associated with the ability of components including wheat gluten, tender jackfruit flour, pretreated pulse, defatted soy flour, as well as oyster mushroom flour to retain water.

Kang *et al*. (2017) reported that defatted soy flour exhibits improved water retention due to the absence of impediments to water absorption along with variety of other functional properties such as solubility, water and oil absorption capacity, emulsifying, swelling, gelling, and foaming. Pretreated oyster mushroom flour shows better water retention and higher swelling capacity (Maray *et al*., 2018).

5.2. Standardisation of breadfruit incorporated meat analogues

Meat analogues were standardised using ingredients such as breadfruit (BF) flour, cowpea (CWP), chickpea (CP), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) with varied proportions in different treatments. The eleven treatments were divided into two sets with T_0 to T_5 using cowpea (CWP), as the first and T_6 to T_{11}using chickpea (CP), as the second set with breadfruit flour (BF), wheat gluten (WG), oyster mushroom flour (OMF) and defatted soy flour (DSF) as common ingredients in varied proportions corresponding to their respective treatments.

To the pretreated pulse, the respective ingredients were added and blended. This blended mass was pressure cooked for 20 minutes at 121^0C and 15 psi which was then cut into cubes with simultaneous immersion in spice broth. They were then dried in cabinet drier for 8 to 12 hours at 65^0C. As the treatment number proceeded, proportion of wheat gluten and tender jackfruit flour increased with simultaneous decrease in the proportion of the pulse (cowpea and chickpea).

The developed meat analogues had appearance and colour similar to dried meat with distinctive 'pulse' flavour. Nisha (2008) reported that green gram meat analogues had acceptable appearance and colour with no distinctive flavour.

5.2.1. Organoleptic evaluation of breadfruit incorporated meat analogues

The eleven treatments were divided into two sets with T_0 to T_5 using cowpea in the first and T_6 to T_{11} using chickpea in the second set. The meat analogues were organoleptically evaluated by replacing meat with meat analogues in standard meat recipe. Meat analogues from each treatment were evaluated for their organolpetic qualities such as appearance, colour, flavour, texture, taste and overall acceptability using nine point hedonic scale by 20 judges.

The treatment (T_4) had the highest total score in organoleptic evaluation followed by T_5, T_3, T_2, T_1 and T_0 as shown in Table 7. Treatment T_4 had the highest scores for all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability and hence was considered to be the best selected treatment. From Table 8, the total score was observed to be the highest in T_{11} and lowest in the control T_6 which is 100 per cent chickpea. Treatment T_{11} was observed to have highest scores for all the sensory attributes including appearance, colour, flavour, texture, taste and overall acceptability and hence was considered to be the best selected treatment. Among all the treatments (T_0 to T_{11}) of breadfruit incorporated meat analogues T_{11} had highest scores for all the sensory attributes including appearance, colour, flavour, taste, texture and overall acceptability.

The best treatment of set I (breadfruit incorporated cowpea meat analogues), T_4, exhibited a greater overall acceptability than best treatment of set II (breadfruit incorporated chickpea meat analogues), T_{11}, implying that participants preferred and accepted chickpea incorporated breadfruit meat analogues more than they did cowpea incorporated meat analogues.

5.2.2. Selection of the best treatment

The best treatment from the two sets of breadfruit incorporated meat analogues of set I (T_0 to T_5) and set II (T_6 to T_{11}) was selected based on the overall acceptability and total score by each treatment in the organoleptic scores. From set I (breadfruit incorporated cowpea meat analogues) and II (breadfruit incorporated chickpea meat analogues), T_4 and T_{11} were the best selected treatments. Statistical analysis by applying Kendall's (W) test showed that Kendall's (W) value was highly significant with regards to all quality attributes.

Breadfruit flour possessed distinctive grainy hand feel with clear, translucent body and therefore adds not only to the texture but appealing colour to the meat analogues. In terms of the sensory quality taste and flavour, breadfruit possesses starchy yet 'grainy' flavour which forms a pliable, firm, viscous mass when mixed with saliva in the mouth due to which it sustains for more time in the mouth before being swallowed. This may be due to its water holding capacity with a marked water absorption capacity of 2.54 to 4.28 g of water per gram breadfruit flour (Ma *et al*., 2012). Moreover, compared to other flour sources like wheat flour, breadfruit flour has showed potential as a substitute flour source with superior water retention abilities. Adding optimal quantity of water could reduce the degradation of starch due to which the capacity to retain water improves thereby making the product shear resistant (Roman *et al.*, 2018). Meat analogues made from breadfruit showed characteristic flavour which could be associated with the presence of higher content of volatile organic acids such as quinic acid, oxalic acid, succinic acid along with triterpenes, limonene and γ- terpinene which make breadfruit nutritionally superior (Soifoini *et al*., 2021).

Breadfruit along with wheat gluten added pliability to the meat analogues with pronounced springiness. When added to the pretreated mass and other ingredients, breadfruit incorporated meat analogues showed better firmness which

may be due to their pasting viscosity profile (Hagenimana *et al.*, 2016). Thermal treatment allows the starch to gelatinize and the optimal gelatinsation of which prevents starch degradation therefore improving the rheological properties of the food system to which they are added (Zhang *et al.*, 2016). When the breadfruit incorporated meat analogues were placed in the spice broth for 20 minutes, no deformation of the structure of the steamed meat analogues was seen which may be attributed to the optimal gelatinsation and the simultaneous improvement in pasting properties (Huang and Bohrer, 2020).

Cut surface of dried breadfruit incorporated meat analogues showed interconnected amorphous network with regular spaces distributed throughout the matrix with very notable grainy appearance within the meat analogue. This could be due to the gelatinsation of the starch in different ingredients specifically such as cowpea or chickpea, breadfruit, defatted soy flour due to which the crystalline structure is lost generating an amorphous agglomerates that give pliability and elasticity to the food system they are added to (Gomez and Martinez, 2016). The interconnected matrix of the meat analogues could be because of the interactions of proteins of wheat gluten namely gliadins and glutenins together with starch both exposed to the uniform thermal processing become denatured and gelatinized respectively. The unfolded protein chains interact with amylose and amylopectin yielding a defining amorphous state (Huang *et al.*, 2020).

The pressure cooked breadfruit meat analogues weighed (224.82 g) higher than the blended batter (176.00g) which could be attributed to the water retention capacity of ingredients such as wheat gluten, breadfruit flour, pretreated pulse, defatted soy flour and oyster mushroom flour. According to Maray *et al.* (2018) pretreated mushroom flour shows higher water retention capacity with higher swelling index.

Soy flour shows poor liquid retention capacity correlating to its higher content of lipids and simultaneous lesser protein showing lesser affinity to water. Both defatted soy flour and mushroom flour add to the functional textural properties such as pliability, springiness, chewiness and meaty flavour. Defatted soy flour, on the other hand, shows better water retention as there is no hindrance for absorption of water accompanied by number of other functional qualities such as solubility, water and oil absorption capacity, emulsifying, swelling, gelling and foaming (Kang *et al*., 2017).

5.3. Quality evaluation of the selected meat analogues on storage

The best treatments and the control of both tender jackfruit and breadfruit meat analogues were selected for further studies. The treatments along with their controls were evaluated for their quality in terms of nutrient studies, *in vitro* studies and self life studies.

5.3.1. Nutrient studies of selected meat analogues

Physicochemical composition of meat analogues are depicted in Figure 1 that reveals the estimation of moisture, total carbohydrate, protein, total fat, total ash, fibre in the control (T_0 and T_6) and the best treatments (T_4, T_5, T_{10} and T_{11}) of meat analogues.

The meat analogues made from breadfruit showed higher retention of moisture than that of jackfruit incorporated meat analogues as shown in Figure 1.The highest moisture content was observed in treatment T_{11} (10.62%) and lowest (9.25%) moisture content was observed in T_0 (100% CWP) as the lowest content. Nisha (2008) reported a moisture range of 8.30 to 8.80 per cent in green gram based meat analogues. Dried meat on the other hand was reported to contain 35 to 40 per cent moisture in the study of Ajiboye *et al*. (2011). This could be associated with the water holding capacity of breadfruit together with wheat gluten exhibiting superior water

retention abilities (Roman *et al*., 2018). Moreover, its swelling and gelatinisation properties give it better moisture regain contributing to the increased cooking yield of the cooked food into which it was incorporated (Yusnita *et al*., 2019). However, the controls showed low moisture content compared to the best treatments which shows the effectiveness of the functional properties of other ingredients like wheat gluten in the food system.

Total carbohydrate content in the controls was higher in the controls (T_0 and T_6) which were completely cowpea or chickpea. From Figure 1, it is clearly seen that with the addition of other ingredients like breadfruit or tender jackfruit, defatted soy flour, wheat gluten and oyster mushroom flour there was decrease in the content of total carbohydrates. The highest content of total carbohydrates of 53.29 g $100g^{-1}$ was seen in T_6 (100 % CP) and the lowest content of total carbohydrates was observed in T_5 (32.46g $100g^{-1}$) of selected meat analogues. Ahmad *et al.* (2018) reported that meat contains as less as 0.80 to 1.21 g $100g^{-1}$ of total carbohydrates. Cutroneo *et al.* (2022) reported total carbohydrate content of 20.40 to 22.10 g 100g-1 in meat analogue cutlets.

Jones *et al.* (2011) reported that the total carbohydrates in breadfruit are majorly composed of starch. Decrease in total carbohydrates in breadfruit incorporated meat analogues could be attributed to decreased pulse by 60 to 50 per cent with increased addition of 20 to 25 per cent breadfruit from the control which usually contains only moderate carbohydrates. The decrease in total carbohydrates in tender jackfruit incorporated meat analogues may be due to the addition of the main ingredient like tender jackfruit which contain the least amount of carbohydrates at tender stage. According to Khan *et al.* (2021), carbohydrates in jackfruit are composed of starch, sugar and dietary fibre in both its flesh and seed. Meat analogues made from tender jackfruit showed a considerable decrease in total carbohydrates content which may be associated with the presence of lower content of total carbohydrates at its tender stage unlike its ripe stage. Proximate analysis at different

stages of fruit maturity reveal that jackfruit at tender stage contains 9.40 to 11.50 g of carbohydrates per 100g of edible portion while at mature stage it was observed to be at a higher range of 16.00 to 25.40 g 100^{-1}g of edible portion (Srivasthava and Singh, 2020). Ingredients such as wheat gluten, defatted soy flour and oyster mushroom flour contain lower carbohydrates accompanied by their addition at lower proportion together resulting in reduction of total carbohydrates content.

Protein is an integral part of the diet and mostly derived from non vegetarian sources that contain an average of 35.67 g $100g^{-1}$ of protein (Ahmed *et al*., 2018). Pulses are rich vegetarian sources of protein, which in this study were refined in their protein content by addition of main ingredients such as tender jackfruit and breadfruit. From proximate analysis, the protein content in the controls (T_0 and T_6) with 100 per cent cowpea and chickpea, protein content varied from 20.79 to 24.96 g $100g^{-1}$ of protein as shown in Figure 1. Compared to cooked meat which has 26 to 36 g $100g^{-1}$ of protein, the protein content of tender jackfruit and breadfruit incorporated cowpea meat analogues showed better protein content which ranged between 34.55 and 38.03 g $100g^{-1}$ respectively, while in chickpea it was 37.13 and 36.61 with the incorporation of the main ingredients tender jackfruit and breadfruit. Meat alternatives in the study of Lakshmy (2011) showed protein content of 29.60 to 34.65 g $100g^{-1}$. Meat was reported to contain protein content in the range of 18.10 to 24.20g $100g^{-1}$ (Ahmad *et al*., 2018).

From the Figure 1, it was observed that protein content increased with increase in the proportion of breadfruit or tender jackfruit, wheat gluten along with other ingredients. Defatted soy flour contains remarkable protein content of 52 to 54 g $100g^{-1}$ while pretreated (dried and powdered) oyster mushroom contains protein content of 25.00 g $100g^{-1}$ (Tolera and Abera, 2017). Tender jackfruit contains 2.00 to 3.42 g $100g^{-1}$ of protein while breadfruit contains protein content of 4.00 g $100g^{-1}$ (Srivasthava and Singh, 2020). Wheat gluten was observed to be the major contributor to the increase as the commercially available wheat gluten was reported to

be 80 per cent protein. Wheat gluten and the base pulse compensate each other for limiting amino acids as wheat gluten and base pulse have cysteine, methionine and lysine, threonine respectively, making a suitable complementary diet (Zhang *et al*., 2022).

As shown in Figure 1, the total fat was found to increase from control treatments to the selected treatments ranging between 1.06 to 1.92 g 100^{-1}g. Fat content in meat analogues made from green gram were reported to be in the range of 1.23 to 2.67 g $100g^{-1}$(Nisha, 2008). While defatted soy flour adds nothing to the lipid profile, tender jackfruit and breadfruit contain 0.64 g 100^{-1}g and 0.23 g $100g^{-1}$ of total fat respectively (Ragone, 2014). Other ingredients such as oyster mushroom flour (1.18 g 100^{-1}g total fat on wet basis) and wheat gluten (2.00 g 100^{-1}g total fat) may be other factors which contributed to the lipid content of the selected meat analogues (Rashidi and Yang, 2016). Pulses like cowpea and chickpea contain moderate fat content of 2.10 and 6.04 g 100^{-1}g respectively (Wallace *et al*., 2016). Decreased proportion of chickpea in T_{11} (1.63 g 100^{-1}g) with increased proportion of breadfruit could be associated with the slight rise compared to the control T_6 (1.20 g 100^{-1}g).

As shown in Figure 1, the total ash content increased in the selected treatments compared to the controls. Total ash content ranged from 2.92 to 5.66 g 100^{-1}g in the meat analogues of the study. Meat analogues made from dry-fractionated pea and oat proteins in the study of De Angelis *et al*. (2020) showed total ash content in the range of 3.21 to 4.25 g $100g^{-1}$. Meat was reported to contain total ash content in the range of 1.04 to 1.95g $100g^{-1}$ (Rahman *et al*., 2012).

Pulses like chickpea contain total ash content of 2.00 to 4.00 per cent (Hirdyani, 2014) and cowpea contain total ash content of 2.97 to 3.47 per cent (Gondwe *et al*., 2019). In both cowpea and chickpea meat analogues, incorporation of tender jackfruit showed an increase in total ash content higher than breadfruit incorporation which may be associated with its prominent total ash content of 3.15 g

$100g^{-1}$ at immature stage (Chandra and Bharati, 2020). Breadfruit has total ash of 2.56 g $100g^{-1}$ and its cowpea meat analogues (T_4) had lower total ash than its chickpea meat analogues (T_{11}) which might be due to the higher proportion of pulse in the latter which had higher total ash than cowpea. Additionally, pretreated oyster mushrooms contain higher amounts of minerals such as phosphorous, calcium, potassium and magnesium therefore having higher total ash content (Anyasi *et al*., 2018).

Figure 1 depicts the remarkable increase in the fibre content of the selected treatments was seen against the controls with an average increase by 2.50 g of fibre in cowpea and 3.60 g of fibre in chickpea in both tender jackfruit and breadfruit incorporated meat analogues. Specifically, the increase in tender jackfruit incorporated in cowpea and chickpea meat analogues was 4.20 and 4.63 g $100g^{-1}$ compared to the controls (T_0 and T_6). However, the increase in breadfruit incorporated in cowpea and chickpea meat analogues was 0.75 and 2.46 g $100g^{-1}$ compared to the controls (T_0 and T_6). In green gram based meat analogues, the fibre content was reported to be in the range of 1.33 to 1.82 g $100g^{-1}$ (Nisha, 2008). Verma and Banerjee (2010) reported that meat contains no fibre content in it. Meat analogues in the form of meat balls were reported to have fibre content of 3.60 to 6.80 g $100g^{-1}$ (Cutroneo *et al*., 2022).

Increase in fibre is more prominent with the addition of tender jackfruit compared to breadfruit as main ingredient. Tender jackfruit contains higher fibre of 2.60 to 3.60 g 100^{-1}g edible portion compared to mature ones which contain only 1.00 to 1.50 g $100g^{-1}$ edible portion (Srivasthava and Singh, 2020). Reduced total carbohydrates with healthier increase in fibre imply that the tender jackfruit and breadfruit meat analogues are nutritionally superior and could be a better alternative to meat in which there is complete absence of fibre.

Fig. 1. Moisture, total carbohydrates, protein, total fat, total ash and fibre content of meat analogues

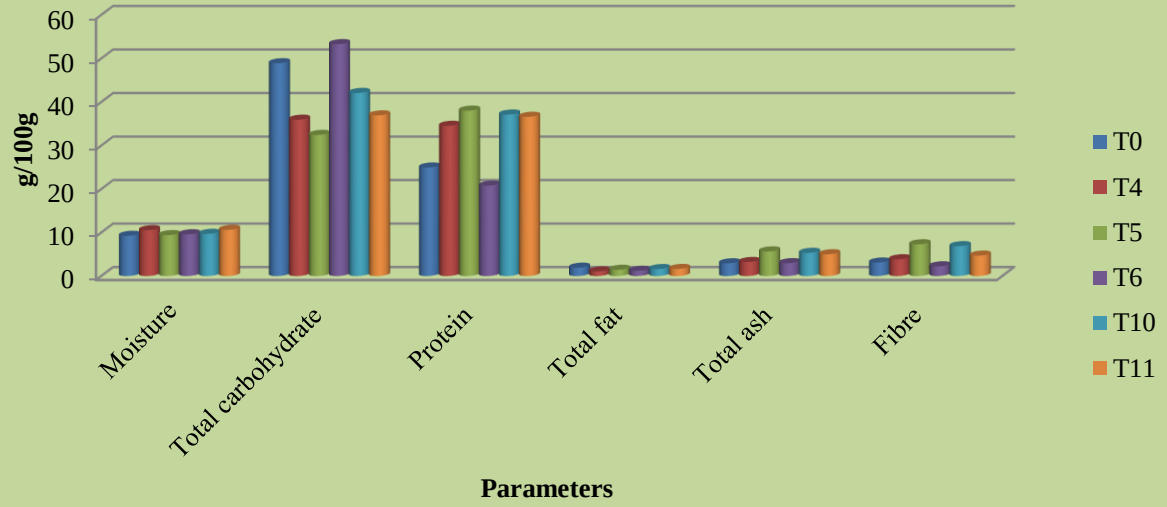
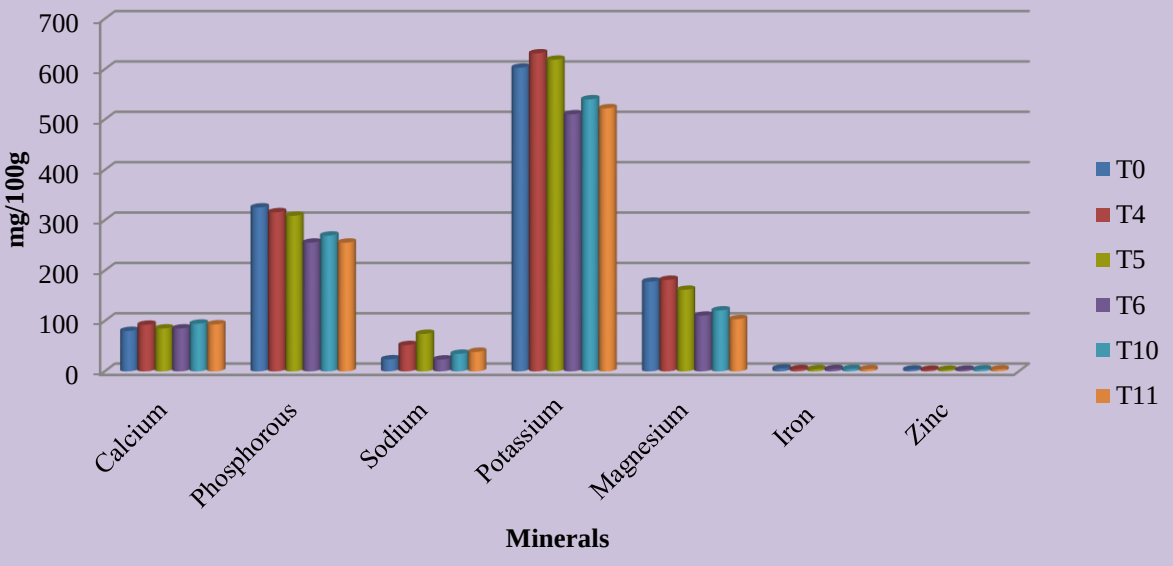


Fig. 2. Mineral composition of meat analogues



All the treatments of meat analogues including controls were shown to be good source of minerals such as phosphorous, sodium, potassium, magnesium, iron and zinc except for calcium (Table 11).

Meat analogues of this study were composed of pulses such as cowpea and chickpea due to which higher mineral content with higher potassium, phosphorous and magnesium was found.

Natural antinutritional factors such as phytates, chelates, tannins and phytochemicals bind to these minerals forming mineral complexes which lead to their losses. According to Singh (2017), pretreatments such as soaking, roasting, blanching, dehusking, cooking and soaking improves the mineral profile of foods.

Calcium content was observed to be the highest in T_{10} (94.67 mg 100g $^{-1}$) and the lowest calcium content (80.25 mg 100g $^{-1}$) was observed in T_0 (100 % CWP). According to Devisetti and Prakash (2020) cowpea and chickpea contain lower calcium content of 96.19 and 97.60 mg 100g^{-1} respectively. Calcium content in green gram based meat analogues ranged from 253.00 to 276.00 mg 100g-^{1} (Nisha, 2008). Calcium content in meat was found to be in the range of 118.00 to 120.00 mg 100g^{-1} (Tasic *et al*., 2017). Phosphorous content was found to be highest (325.46 mg 100g $^{-1}$) in T_0 and lowest phosphorous content was observed in T_{11} (255.62 mg 100g $^{-1}$) which could be attributed to the high phosphorous content in the pulses and pretreatments. Phosphorous content in green gram based meat analogues was reported to be in the range of 177.94 to 190.85 mg 100g^{-1} (Nisha, 2008). Phosphorous content in meat was found to be in the range of 164.00 to 218.00 (Ahmad *et al*., 2018). This gives the inference that the phosphorous content of the meat analogues in the present study was comparatively higher.

Soaking and cooking improve the mineral content as they contribute to the reduction of phytic acid, tannins and lectins which chelate the minerals like phosphorous leading to its loss (Grases *et al*., 2017).

Sodium content ranged between 23.52 to 74.43 mg $100g^{-1}$ in the meat analogues while the potassium content ranged from 510.49 to 631.50 mg $100g^{-1}$. The range of magnesium content in the selected meat analogues was between 103.64 to 181.69 mg $100g^{-1}$. Meat on the other hand contains 51.00 to 71.00, 344.00 to 365.00 and 25.00 to 28.00 mg $100g^{-1}$ of sodium, potassium and magnesium respectively (Williams, 2007).

The potassium content of meat alternatives developed by Lakshmy (2011) ranged from 534.02 to 602.70 mg $100g^{-1}$. According to Fresan *et al*. (2019), meat analogues developed only from plant sources showed sodium content of 235.28 mg $100g^{-1}$.

The major contributors to increase in the minerals sodium, potassium and magnesium may be associated with pulses and ingredients added to it such as tender jackfruit, breadfruit and oyster mushroom flour. Swami *et al*. (2012) reported that tender jackfruit contains notable content of sodium in the range of 3.00 to 35.00 mg $100g^{-1}$ and it possesses remarkable potassium content in the range of 287.00 to 323.00 mg $100g^{-1}$. Breadfruit contains sodium content of 19.40 mg $100g^{-1}$ and potassium was found to be 376.7 mg $100g^{-1}$ (Ragone, 2014). Higher potassium content of meat analogues could be associated with higher potassium profile of all the ingredients in the meat analogues. Bawa and Webb (2016) reported higher magnesium content in mature breadfruit of 25.00 mg $100g^{-1}$. On the contrary, magnesium was reported to be not present in tender jackfruit at its young stage (Srivasthava and Singh, 2020).

Additionally, pretreated oyster mushroom contains sodium and potassium content of 9.76 to 15.32 mg $100g^{-1}$and 226.50 to 299.50 mg $100g^{-1}$ respectively. Oyster mushroom contains magnesium in the range of 254.00 to 297.00 mg $100g^{-1}$ after its pretreatment (Maray *et al*., 2018).

Iron and zinc are vital microminerals that perform wide variety of physiological and biochemical functions in human body. The content of the iron was observed to be highest (5.73 mg $100g^{-1}$) in T_0 and the lowest iron content was observed (4.17 mg $100g^{-1}$) in T_5 while the zinc content ranged from (3.17 to 3.96 mg $100g^{-1}$) in the meat analogues. Adeyi *et al.* (2015) reported that meat contains iron and zinc in the range of 1.10 to 3.30 and 3.90 to 4.60 mg $100g^{-1}$ respectively. Meat analogues developed from green gram showed iron content in the range of 1.77 to 1.89 mg $100g^{-1}$ (Nisha, 2008). Lakshmy (2011) reported zinc content in meat alternatives to be in the range of 3.57 to 4.41 mg $100g^{-1}$. Swami *et al*. (2012) reported that tender jackfruit contains notable content of iron in the range of 0.40 to 1.90 mg $100g^{-1}$. Breadfruit contains iron content of 0.50 mg $100g^{-1}$ and zinc was found to be 0.10 mg $100g^{-1}$ (Ragone, 2014). Tender jackfruit also possesses pronounced zinc content of 0.13 mg $100g^{-1}$ (Nansereko and Muyonga, 2022).

Natural antinutritional factors such as phytic acid are structured with negative charge due to which they exert interaction with positively charged metal ions such as zinc, iron, magnesium and calcium forming chelates. Iron and zinc are major minerals which are lost due to the anti nutritional factors in foods specifically phytic acid and oxalates due to which they are considered as a major cause of mineral deficiencies in human nutrition (Chen *et al*., 2012). All incorporated ingredients of the selected meat analogues were pretreated which may be associated with increase in the content of all the minerals. This was supported by Mustafa and Adem (2014) according to whom pretreatments such as blanching, soaking and cooking showed reduced level of antinutritional factors increasing the level of total free (unbound)

mineral content. Grases *et al*. (2017) reported that soaking for two to 12 hours led to the reduction of phytic acid by 47.45 to 55.71 per cent.

5.3.2. *In vitro* studies of selected meat analogues

In vitro digestibility is the bioaccessibility of nutrients in a physiological system and determines the proportion of ingested nitrogen or amino acid made available to the system after digestion and absorption (Tome, 2013). The *in vitro* digestibility of protein in the controls was found to be in the range of 62.12 to 63.76 per cent as shown in Figure 5. In the selected treatments, increase was observed with *in vitro* digestibility of protein in the range between 65.43 and 80.30 per cent as shown in Figure 3. Meat alternatives in the study of Lakshmy (2011) showed protein *in vitro* digestibility in the range of 71.43 to 88.98 per cent. Animal proteins such as meat have the *in vitro* protein digestibility as high as 95 per cent and more, whilst plant proteins like wheat gluten and soy protein isolate were reported to have *in vitro* protein digestibility in the range of 50 to 80 per cent (Wu, 2016).

In vitro protein digestibility of plant based foods is limited by factors such as antinutritional compounds, food processing techniques and heat treatments applied specifically to the food system. Natural plant secondary metabolites like protease inhibitors, tannins, phytates and lectins exhibit inhibitory properties towards protein *in vitro* digestibility (Bora, 2014). At molecular level, these compounds hinder the activity of enzymes through catalytic mode by blocking all the active sites of the enzymes. On the other hand tannins have the ability to form reversible and irreversible tannin – protein complexes between the hydroxyl group of tannins and carbonyl group of protein leading to reduction of essential amino acids thereby decreasing the overall protein digestibility (Raes *et al*., 2014). Other compounds such as phytic acid, inhibit the activity of those intrinsic enzymes which degrade the protein and amino acids in digestion (Al Hasan *et al*., 2016). Additionally, saponins found in plant foods were shown to exhibit inhibitory effect toward proteolytic

enzymes such as trypsin and chymotrypsin by blocking their active sites. Some plant based proteins also show chelating property towards protein thereby forming insoluble complexes leading to their loss (Kregiel *et al*., 2017).

Pretreatments like soaking, cooking, pressure cooking, roasting, blanching and autoclaving were found to be effective in reducing the antinutritional factors in plant based foods. In black gram, pressure cooking was found to reduce the tannin content which showed positive correlation with its protein digestibility (Shah, 2001). Coulibaly *et al*. (2011) reported that soaking reduced the phytic acid content and this reduction was associated with increase in protein availability in plant foods like legumes and nuts. Boiling plant food sources in water for 40 minutes was reported to reduce the oxalate levels by 47 per cent which was effective in preventing the loss of amino acids (Savage and Martensson, 2010). Torres *et al*. (2016) reported that autoclaving, soaking and cooking legumes showed significant reduction in several antinutritional factors. Pretreatments such as roasting showed decreased trypsin inhibitor activity significantly in soybean meal resulting in improved *in vitro* amino acid profile (Vagadia *et al*., 2017). The *in vitro* protein digestibility of meat analogues, both in controls and selected treatments is in accordance with the findings of Drulyte and Orlien (2019).

The *in vitro* bioavailability of a mineral is defined as its measure of proportion of the total minerals in food or a meal that is utilized for regular bodily processes. The amount absorbed from the gut is a major factor of bioavailability, and it varies substantially amongst minerals (Shah, 2001). Mineral absorption is controlled by dietary components such as phytates, tannins, oxalates, and vitamins such as Vitamin C, which either hinder or boost absorption rates (Sandberg, 2002).

Calcium *in vitro* availability was observed to be the highest (87.62 %) in T_4 and lowest *in vitro* calcium availability was observed in T_6 (34.43 %) from Table 13. Phosphorous *in vitro* availability was observed to be the lowest (47.62 %) in T_6 (100

% CP) with the highest (71.43 %) observed in T_5. According to Samtiya *et al*.(2020), Oxalic acid and phytates are the chelating factors responsible for the loss of *in vitro* availability of the minerals in foods, which when soaked, pressure cooked and roasted leads to the reduction in the the antinutritional compounds and increased *in vitro* availability.

Sodium *in vitro* availability ranged between 57.66 to 77.20 per cent in the selected meat analogues. The treatment T_5 (82.85 %) had the highest potassium *in vitro* availability and lowest (62.54 %) was seen in T_{11} (Table 13). The range of *in vitro* availability of magnesium in the selected meat analogues was between 54.40 to 63.73 per cent with the lowest *in vitro* magnesium availability observed in T_5 and highest observed in T_4.

Autoclaving or pressure cooking was found to be the most effective way to increase the bioavailability of minerals specifically sodium, potassium and magnesium. The risk of mineral loss was reduced by pretreating the plant based ingredients through soaking followed by pressure cooking which reduced the content of chelating agents such as phytic acid, tannins and oxalates (Torres *et al*., 2016). Reduction in these compounds was associated with increased mineral availability as the level of bound minerals decreased releasing the unbound contents into the food system through leaching (Maphosa and Jideani, 2017). Significant reduction in tannins improved the overall mineral profile of plant based foods leading to better bioavailability (Marinangeli *et al*., 2017).

The *in vitro* availability of iron was observed to be highest (73.32 %) in $T_{11}$and the lowest (52.40 %) in T_0 (100 % CWP). Zinc *in vitro* availability was found to be highest (64.28 %) in T_{11} and the lowest (55.89 %) was reported in T_0 (100 % CWP). Iron and zinc are the micronutrients whose availability could be improved with apt pretreatment and addition of absorption enhancing factors. Maphosa and Jideani (2017) also reported an increase in iron bioavailability with pretreatments such as

Fig. 3. *In vitro* protein digestibility of meat analogues

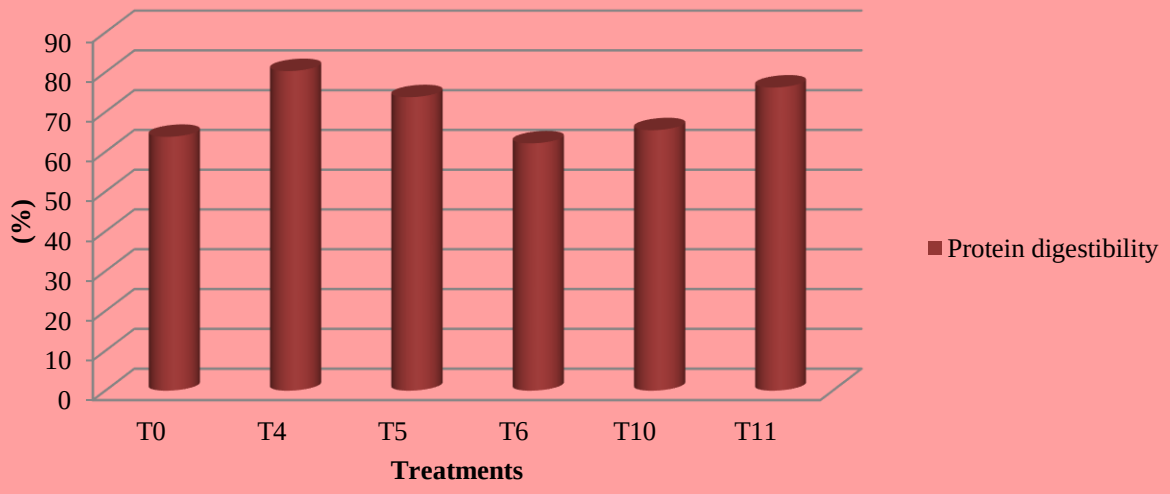
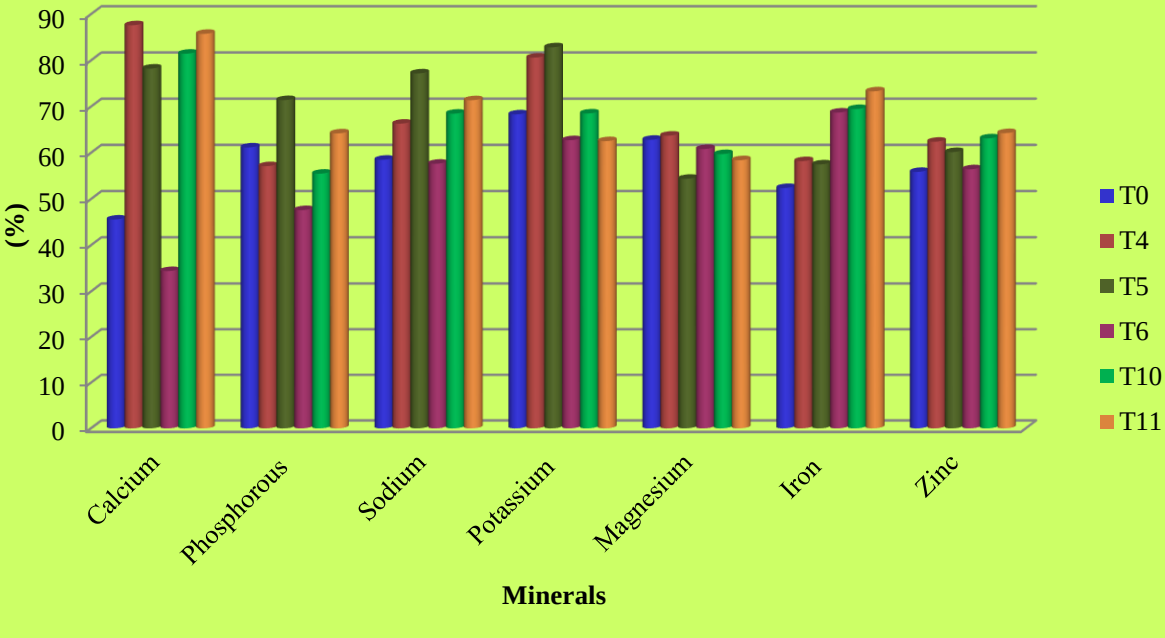


Fig. 4. *In vitro* mineral availability of meat analogues



soaking and pressure cooking which were a part of developing all the meat analogues in the study.

5.4. Shelf life studies

Production of foods consistently acceptable quality with shelf lives adequate for their intended uses, together with correct communication to the consumer of their durability is important of the manufacturer, retailer and the consumer. The consideration which is most important is that, the food must reach the consumer in good condition and retain its quality for the period expected (Man and Jones, 2000).

The selected meat analogues were packed in food grade HDPE covers (250 gauge) and kept at both ambient and refrigerated conditions for a period of three months. At monthly intervals, aspects such as organoleptic qualities, enumeration of total microflora, insect infestation were studied. Nutrient and *in vitro* studies were conducted initially and the end of storage period.

5.4.1. Organolpeptic evaluation of selected meat analogues on storage

Table 14 (a), 14 (b) and 15 (a), 15 (b) shows the mean scores of all the sensory attributes of the meat analogues stored both in ambient and refrigerated conditions. The mean scores for all the sensory attributes showed a gradual decrease in both the storage conditions as the duration of storage progressed. The organoleptic mean scores of meat analogues stored in refrigerated condition was observed to be better than that of ambient conditon. Inspite of the decrease, the meat analogues showed mean scores above 6.00 which indicates that the meat analogues stored at both conditions were acceptable at the end of storage period.

Initially, highest (8.64) organoleptic score for appearance was found in T_{10} while lowest (7.50) score was found in treatment T_6. At the end of third month of ambient storage, treatment T_{10} had highest (8.20) organoleptic score while the lowest (6.45) score was seen in T_0. At the end of third month of refrigeration storage, treatment T_5 had highest (8.44) organoleptic score while the lowest (7.21) score was

seen in T_6. The sensory attribute colour initially had the highest score (8.45) in treatments T_5 and T_{11} while treatment T_6 had lowest score (7.78). At the end of third month, organoleptic score for colour had highest (8.09) in treatment T_{11} and lowest score (6.43) was found in T_6 of the meat analogues stored at ambient condition. At the end of third month, organoleptic score for colour had highest (8.45) in treatment T_{11} and lowest score (7.33) found in T_6 of the meat analogues under refrigerated condition. However, prominent changes were not observed at the end of third month of storage which may be associated with effective air tight packaging in HDPE (250 guage) covers which could prevent interaction of the food product with oxygen which might initiate changes in appearance and colour.

Similar decreasing trend in the sensory attributes appearance and colour was seen in green gram based meat analogues (Nisha, 2008). This change was in accordance with the findings of McClements *et al.* (2021) who reported change in appearance and colour in meat analogues with increase in storage and attributed it to changes in the proximate composition.

Molu (2018) reported decrease in the mean scores of sensory attributes appearance and colour of nutri spreads stored at both ambient and refrigerated conditions. According to Lawrence and Kropf (2018), cold preservation like refrigeration minimizes the degradation of pigments or substances in food that may lead to undesirable appearance or colour formation.

Initially, lowest (7.27) organoleptic score for flavour was found in treatment T_0 while the highest score (8.53) for flavour was found in treatment T_5. At the end of third month of storage, the sensory attribute flavour was highest (8.23) in T_4 and lowest (6.30) organoleptic score for flavour was seen in treatment T_6 under ambient condition. At the end of third month of storage, the sensory attribute flavour was highest (8.47) in T_5 and lowest (7.15) organoleptic score for flavour was seen in treatment T_6 under refrigerated condition. The sensory quality taste was found to be

highest (8.64) in treatment T_{10} and lowest score (7.50) for taste was seen in treatment T_0 initially. At the end of ambient storage, the sensory attribute taste was found to be highest (8.11) in T_5 with lowest (6.94) in T_6. At the end of refrigerated storage, the sensory attribute taste was found to be highest (8.46) in T_5 with lowest (7.19) in T_6.

This decreasing trend of mean scores of flavour and taste in relation to ambient storage is in accordance with the findings of Nisha (2008) who reported similar decreasing trend in the flavour and taste attributes of green gram based meat analogues. However, there was no development of off flavours in the meat analogue at the end of third month of storage which may be associated with effective processing techniques such as optimal heat treatments and drying (Zhang *et al*., 2021). These food processing techniques lead to protein denaturation that changes the number and distribution of their flavour binding sites leading to reduced chances of off flavour and off taste production in storage (Wang *et al*., 2022).

Lakshmy (2011) reported similar decreasing trend in the flavour and taste of *tempeh* at stored at refrigerated condition as the storage period progressed. According to Kumar (2017), off flavours are usually developed in food when its intrinsic constituents such as proteins, lipids, enzymes and phospholipids interact with extrinsic factors such as oxygen and release volatile compounds such as hexanal, 1-octen-3-ol, 1-hexanol, and 2-pentylfuran producing off flavours and undesirable taste. El-Hay (2022) reported that refrigeration together with air tight packaging retains sensory qualities of foods by reducing oxygen interactions and inhibiting microbial growth.

Initially treatment T_0 had lowest score (6.40) for texture and the treatment T_5 had highest score (8.78). Texture was found to be highest (8.69) in T_4 and the lowest organoleptic score (5.45) for texture was found in T_0 at the end of ambient storage. Texture was found to be best (8.69) in T_5 and the lowest organoleptic score (6.09) for texture was found in T_6 at the end of storage period at refrigerated condition. This

trend of decrease in the sensory attribute texture was also observed in green gram incorporated meat analogues stored at ambient temperature (Nisha, 2008). Lakshmy (2011) reported a similar decrease in texture of *tempeh* under refrigerated condition. Texture in meat analogues is largely dependant on wheat gluten and its interaction with other ingredients such as breadfruit and tender jackfruit. These ingredients show higher moisture retention which could interact with the intermolecular linkages of wheat gluten leading to its changes in its associations with other ingredients (Berk, 2018). This could be associated with the notable difference in the sensory quality texture.

The observation in textural quality of meat analogues in storage in the present study can be correlated with the increase in moisture content of meat analogues stored at ambient temperature (Table 17). Berk (2018) reported that the minimal change in the textural quality of meat analogues stored at refrigerated condition may be associated with minimal water retention at refrigerated storage which would keep the interlinks of wheat gluten proteins and starch molecules intact due to which textural properties such as elasticity, springiness and chewiness would be retained.

Overall acceptability score and total score is the reflection of the scores of all other attributes. Decrease in overall acceptability and total score can be thus attributed to the corresponding decrease in the mean scores of sensory attributes such as appearance, colour, flavour, texture and taste. The initial overall acceptability score of meat analogues was found to be highest (8.81) in both T_5 and T_{11} while the lowest score (6.54) was seen in treatment T_0. The overall acceptability of meat analogues at the end of third month of storage was found to be lowest (6.04) in T_6 and highest (8.12) score was observed in treatment T_{11} at ambient storage. At refrigerated condition, the overall acceptability of meat analogues at the end of third month of storage was found to be lowest (6.23) in T_0 and highest (8.72) score was observed in treatment T_5. Initially the total score for the meat analogues was highest (48.43) in

T_{11} while lowest (43.26) total score was found in treatment T_0. The total score for the meat analogues at the end of third month of ambient storage was highest (48.43) in treatment T_{11} and the lowest total score (38.27) was seen in treatment T_6. The total score for the meat analogues at the end of third month of storage was highest (51.18) in treatment T_5 and the lowest total score (41.98) was seen in treatment T_6 at refrigerated condition.

This decreasing trend in both overall acceptability and total score of *tempeh* stored at the ambient and refrigerated condition was in accordance with the findings of Lakshmy (2011). Malganji *et al*. (2016) reported that the sensory qualities did not show much variation under refrigerated storage. Total scores in the present study at the end of third month of storage were higher in refrigerated condition compared to ambient condition in all the treatments. According to Dekkers *et al*. (2018) storage at refrigerated temperature preserves the pigments and proximate composition of meat analogues because of which colour, flavour, taste and textural changes are minimal.

Packaging conditions were reported to play a huge role in retaining the sensory qualities of food, especially air tight packing like HDPE (250 guage) (Lawrence and Kropf, 2018). Air tight packaging prior to refrigerated storage contributes to the extended shelf life along with minimizing factors such as microbial invasion, browning and oxidation that may lead to development of undersirable sensory characteristics to the food (Bassey *et al*., 2022).

Singh *et al*. (2009) reported that all the sensory attributes of aerobically packed dehydrated meat products decreased with increase in storage period.

5.4.2. Effect of storage conditions on the nutrient composition of selected meat analogues

Meat analogues were stored in HDPE covers (250 gauge) for three months in ambient and refrigerated conditions. The changes in the nutrient composition and *in*

vitro studies of meat analogues were estimated at the end of storage period. All the meat analogues showed a declining trend in nutrient and mineral composition which was more significant at ambient storage.

Table 17 shows the increase in moisture content at both ambient and refrigerated conditions after three months of storage. In both the storage conditions, an increase in the moisture content was observed on storage. Initially, meat analogues showed moisture content in the range of 9.25 to 10.62 per cent. At the end of third month of storage, the range of moisture at ambient storage was between 10.67 to 12.41 per cent while at refrigerated condition the observed range was 9.71 to 11.34 per cent. Similar results were seen in the study of Nisha (2008) where the increase in moisture content was higher in meat analogues stored at ambient condition compared to their refrigerated counterpart. According to Kyriakopoulou *et al.* (2019) at refrigeration temperature, the water activity of meat analogues increase due to which unbound water is less compared to the meat analogues stored at ambient condition. According to Pérez-Santaescolastica *et al*. (2021), increase in moisture content on storage is seen due to the interaction of air with the food and that overtime in the meat analogues the textural gluten protein and starch linkages relax during which the water molecules are released back into the food system.

A decrease in total carbohydrate content was observed in all the treatments at the end of storage at both ambient and refrigerated conditions. The total carbohydrate content of meat analogues was found to be in the range of 32.46 to 53.29 g $100g^{-1}$ initially. At the end of storage third month of at ambient condition, the range of total carbohydrates was between 29.05 to 49.92 g $100g^{-1}$. At refrigerated condition, the total carbohydrate content of the meat analogues was between 31.72 to 51.24 g $100g^{-1}$at the end of storage (Table 18). Lakshmy (2011) reported similar decrease in total carbohydrates in *tempeh*. Frančáková *et al*. (2021) reported that simple carbohydrates increase during storage. In the present study, increase in

microbial activity was observed which may be associated with the decrease in total carbohydrate content of meat analogues.

The protein content in all the treatments showed a decreasing trend while on storage in both ambient and refrigerated conditions. The protein content of meat analogues was found to be in the range of 20.79 to 38.03 g 100 g^{-1} initially. At the end of third month of storage at ambient condition, the range of protein was between 17.45 to 34.20 g $100g^{-1}$. At refrigerated condition, the protein content of the meat analogues was between 19.39 to 35.86 g $100g^{-1}$at the end of storage (Table 19). Similar decreasing trend was observed in protein content in *tempeh* on storage (Lakshmy, 2011). According to Liu (2022), oxidation of proteins in peanuts may be the cause of the decrease in peanut proteins during storage. He further stated that the active groups or oxidation products created by lipid oxidation could interact with proteins, causing protein breakdown or polymerization, which could harm the natural structure of proteins and affect their functional capabilities.

The total fat content in most of the treatments showed a decreasing trend while on storage in both ambient and refrigerated conditions. The total fat content of meat analogues was found to be in the range of 1.20 to 1.92 g $100g^{-1}$ initially. At the end of third month of storage at ambient condition, the range of total fat was between 0.30 to 1.54 g $100g^{-1}$. At refrigerated condition, the total fat content of the meat analogues was between 0.57 to 1.60 g $100g^{-1}$at the end of storage. Lakshmy (2011) reported a decreasing range of total fat in storage in *tempeh*. Liu *et al*. (2022) reported significant change in total carbohydrates, protein, total fat and moisture in peanuts with relation to storage. Liu *et al*. (2022) reported that fatty acid composition changed significantly during storage at different temperatures while high storage temperatures may lead to high degree of lipid oxidation and nutrient loss. This was in accordance with the present study in which there was significant decrease in fat content in some of the meat analogues stored at ambient and refrigerated conditions.

The total ash content in all the treatments showed a decreasing trend while on storage in both ambient and refrigerated conditions. The total ash content of meat analogues was found to be in the range of 2.92 to 5.66 g $100g^{-1}$ initially. At the end of third month of storage at ambient condition, the range of total ash was between 1.80 to 4.62 g $100g^{-1}$. At refrigerated condition, the total ash content of the meat analogues was between 1.68 to 4.70 g $100g^{-1}$at the end of storage. Molu (2018) reported a decrease in total ash content in nutri spreads stored at both ambient and refrigerated conditions. The author also reported the decrease in nutri spreads stored at refrigerated condition was less significant compared to ambient condition. The results of the present study were also in accordance with the findings of Molu (2018).

The fibre content in all the treatments showed a decreasing trend while on storage in both ambient and refrigerated conditions. The fibre content of meat analogues was found to be in the range of 2.23 to 7.30 g $100g^{-1}$ initially. At the end of third month of storage at ambient condition, the range of fibre was between 2.32 to 5.25 g $100g^{-1}$. At refrigerated condition, the fibre content of the meat analogues was between 2.02 to 6.02 g $100g^{-1}$ at the end of storage. Nisha (2008) reported a decrease in fibre content in green gram based meat analogues on storage. According to Frančáková *et al*. (2021), complex carbohydrates decrease during storage. In the present study, the decrease in fibre content at ambient storage was more significant compared to refrigerated condition. Nadarajah and Thevaki (2015) also reported a significant decrease in the fibre content of stored biscuits at ambient condition and reported that it may be due to the increasing temperature breaks the weak points between polysaccharide chains.

The calcium content in all the treatments showed a decreasing trend while on storage at ambient condition (Table 23). A decrease in calcium content was observed in all the treatments stored under ambient condition. Calcium content ranged from 78.54 to 93.24 mg $100g^{-1}$ at final ambient storage while it was 80.11 to 94.11 mg 100 g^{-1} at final refrigerated storage with values of all treatments under refrigerated

condition showing no significant difference from initial values. All the treatments differed significantly in the calcium content after three months of storage under ambient condition. Molu (2018) reported similar decrease in calcium content in nutri spreads stored at both storage conditions. Sulieman *et al*. (2012) reported no significant change in calcium content in mozzarella cheese during refrigerated storage. According to Amit *et al*. (2017) increased storage period leads to increased moisture transfer which increases the moisture content of food which may result in proportionate reduction in other mineral constituents such as calcium.

The phosphorous content in all the treatments showed a decreasing trend while on storage at ambient condition (Table 24) with significant difference in the phosphorous content in most of the treatments in ambient condition while the change in phosphorous content was not significant in all the treatments of meat analogues stored at refrigerated condition after three months of storage. The range of phosphorous content at the initial stage of storage was 255.62 to 325.46 mg 100 g^{-1} while after three months of ambient temperature it ranged from 254.97 to 324.16 mg 100 g^{-1}. The meat analogues stored in refrigerated temperature reported a phosphorous content ranging from 255.17 to 325.22 mg 100 g^{-1}. Lakshmy (2011) reported similar decreasing trend of phosphorous content in storage in *tempeh*. Rousseau *et al*. (2020) reported storage condition and time period to be major determinants of changes in phosphorous content in the food which may be due to factors such as microbial growth, oxygenation and intrinsic biomolecular interactions in food.

The sodium content in all the treatments showed change while on storage in both ambient and refrigerated conditions. The sodium content of meat analogues was found to be in the range of 23.52 to 74.43 mg 100 g^{-1} initially. At the end of third month of storage at ambient condition, the range of sodium was between 23.02 to 73.03 mg 100 g^{-1}. At refrigerated condition, the sodium content of the meat analogues was between 23.19 to 74.15 mg 100 g^{-1} at the end of storage and did not show any

significant change statistically (Table 25). Molu (2018) reported similar change in the content of sodium under both ambient and refrigerated storage in nutri spreads. Veldhuizen *et al.* (2020) reported that changes in sodium content may be due to lipid oxidation, oxygenation or browning, however at only in prolonged storage conditions.

The potassium content in all the treatments showed a decreasing trend while on storage at ambient condition. . The range of potassium content at the initial stage of storage was 510.49 to 631.50 mg $100g^{1}$ while after three months of ambient temperature it declined to 509.03 to 630.97 mg $100g^{1}$. The meat analogues stored in refrigerated temperature reported potassium content ranging from 520.89 to 631.35 mg 100 g^{1} respectively (Table 26). Similar change in potassium content by the end of storage was found in accordance with the findings of Molu (2018). Sulieman *et al*. (2012) detected a considerable change in potassium content in mozzarella cheese during ambient storage which was not observed at refrigerated storage. He also reported that the result of the interaction of microbial and food biomolecules could be associated with such change.

All the treatments differed significantly in the magnesium content after three months of ambient storage. The change in magnesium content in refrigerated condition showed no significance in all the treatments in the content of magnesium after three months storage period. Magnesium content of meat analogues ranged from 103.64 to 181.69 mg $100g^{1}$ initially and it ranged from 100.34 to 180.94 mg $100g^{1}$ at final month of ambient storage respectively. The range of magnesium content of selected meat analogues at refrigerated storage was between 103.25 to 181.14 mg $100g^{1}$ at the end of storage. Nisha (2008) reported similar change of magnesium content in green gram based meat analogues at ambient storage. According to Bouzari *et al.* (2015), non significant change in magnesium in storage

may be due to the changing composition of food product due to oxidation or biomoleular degradation which is more prominent on storage.

Most of the treatments differed significantly with regards to change in iron and zinc content after three months storage at ambient condition. In refrigerated condition, all the treatments showed no significance in the initial and final content of iron and zinc content after three months of storage. Iron content ranged from 4.01 to 4.73 mg $100g^{1}$ at final ambient storage while it was 4.18 to 5.63 mg $100g^{1}$ at final refrigerated condition (Table 28). The range of zinc content at the initial stage of storage was observed to be from 3.17 to 3.96 mg 100^{-1} while at final stage of ambient storage it was 2.93 to 3.12 mg 100^{-1}g. In refrigerated storage, the zinc content in meat analogues ranged from 3.13 to 3.94 mg 100^{-1}g at the end of storage period (Table 28). Significant change was not observed at storage at refrigerated condition. Lakshmy (2011) reported similar change of iron content in *tempeh* under storage. Molu (2018) reported similar decreasing trend of zinc content in *tempeh* under storage. Kuong *et al*. (2016) reported non significant loss of both iron and zinc of fortified rice premix during storage. The authors also suggested the inclusion of iron as iron EDTA (Ethylene - di-amine-tetra-acetic acid) would stabilize the content of iron preventing its loss. Zahara *et al*. (2020) also observed a non significant decrease trend of iron in fruit bars under storage for a period of 60 days.

The use of mineral content enhancing pretreatments such as soaking and processing approaches such as autoclaving reduces antinutritional components such as phytic acid, tannins, oxalates, and lectins that exhibit mineral chelating properties (Marinangeli *et al*., 2017). Furthermore, proper packing, such as air tight HDPE container and clean, dry, cool, and refrigerated storage conditions, may be associated with less significant changes in mineral content of meat analogues (Gharibzahedi and Jafari, 2017).

5.4.2.1. *In vitro* protein digestibility of selected meat analogue on storage

There was a declining trend in the *in vitro* availability of protein in the meat analogues while on storage at both ambient and refrigerated conditions. The protein *in vitro* digestibility of meat analogues was found to be in the range of 62.12 to 80.30 per cent. At the end of third month of storage at ambient condition, meat analogues showed *in vitro* protein digestibility in the range of 61.22 to 80.07 per cent. At refrigerated condition, the *in vitro* digestibility of protein in the meat analogues was between 62.04 to 80.21 per cent at the end of storage and was less significant. Similar decrease in the protein *in vitro* digestibility in *tempeh* by the end of storage was found in accordance with the findings of Lakshmy (2011). However, prominent decrease was not observed in the *in vitro* protein digestibility initially and at the end of third month of storage at refrigerated condition. This was supported by Shaghaghian *et al*. (2022) who reported that choosing appropriate plant protein like soy protein and wheat gluten accompanied by apt processing technology and air tight packaging retains the *in vitro* protein digestibility up to three to four months.

5.4.2.2. *In vitro* mineral digestibility of selected meat analogue on storage

All the minerals including calcium, phosphorous, sodium, potassium, magnesium, iron and zinc showed change in terms of their bioavailability in the meat analogues while on storage at both ambient and refrigerated conditions. However, the change was not significant in most of the treatments stored at refrigerated condition at the end of storage. The *in vitro* availability of calcium in meat analogues was found to be in the range of 34.43 to 87.62 per cent. At the end of third month of storage at ambient condition, meat analogues showed *in vitro* availability of calcium in the range of 32.19 to 86.81 per cent. At refrigerated condition, the *in vitro* availability of calcium in the meat analogues was between 33.78 to 86.91 per cent at the end of storage (Table 31). The *in vitro* availability of phosphorous in meat analogues was found to be in the range of 47.62 to 71.43 per cent (Table 32). At the end of third

month of storage at ambient condition, meat analogues showed *in vitro* availability of phosphorous in the range of 46.17 to 69.53 per cent. At refrigerated condition, the *in vitro* availability of phosphorous in the meat analogues was between 46.17 to 70.12 per cent at the end of storage. Range of sodium *in vitro* availability at ambient storage showed variance 57.66 to 77.20 per cent initially to 55.82 and 73.74 per cent after three months of ambient storage. Range of sodium *in vitro* availability after three months of storage at refrigerated storage ranged between 57.93 and 76.84 per cent (Table 33). The change in both phosphorous and sodium was not significant in most of the treatments stored under refrigerated conditions.

Initial *in vitro* availability of potassium ranged from 62.54 to 82.85 per cent which was 59.98 to 80.04 per cent at the end of storage at ambient condition. The *in vitro* availability of potassium ranged between 61.51 and 81.81 per cent at end of storage at refrigerated condition (Table 34). Range of magnesium *in vitro* availability at ambient storage showed variance from 54.40 to 63.73 per cent at initial stage and 53.02 to 65.63 per cent at final stage respectively. The magnesium *in vitro* availability of the treatments was observed to be in the range of 54.06 to 67.91 per cent at the end of storage at refrigerated condition (Table 35). Iron *in vitro* availability ranged from 51.66 to 72.24 per cent at final ambient storage while it was 51.92 to 73.30 per cent at final stage of refrigerated storage (Table 36). The range of zinc *in vitro* availability at the initial stage of storage was between 55.89 and 64.28 per cent while at final stage of ambient temperature it was between 54.34 and 63.41 per cent. The *in vitro* zinc availability after three months of refrigerated storage ranged from 55.44 to 64.79 per cent (Table 37). Similar changes in bioavailability of minerals at different storage conditions were seen in the findings of Drago (2022). Application of mineral content enhancing pretreatments like soaking and processing techniques like autoclaving decreases the antinutritional factors like phytic acid, tannins, oxalates and lectins which exhibit mineral chelating property. Additionally, appropriate packaging like air tight HDPE packaging and clean, dry, cool and

refrigerated storage condition could be associated with less or no significant changes in mineral *in vitro* bioavailability of food products (Gharibzahedi and Jafari, 2017).

5.4.3. Enumeration of total microflora in selected meat analogues on storage

Acceptability of processed foods is largely dependant in its microbiological safety. Convenience foods should have shelf life on storage and the microbiological safety of these products should be ensured. Microbiological criteria provide guidance on the acceptability of food stuffs and their manufacturing processes.

According to Toth *et al*. (2021) different factors such as quality of raw materials, storage temperature, processing temperature, storage containers, processing technique and the environment in which it is processed have an effect on the microbial quality of processed foods. In the present study, the total bacterial count in selected treatments and controls of meat analogues ranged from 3.00 to 3.33×10^5cfu/g initially. After three months of storage at ambient temperature, an increase in the total bacterial count was observed in all the meat analogues which varied from 5.00 to 7.00 $\times10^5$cfu/g. After three months of storage at refrigerated temperature, an increase in the total bacterial count was observed in all the meat analogues which varied from 4.00 to 5.00 $\times10^5$cfu/g. Nisha (2008) reported an increase in total bacterial count while on storage and recorded a count of 1.33 $\times10^6$cfu/g at the end of third month of storage in green gram based meat analogues.

In the present study, initially, the total fungal count was not detected in most of the treatments of meat analogues. After three months of storage at ambient temperature, an increase in the total fungal count was observed in all the meat analogues which varied from 2.33 to 3.88 $\times10^3$cfu/g. After three months of storage at refrigerated temperature, an increase in the total fungal count was observed in all the meat analogues which varied from 0.29 to 2.33 $\times10^3$ cfu/g. Nisha (2008) reported an increase in total fungal count while on storage in ambient condition with a recorded

count of 1.66 $\times 10^3$ cfu/g at the end of third month in green gram based meat analogues.

In the present study, initially, the total yeast count was not detected in all the treatments of meat analogues. After three months of storage at ambient temperature, an increase in the total yeast count was observed in all the meat analogues which varied from 0.22 to 0.88 $\times 10^3$cfu/g. After three months of storage at refrigerated temperature, an increase in the total fungal count was observed in all the meat analogues which varied from 0.11 to 0.55 $\times 10^3$ cfu/g. Nisha (2008) reported an increase in total yeast count while on storage with a recorded count of 0.71 $\times 10^3$cfu/g at the end of third month in green gram based meat analogues.

In the present study, fungal growth in storage was more in ambient condition compared to refrigerated condition. The low microbial load observed in the products of the present study may be associated with effective processing including high temperature treatment and cabinet drying followed by suitable air tight packaging. Microbial activity in meat analogues stored at ambient temperature is higher than refrigerated stored meat analogues. This could be attributed to the refrigeration temperature at which unbound water is unavailable for the growth of microbes. Maheswarappa *et al.* (2016) reported marked increase in the shelf life of meat products have been documented upto 80 days of refrigerated storage. According to Kyriakopoulou *et al.*(2019) suitable processing and packaging would retard the microbial growth which could extends the normal shelf life of food products by two to three times. Depending on the intrinsic ingredients in a food system and packaging conditions, storing the meat analogues at suitable condition can extend its shelf life from several days to a month (Domínguez *et al*., 2021). Low microbial load may also be associated with the use of spices in spice broth such as turmeric and pepper which contain compounds such as *curcumin, sabinene, α-pinene, β-pinene, limonene, β-caryophyllene, caryophyllene* that exhibit antibacterial, antifungal and antimicrobial properties *(*Mashabela *et al*., 2022*)*. Low microbial activity in meat analogues stored

at refrigerated temperatures may be due to the fact that the rate of enzyme actions in microorganisms are decreased which retards their growth (Frazier and Westhoff, 1995). According to them cooler temperatures will prevent the growth of microbes, but slow metabolic activity may be continue.

5.4.4. Insect infestation in selected meat analogues on storage

Selected treatments of tender jackfruit and breadfruit incorporated meat analogues and the controls of the study showed absolute absence of insect infestation initially and at the end of the third month of storage at both ambient and refrigerated storage. Similar results were seen in the study of Lakshmy (2011) in meat alternatives. Campbell *et al*. (2012) reported that safe post harvest practices during storage and transport of food produce would minimize the risk of insect infestation. Following safe food practices such as thorough washing, storage at clean, dry place along with processing the food at high temperature could eliminate chances for any infestation of insects (Hagstrum and Philips, 2017). Kumar (2017) also reported that storage in air tight packaging such as HDPE covers would minimize the contact of food with extrinsic factors that lead to insect infestation.

5.5. Cost benefit analysis

The cost of production of the selected meat analogues and their controls were estimated per 100g of the finished products. The cost for the controls ranged from Rs. 34.00 to Rs. 39.00/100g for 100 per cent cowpea and chickpea respectively. The cost varied from Rs. 57.00 to 60.00/100g in the selected treatments of meat analogues. The cost incurred for the production of tender jackfruit incorporated cowpea meat analogues was Rs. 57.00/100g and for tender jackfruit incorporated chickpea meat analogues it was Rs. 60.00/100g. The cost of production of breadfruit incorporated cowpea meat analogues approximated to Rs. 58.00/100g and for breadfruit incorporated chickpea meat analogues it was Rs. 59.00/ 100g. The market price of different meat analogues was found to be in the range of Rs. 58.00 to 100.00

per 100g. The market price of meat varied in the range of Rs. 37.00 to 76.00/ 100g. The cost of developed meat analogues in this study were comparable to the market price of various types of meats. The present study found that meat analogues with better organoleptic properties, nutritional profile and shelf stable could be developed with indigenous plant foods such as tender jackfruit and breadfruit which could be a better, healthier, cost effective and versatile option compared to other commercial meat analogues and meat.

Summary

6. SUMMARY

In the study entitled "Standardisation of jackfruit and breadfruit incorporated meat analogues" meat analogues using cowpea (CWP), chickpea (CP), tender jackfruit (TJ), breadfruit (BF), wheat gluten (WG), defatted soy flour (DSF) and oyster mushroom flour (OMF) were formulated, developed and standardised. Also, the organoleptic, nutritional and shelf life qualities were studied as part of quality evaluation of the developed meat analogues.

In the first experiment of the study, tender jackfruit incorporated cowpea and chickpea meat analogues were developed in different combinations. As the number of treatments proceeded, the percentage of tender jackfruit and wheat gluten increased, with corresponding decline in the percentage of pulses (cowpea and chickpea). In the second experiment, breadfruit incorporated cowpea and chickpea meat analogues were developed in different combinations. As the number of treatments increased, the amount of breadfruit and wheat gluten increased, but the quantity of pulses (cowpea and chickpea) decreased. Defatted soy flour (DSF) and oyster mushroom flour (OMF) were added to all the treatments except T_0 (100% CWP) and T_6 (100% CP).

The developed fresh meat analogue cubes were soft, springy with distinctive 'grainy' texture which in cut surface showed defined interconnected inner framework of its ingredients.

Organleptic evaluation was conducted in which sensory attributes such as appearance, colour, flavour, taste, texture and overall acceptability of tender jackfruit and breadfruit incorporated cowpea and chickpea meat analogues were evaluated. In tender jackfruit incorporated cowpea and chickpea meat analogues, the treatments T_5 (40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF) and T_{10} (50 % CP + 20 % TJ+ 20 % WG + 5 % DSF + 5 % OMF) were selected respectively as best treatments based on their organoleptic evaluation score. Treatments T_4 (50 % CWP +

20 % BF+ 20 % WG + 5 % DSF + 5 % OMF) and T_{10} (40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF) showed higher scores for overall acceptability and total scores in breadfruit incorporated cowpea and chickpea meat analogues respectively due to which they were chosen as the best treatments.

The selected treatments from both experiments along with their controls (T_0 - 100% CWP and T_6 - 100% CP) were evaluated for their quality attributes such as nutritional studies, *in vitro* investigations, and shelf life studies. Nutrient studies of the selected meat analogues and their controls included estimation of moisture, total carbohydrate, protein, total fat, total ash, fibre and minerals such as calcium, sodium, phosphorous, magnesium, iron, zinc and potassium. The range of moisture content in the meat analogues was from 9.25 to 10.62 per cent. Meat analogues of this study were observed to contain total carbohydrate, protein, total fat, total ash and fibre content in the range of 32.46 to 53.29, 20.79 to 38.03, 1.06 to 1.92, 2.92 to 5.55 and 2.23 to 7.30 g 100^{-1} respectively.

Proximate analysis also showed that meat analogues were abundant in minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc whose contents ranged from 80.25 to 94.67, 255.62 to 325.46, 23.52 to 74.43, 510.49 to 631.50, 103.64 to 181.69, 4.17 to 5.73 and 3.1 to 3.96 mg $100g^{-1}$ respectively. The protein *in vitro* digestibility of meat analogues was in the range of 62.12 to 80.30 per cent. The *in vitro* availability of minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc of the selected treatments and the controls was in the range of 34.43 to 87.62, 47.62 to 71.43, 57.66 to 77.20, 62.54 to 82.85, 54.40 to 63.73, 52.40 to 73.32, 55.89 to 64.28 per cent respectively.

The *in vitro* protein digestibility of the meat analogues was highest in T_4 (80.30 %) and the control T_6 (100% CP) had the lowest protein *in vitro* digestibility of 62.12 per cent. The *in vitro* availability of minerals such as calcium, phosphorous,

sodium, potassium, magnesium, iron and zinc were observed to be in the range of 34.43 to 87.62, 47.62 to 64.22, 27.66 to 77.20, 32.70 to 82.85, 19.84 to 63.73, 18.73 to 73.32, and 15.89 to 63.14 per cent respectively in the meat analogues.

The selected meat analogues and the controls were packed in food grade HDPE covers (250 gauge) and kept at both ambient temperature and refrigerated conditions for a period of three months. At monthly intervals, aspects such as organoleptic qualities, enumeration of total microflora and insect infestation were studied. Nutrient and *in vitro* studies were conducted initially and the end of storage period.

Organoleptic evaluation of meat analogues at monthly intervals showed a decreasing trend in the organoleptic qualities in all the treatments stored at ambient temperature with the increase in time of storage. At the initial period all the six treatments had higher scores in all the sensory attributes such as appearance, colour, flavour, taste, texture and overall acceptability. At the end of third month of ambient storage, highest (48.43) total score was observed in T_{11} while the lowest (38.27) was seen in control T_6. At the end of third month of refrigerated storage, highest total score (51.18) was observed in treatment T_5 while the lowest (41.98) was seen in treatment T_6. In both ambient and refrigerated conditions, there was reduction in organoleptic scores, for instance, T_0 (100% CWP) had the total score of 43.26 which at the end of storage decreased to 39.00 (with difference of 5.26) in ambient condition. However, in refrigerated condition the same showed a difference of only 0.86 with final total score of 42.40. This implies that meat analogues stored under refrigerated condition showed better sensory qualities than their ambient stored counterparts throughout storage.

The meat analogues stored at ambient and refrigerated storage were analysed for their nutritional and *in vitro* aspects. In both the storage conditions, an increase in the moisture content was observed on storage. There was a general decrease in the total carbohydrate content, protein, total fat, total ash and fibre in meat analogues

stored at ambient and refrigerated conditions. However, this change was less significant in meat analogues stored in refrigerated condition when compared to ambient storage. A change in the contents of calcium, phosphorous, sodium, potassium, magnesium, iron and zinc was observed in all the treatments stored under ambient condition. The change in most of the treatments with respect to their mineral content was not significant at refrigerated storage.

Protein *in vitro* digestibility was shown to significantly differ in the selected treatments of meat analogues along with the controls from the initial and final storage at both ambient and refrigerated conditions of storage. The *in vitro* digestibility of protein of meat analogues stored in ambient condition showed a general trend of decrease in all the treatments. The initial *in vitro* protein digestibility ranged from 62.12 to 80.30 per cent and after storage for three months the *in vitro* protein availability ranged from 61.22 to 80.07 per cent after three months of ambient storage. The *in vitro* digestibility of meat analogues stored in refrigerated condition for three months ranged between 62.04 to 80.21 per cent. The *in vitro* availability of the minerals calcium, phosphorous, sodium, potassium, magnesium, iron and zinc were observed to change while on storage at both ambient and refrigerated conditions. However, the change of *in vitro* mineral availability of most of the meat analogues stored at refrigerated temperature was not significant after three months of storage.

Under microbial studies, micro organisms such as bacteria, fungi and yeast were enumerated in meat analogues (control and best treatments) throughout the storage period of three months under two different conditions (ambient and refrigerated storage). At both ambient and refrigeration storage conditions, bacterial colonies increased with increase in storage time. However, viable bacterial count was higher in treatments of meat analogues under ambient condition against refrigerated condition. Initially, most of the treatments showed no fungal or yeast growth. However, there was an observed growth of fungal and yeast colonies with increase in

storage period at both ambient and refrigerated conditions. However, the fungal and yeast growth was higher in treatments of meat analogues stored under ambient condition against refrigeration condition.

During storage of three months, insect infestation was not observed in any of the different treatments of meat analogues stored in both ambient and refrigerated temperature.

The cost of production of the selected meat analogues and their controls were estimated per 100g of the finished products. The cost for the controls ranged from Rs. 34.00 to Rs. 39.00/100g for 100 per cent cowpea and chickpea respectively. The cost varied from Rs. 57.00 to 60.00/ 100g in the selected treatments of meat analogues. The cost of developed meat analogues in this study were lower and are also within the range of market price of various types of meat.

The current research found that meat analogues with improved organoleptic properties, nutritional profiles, and shelf stability could be developed using indigenous plant foods such as tender jackfruit and breadfruit, which could be a better, healthier, more cost-effective, and versatile alternative to other commercial meat analogues and meat.

Future line of work

- Utilization, modifying and formulating novel proteins from plant sources to create desirable textural properties alike meat
- Development of meat analogues from non – plant sources such as algae, yeast, mushroom, and bacteria
- Development of other types of meat analogues using indigenous crops
- Introduction of compounds that mimic appearance, flavour and taste of meat into meat analogues
- Introduction of novel meat analogues production technologies

References

REFERENCES

A.O.A.C. 1980. *Official and tentative methods of analysis* (13th Ed.). Association of Official analytical chemists, Washington, D.C, 1018p.

Acevedo-Juarez, S., Guajardo-Flores, D., Heredia-Olea, E., and Antunes-Ricardo, M. 2022. Bioactive peptides from nuts: A review. *Int. J. Food Sci. Technol.* 57(4): 2226-2234.

Adeyi, A. M., Osunde, Z. D., Ogunjirin, O. A., Awu, J. I., and Ibrahim, A. 2015. Effect of drying on nutritional quality of dried meat (Kilishi). *J. Agric. Eng. Technol.* 23(1): 14-34.

Adhikari, U., Mateu, C. G., Chattopadhyay, K., Pujol, C. A., Damonte, E. B., Ray, B. 2006. Structure and antiviral activity of sulfated fucans from *Stoechospermum marginatum*. *Phytochem.* 67: 2474-2482.

Agarwal, G. P. and Hasija, S. K. 1986. *Microorganisms in the Laboratory*. Print house India Ltd., Lucknow, 155p.

Aguilar, E. G., Albarracin, G. J., Unates, M. A., and Piola, H. D. 2015. Evaluation of the nutritional quality of the grain protein of new amaranths varieties. *Plant Foods Hum. Nutr*. 70(1): 21-26.

Ahmad, R. S., Imran, A., and Hussain, M. B. 2018. Nutritional composition of meat. *Meat Sci. Nutr.* 61(10): 57-72.

Ajiboye, E. A., Alhassan, S., Adedayo, R. M., Kolawole, M. O., and Oladosu, O. T., 2011. Physicochemical properties and microorganisms isolated from dried meat obtained in Oja-Oba market in Ilorin, Nigeria. *Pelagia Res. Libr.* 2(4): 391-400.

Al Hasan, S. M., Hassan, M., Saha, S., Islam, M., Billah, M., and Islam, S. 2016. Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh: A cross-sectional study. *BMC Nutr.* 2(1): 1-10.

Amit, S. K., Uddin, M., Rahman, R., Islam, S. M., and Khan, M. S. 2017. A review on mechanisms and commercial aspects of food preservation and processing. *Agric. Food Secur.* 6(1): 1-22.

Anand, K., Kant, S., and Kapoor, S. K. 1999. Nutritional status of adolescent school children in rural north India. *Indian Pediatr.* 36(8): 810-816.

Annor, G. A., Tyl, C., Marcone, M., Ragaee, S., and Marti, A. 2017. Why do millets have slower starch and protein digestibility than other cereals?. *Int. J. Food Sci. Technol*. 66: 73-83.

Anyasi, T. A., Jideani, A. I., and Mchau, G. R. 2018. Phenolics and essential mineral profile of organic acid pretreated unripe banana flour. *Food Res. Int.* 104: 100-109.

Bajpai, V. K., Rather, I. A., Lim, J., and Park, Y. H. 2014. Diversity of bioactive polysaccharide originated from marine sources: A review. *Indian J. Geo-Mar Sci.* 43: 1857-1869.

Baliga, M. S., Shivashankara, A. R., Haniadka, R., Dsouza, J., and Bhat, H. P. 2011. Phytochemistry, nutritional and pharmacological properties of *Artocarpus heterophyllus* Lam (jackfruit): A review. *Int. Food Res. J.* 44(7): 1800-1811.

Bassey, A. P., Chen, Y., Boateng, E. F., Zhang, Y., Diao, X., Nasiru, M. M., Tang, C., Ye, K., Li, C., and Zhou, G. 2022. Evaluation of physicochemical, microbiological, and sensory profiles of vacuum-packed cooked low-salt pork

belly under refrigeration and room-temperature storage. *LWT Food Sci. Technol.* 167: 113847.

Bawa, S. H. and Webb, M. 2016. Nutritional and health effects of the consumption of breadfruit. *Trop Agr. (Trin.)*. 93: 52-69.

Bax, M. L., Buffiere, C., Hafnaoui, N., Gaudichon C., Savary-Auzeloux, I., Dardevet, D., Sante-Lhoutellier, V., and Remond, D. 2013. Effects of meat cooking, and of ingested amount, on protein digestion speed and entry of residual proteins into the colon: A study in minipigs. *PLoSONE* 6(4): e61252.

Berk, Z. 2018. *Food process engineering and technology*. Academic press, London, 689p.

Bernat, N., Chafer, M., Rodríguez-García, J., Chiralt, A., and González-Martínez, C. 2015. Effect of high pressure homogenisation and heat treatment on physical properties and stability of almond and hazelnut milks. *LWT Food. Sci. Technol*. 62(1): 488-496.

Bernstein, A. M., Sun, Q., Hu, F. B., Stampfer, M. J., Manson, J. E., and Willett, W. C. 2010. Major dietary protein sources and risk of coronary heart disease in women. *Circulation* 122(9): 876-883.

Bessada, S. M., Barriera, J. C., and Oliveira, M. B. P. 2019. Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. *Trends Food Sci. Technol*. 93: 53-68.

Bohrer, B. M. 2019. An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Sci. Hum. Wellness* 8 (4): 320-329.

Boland, M. J., Rae, A. N., Vereijken, J. M., Meuwissen, M. P. M., Fischer, A. R. H., Boekel, M. A. J. S., Rutherfurd, S. M., Gruppen, H., Moughan, P. L., and

Hendriks, W. H. 2013. The future supply of animal derived protein for human consumption. *Trends Food. Sci. Technol.* 29: 62-73.

Bora, P. 2014. Anti-nutritional factors in foods and their effects. *J. Acad. Ind. Res*. 3(6): 285-290.

Boukid, F. 2021. Plant-based meat analogues: From niche to mainstream. *Eur. Food Res. Technol*. 247(2): 297-308.

Bouvard, V., Loomis, D., Guyton, K. Z., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Mattock, H., and Straif, K. 2015. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol.* 16(16): 1599-1600.

Bouzari, A., Holstege, D., and Barrett, D. M. 2015. Mineral, fiber, and total phenolic retention in eight fruits and vegetables: a comparison of refrigerated and frozen storage. *J. Agric. Food Chem.* 63(3): 951-956.

Campbell, J. F., Perez-Mendoza, J., and Weier, J., 2012. 19 Insect Pest Management Decisions in Food Processing Facilities. In: Hangstrum, D. W., Phillips, T. W., and Cuperus, G. (eds), *Stored product protection*, K-STATE Research and Extension, Kansas, pp. 225-239.

Campbell, C. L., Wagoner, T. B., and Foegeding, E. A. 2017. Designing foods for satiety: The roles of food structure and oral processing in satiation and satiety. *Food Struct.* 13:1-12.

Cavazos A. and de Mejia, G. G. E. 2013. Identification of bioactive peptides from cereal storage proteins and their potential role in prevention of chronic diseases. *Compr. Rev. Food Sci. Food Saf.* 12: 364-380.

Chambers, E, V., Chambers, E. IV., and Castro, M. 2018. What is “natural”? Consumer responses to selected ingredients. *Foods* 7(4): 65.

Chandra, E. and Bharati, P. 2020. Physical and nutritional properties of jackfruit at different stages of maturity. *Pharma Innov. J.* 9(12): 354-357.

Chen, W., Hira, T., Nakajima, S., Tomozawa, H., Tsubata, M., Yamaguchi, K., and Hara, H. 2012. Suppressive effect on food intake of a potato extract (Potein®) involving cholecystokinin release in rats. *Biosci. Biotechnol. Biochem.*76(6): 1104-1109.

Chereau, D., Videcoq, P., Ruffieux, C., Pichon, L., Motte, J. C., Belaid, S., Ventureira, J., and Lopez, M. 2016. Combination of existing and alternative technologies to promote oilseeds and pulses proteins in food applications. *OCL* 23(4): D406.

Chiang, J. H., Loveday, S. M., Hardacre, A. K., and Parker, M. E. 2019. Effects of soy protein to wheat gluten ratio on the physicochemical properties of extruded meat analogues. *Food Struct*. 19: 100-103.

Chopra, S. L. and Kanwar, J. S. 1978. *Analytical Agricultural Chemistry*. Kalyani Publishers, Ludhiana, 210p.

Churchward-Venne, T. A., Pinckaers, P. J., van Loon, J. J., and van Loon, L. J. 2017. Consideration of insects as a source of dietary protein for human consumption. *Nutr. Rev.* 75(12): 1035-1045.

Coelho, M. S., Salas-Mellado, M., and de las, M. 2018. How extraction method affects the physicochemical and functional properties of chia proteins. *LWT Food Sci. Technol.* 96: 26-33.

Cook, L., Munier, C. M. L., Seddiki, N., van Bockel, D., Ontiveros, N., Hardy, M. Y., Gillies, J. K., Levings, M. K., Reid, H. H., Petersen, J., and Rossjohn, J. 2017. Circulating gluten-specific FOXP3+ CD39+ regulatory T cells have impaired

suppressive function in patients with celiac disease. *J. Allergy Clin. Immunol*. 140(6): 1592-1603.

Cornet, S. H. V., van der Goot, A. J., and van der, S. R. G. M. 2020. Effect of mechanical interaction on the hydration of mixed soy protein and gluten gels. *Curr. Res. Food Sci.* 3: 134-145.

Couch, C., Franxman, T., and Greenhawt, M. 2017. Characteristics of tree nut challenges in tree nut allergic and tree nut sensitized individuals. *Ann Allergy Asthma Immunol*. 118: 591-596.

Coulibaly, A., Kouakou, B., and Chen, J. 2011. Phytic acid in cereal grains: Structure, healthy or harmful ways to reduce phytic acid in cereal grains and their effects on nutritional quality. *Am. J. Plant Nutr. Fertilization Technol*. 1(1): 1-22.

Cumashi, A., Ushakova, N. A., Preobrazhenskaya, M. E., D'Incecco, A., Piccoli, A., Totani, L., Tinari, N., Morozevich, G. E., Berman, A. E., and Bilan, M. I. A. 2007. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiol.* 17: 541-552.

Cunsolo V., Muccilli V., Saletti R., and Foti, S. 2012. Mass spectrometry in the proteome analysis of mature cereal kernels. *Mass Spectrom. Rev.* 31: 448-465.

Cutroneo, S., Angelino, D., Tedeschi, T., Pellegrini, N., Martini, D., and Sinu, Y. 2022. Nutritional quality of meat analogues: results from the Food Labelling of Italian Products (FLIP) Project. *Front. Nutr.* [e-journal] 9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9090485. [26 Apr. 2022]

D'Alessandro, C., Piccoli, G. B., and Cupisti, A. 2015. The "phosphorus pyramid": a visual tool for dietary phosphate management in dialysis and CKD patients. *BMC Nephrol*. 16(9): 1-6.

Daliri, E. B. M., Oh, D. H., and Lee, B. H. 2017. Bioactive Peptides. *Foods* 6(5): 32-53.

Day, L., Cakebread, J. A., and Loveday, S. M. 2021. Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends Food Sci.Technol.* 119: 428-442.

De Angelis, D., Kaleda, A., Pasqualone, A., Vaikma, H., Tamm, M., Tammik, M. L., Squeo, G., and Summo, C. 2020. Physicochemical and sensorial evaluation of meat analogues produced from dry-fractionated pea and oat proteins. *Foods* 9(12): 1754.

Dekkers, B. L., Boom, R. M., and van der, G. A. J. 2018. Structuring processes for meat analogues. *Trends Food Sci. Technol.* 81: 25-36.

Delgado, C. L. 2003. Rising consumption of meat and milk in developing countries has created a new food revolution. *J. Nutr.* 133: 3907S-3910S.

Devisetti, R. and Prakash, J. 2020. Comparative assessment of organic and non-organic–chickpea and cowpea, nutritional composition and antinutrients upon germination. *World J. Adv. Res. Rev*. 8(2): 262-270.

Domínguez, R., Bohrer, B., Pateiro, M., Munekata, P. E. S., and Lorenzo, J. M. 2021. Packaging systems. In: Lorenzo, J., Munekata, P., and Barba, F. (eds), *Sustainable Production Technology in Food*. Academic Press, London, pp. 49-69.

Drago, S. R. 2022. Minerals. In: Galanakis, C. (ed.), *Nutraceutical and functional food components*. Academic Press, London, pp. 153-181.

Drulyte, D. and Orlien, V. 2019. The effect of processing on digestion of legume proteins. *Foods* 8(6): 224-233.

Duhan, A., Khetarpaul, N., and Bisnoi, S. 2001. HCL extractability of zinc and copper as affected by soaking, dehulling, cooking and germination of high yielding pigeon pea cultivars. *J. Food. Sci. Technol*. 17(5): 279-304.

Ekunseitan, O. F., Obadina, A. O., Sobukola, O. P., Omemu, A. M., Adegunwa, M. O., and Kajihausa, O. E. 2017. Nutritional composition, functionality and pasting properties of wheat, mushroom and high quality cassava composite flour. *J. Food Process. Preserv.* 41(5): 130-135.

El-Hay, A. M. M. 2022. Processing and preparation of fish. In: Jafari, S. M. (ed.), *Postharvest and Postmortem Processing of Raw Food Materials*. Woodhead Publishing, London, pp. 315-342.

Emin, M. A., Quevedo, M., Wilhelm, M., and Karbstein, H. P. 2017. Analysis of the reaction behavior of highly concentrated plant proteins in extrusion-like conditions. *Innov. Food Sci. Emerg. Technol.* 44: 15-20.

FAO [Food and Agricultural Organization]. 2010. *The State of Food Insecurity in the World, Addressing Food Insecurity in Proctracted Crises.* FAO, Rome, Italy, 120p.

FAO [Food and Agricultural Organization]. 2010. *The State of Food Insecurity in the World, Addressing Food Insecurity in Protracted Crises.* FAO, Rome, Italy, 450p.

FAO [Food and Agricultural Organization]. 2010. *The State of Food Insecurity in the World, Addressing Food Insecurity in Protracted Crises.* FAO, Rome, Italy, 455p.

Fardet, A. 2017. New concepts and paradigms for the protective effects of plant-based food components in relation to food complexity. In: Mariotti, F. (ed.), *Vegetarian and plant-based diets in health and disease prevention*. Academic Press, London, pp. 293-312.

Ferreira, H., Vasconcelos, M., Gil, A. M., and Pinto, E. 2021. Benefits of pulse consumption on metabolism and health: A systematic review of randomized controlled trials. *Crit. Rev. Food Sci. Nutr.* 61(1): 85-96.

Frančáková, H., Líšková, M., Bojňanská, T., and Mareček, J. 2021. Changes of carbohydrates complex influenced by the storage time. *J. Microbiol. Biotechnol. Food Sci*. 1(3): 446-454.

Frazier, W. C. and Westhoff, D. C. 1995. *Food Microbiology*. Tata McGraw-Hill Pub. Co. Ltd., New Delhi, 508p.

Fresan, U., Mejia, M. A., Craig, W. J., Jaceldo-Siegl, K., and Sabate, J. 2019. Meat analogs from different protein sources: a comparison of their sustainability and nutritional content. *Sustain.* 11(12): 3231.

Geada, P., Moreira, C., Silva, M., Nunes, R., Madureira, L., Rocha, C. M., Pereira, R. N., Vicente, A. A., and Teixeira, J. A. 2021. Algal proteins: Production strategies and nutritional and functional properties. *Bioresour. Technol.* 332: 125125p.

Geerts, M. E. J., Dekkers, B. L., van der, P. A., and van der, G. A. J. 2018. Aqueous fractionation processes of soy protein for fibrous structure formation. *Innov. Food Sci. Emerg. Technol.* 45: 313-319.

Ghanghas, N., Mukilan, M. T., Sharma, S., and Prabhakar, P. K. 2022. Classification, composition, extraction, functional modification and application of rice (*Oryza sativa*) seed protein: A comprehensive review. *Food Rev. Int.* 38(4): 354-383.

Gharibzahedi, S. M. T. and Jafari, S. M. 2017. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. *Trends Food Sci. Technol.* 62: 119-132.

Gomez, M. and Martinez, M. M. 2016. Changing flour functionality through physical treatments for the production of gluten-free baking goods. *J. Cereal Sci.* 67: 68-74.

Gondwe, T. M., Alamu, E. O., Mdziniso, P., and Maziya-Dixon, B. 2019. Cowpea (*Vigna unguiculata* (L.) Walp) for food security: an evaluation of end-user traits of improved varieties in Swaziland. *Sci. Rep*. 9(1): 15991.

Grases, F., Prieto, R. M., and Costa-Bauza, A. 2017. Dietary phytate and interactions with mineral nutrients. In: Gutierrez, O. M., Zadeh, K. K., and Mehrothra, R. (eds), *Clinical aspects of natural and added phosphorus in foods.* Springer, New York, pp. 175-183.

Hagenimana, A., Ding, X., and Fang, T. 2016. Evaluation of rice flour modified by extrusion cooking. *J. Cereal Sci.* 43: 38-46.

Hagstrum, D. W. and Phillips, T. W. 2017. Evolution of stored-product entomology: protecting the world food supply. *Annu. Rev. Entomol.* 62: 379-397.

Harvey, F. and Phillips, D., 2020. A fifth of Brazilian soy in Europe is result of deforestation. *THE GUARDIAN*. https://www.Theguardian. com/environment/2020/jul/16/afifth–of-brazilian-soy-in-europe-is-result-of-deforestation-amazon-jair-bolsonaro.

Havlik, J., Vladimir, P., Javier, F., and Vojtech, R. 2010. Dietary purines in vegetarian meat analogues. *J. Sci. Food Agric.* 90: 2352-2357.

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., and Tiwari, B. 2017. Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods* 6(7): 53.

Hernandez-Ledesma, B., del Mar, C. M., and Recio, I. 2011. Antihypertensive peptides: Production, bioavailability and incorporation into foods. *Adv. Colloid Interface Sci*. 165(1): 23-35.

Hirdyani, H. 2014. Nutritional composition of Chickpea (Cicerarietinum-L) and value added products -a review. *Indian J. Comm. Health* 26(2): 102-106.

Hoppe, C, Molgaard, C., and Michaelsen, K. F. 2006. Cow's milk and linear growth in industrialized and developing countries. *Annu. Rev. Nutr.*26: 131-173.

Howse, G., Sidhu, K., and Grex, D. 2015. Methods of Preparing Meat Analogues, Meat Analogues, and Foodstuffs Comprising Meat Analogues. United States Patent No. 20050008758A1. Available: https://patents.google.com/patent/US20050008758A1/en [20 Dec. 2022].

Huang, S. and Bohrer, B. M. 2020. The effect of tropical flours (breadfruit and banana) on structural and technological properties of beef emulsion modeling systems. *Meat Sci.* 163: 108082.

Huang, S., Roman, L., Martinez, M. M., and Bohrer, B. M. 2020. Modification of physicochemical properties of breadfruit flour using different twin-screw extrusion conditions and its application in soy protein gels. *Foods* 9(8):1071.

Hurrell, R. and Egli, I. 2006. Iron bioavailability and dietary reference values. *Am. J. Clin. Nutr.* 91: 1461S-1467S.

IARC [International Agency for Research on Cancer]. 2015. *Q and A on the carcinogenicity of the consumption of red meat and 640 processed meat*. International Agency for Research on Cancer, World Health Organization, Geneva, 641p.

IFIF [International Feed Industry Federation]. 2009. *Annual Report 2008-2009*. International Feed Industry Federation, Wiehl, Germany, 186p.

IIPS. and ICF. 2022. *India National Family Health Survey (NFHS-5) 2019-21*. https://www.dhsprogram.com/pubs/pdf/FR374/FR374.pdf

Ismail, B. P., Senaratne-Lenagala, L., Stube, A., and Brackenridge, A. 2020. Protein demand: Review of plant and animal proteins used in alternative protein product development and production. *Anim. Front.* 10(4): 53-63.

Jackson, M. L. 1973. *Soil chemical Analysis*. Prentice Hall of India, New Delhi, 299p.

Jayathilake, C., Visvanathan, R., Deen, A., Bangamuwage, R., Jayawardana, B. C., Nammi, S., and Liyanage, R. 2018. Cowpea: an overview on its nutritional facts and health benefits. *J. Sci. Food Agric.* 98(13): 4793-4806.

Jellinek, G. 1985. *Sensory Evaluation of Food: Theory and Practice*. Ellis Horwood, Chichester, England, 596p.

Jones, A. M. P., Ragone, D., Bernotas, D. W., and Murch, S. J. 2011. Beyond the Bounty: Breadfruit (*Artocarpus altilis*) for food security and novel foods in the 21st Century. *Ethnobot. Res. Appl.* 9: 129-149.

Joshi, V. K. and Kumar, S. 2015. Meat analogues: Plant based alternatives to meat products – A review. *Intl. J. Food. Ferment. Technol.* 5(2): 107-119.

Juturu, V. 2014. Polyphenols and cardiometabolic syndrome. In: Watson, R. R., Zibadi, S., and Preedy, V. R. (eds), *Polyphenols in human health and disease*. Academic Press, London. pp. 1067-1076.

Kang, S. W., Rahman, M. S., Kim, A. N., Lee, K. Y., Park, C. Y., Kerr, W. L., and Choi, S. G. 2017. Comparative study of the quality characteristics of defatted soy flour treated by supercritical carbon dioxide and organic solvent. *J. Food Sci. Technol.* 54(8): 2485-2493.

Khan, A.U., Ema, I. J., Faruk, M., Tarapder, S. A., Khan, A. U., Noreen, S., and Adnan, M. 2021. A review on importance of Artocarpus heterophyllus L. (Jackfruit). *J. Multidiscip. Appl. Nat. Sci*. [e-journal]. Available at SSRN: https://ssrn.com/abstract=3897229 [02 Sept. 2021].

Klunder, H. C., Wolkers-Rooijackers, J., Korpela, J. M., and Nout, M. R. 2012. Microbiological aspects of processing and storage of edible insects. *Food Control* 26(2): 628-631.

Kolodziejczak, K., Onopiuk, A., Szpicer, A., and Poltorak, A. 2021. Meat analogues in the perspective of recent scientific research: A review. *Foods* 11(1): 105-116.

Kregiel, D., Berlowska, J., Witonska, I., Antolak, H., Proestos, C., and Babic, M. 2017. Saponin-based, biological-active surfactants from plants. In: Najjar, R. (ed.), *Application and characterization of surfactants*. Intech publishers, Croatia, pp. 183-205.

Krintiras, G. A., Gobel, J., Van Der, G. A. J., and Stefanidis, G. D. 2015. Production of structured soy-based meat analogues using simple shear and heat in a Couette Cell. *J. Food Eng.* 160: 34-41.

Kumar, K. 2014. Cold Preservation and Processing. In: Mudgil, D. and Mudgil, S. (eds), *Food Processing and Preservation*. Scientific Publisher, Jodhpur, Rajasthan, pp. 23-35.

Kumar, P., Chatli, M. K., Mehta, N., Singh, P., Malav, O. P., and Verma, A. K. 2017. Meat analogues: Health promising sustainable meat substitutes. *Crit. Rev. Food Sci Nutr.* 57(5): 923-932.

Kumar, R. 2017. *Insect pests on stored grain: Biology, behavior, and management strategies. AAP, Waretown.* Apple Academic Press, Canada, 412p.

Kuong, K., Laillou, A., Chea, C., Chamnan, C., Berger, J., and Wieringa, F. T. 2016. Stability of Vitamin A, iron and zinc in fortified rice during storage and its impact on future national standards and programs-Case study in Cambodia. *Nutr.* 8(1): 51.

Kyriakopoulou, K., Dekkers, B., and Van der G. A. J. 2019. *Sustainable meat production and processing.* Academic Press, Pittsburgh, 126p.

Kyriakopoulou, K., Keppler, J. K., and Van der G. A. J. 2021. Functionality of ingredients and additives in plant based meat analogues. *Foods* 10(3): 600.

Lakshmy, P. S. 2011. Standardisation and quality evaluation of 'tempeh' and tempeh based instant soup mixed. Ph.D. (Home Sci.) thesis, Kerala Agricultural University, Thrissur, 208p.

Langelaan, M. L. P., Boonen, K. J. M., Polak, R. B., Baaijens, F. P. T., Post, M. J., and van der, S. D. W. J. 2010. Meet the new meat: Tissue engineered skeletal muscle. *Trends Food Sci. Technol.* 21: 59-66.

Lawrence, T. E. and Kropf, D. H. 2018. *Vacuum Packaging of Meat*.

Lazou, A. E., Michailidis, P. A., Thymi, S., Krokida, M. K., and Bisharat, G. I. 2007. Structural Properties of Corn-Legume Based Extrudates as a Function of Processing Conditions and Raw Material Characteristics. *Int. J. Food Prop.* 10: 721-738.

Lee, H. J., Yong, H. I., Kim, M., Choi, Y. S., and Jo, C. 2020. Status of meat alternatives and their potential role in the future meat market - A review. *Asian-australas. J. Anim. Sci*. 33(10): 1533.

Li, S. S., Blanco-Mejia, S., Lytvyn, L., Stewart, S. E., Viguiliouk, E., Ha, V., de Souza, R. J., Leiter, L. A., Kendall, C. W. C., and Jenkins, D. J. A. 2017. Effect of plant protein on blood lipids: A systematic review and meta-analysis of randomized controlled trials. *J. Am. Heart Assoc.* 6(12): e006659.

Li, T., Guo, X. N., Zhu, K. X., and Zhou, H. M. 2018. Effects of alkali on protein polymerization and textural characteristics of textured wheat protein. *Food Chem.* 239: 579-587.

Lin, Y., Mouratidou, T., Vereecken, C., Kersting, M., Bolca, S., de Moraes, A. C. F., Cuenca-Garcia, M., Moreno, L. A., Gonzalez-Gross, M., and Valtuena, J. 2015. Dietary animal and plant protein intakes and their associations with obesity and cardio-metabolic indicators in European adolescents: The HELENA cross-sectional study. *Nutr. J.* 14(10): 14-10.

Liu, Y., Liu, K., and Zhao, Y. 2022. Effect of storage conditions on the protein composition and structure of peanuts. *ACS Omega* 7(25): 21694-21700.

Longvah, T., Anantan, I., Bhaskarachary, K., Venkaiah, K., and Longvah, T. 2017. *Indian food composition tables*. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, 58p.

Lopez, D. N., Galante, M., Robson, M., Boeris, V., and Spelzini, D. 2018. Amaranth, quinoa and chia protein isolates: Physicochemical and structural properties. *Int. J. Biol. Macromol.* 109: 152-159.

Ma, H., Pan, Z., Li, B., Atungulu, G. G., Olson, D. A., Wall, M. M., and McHugh, T. H. 2012. Properties of extruded expandable breadfruit products. *LWT Food Sci. Technol*. 46(1): 326-334.

Maheswarappa, N. B., Mohan, K., and Jagadeesh, D. S. 2016. *Meat Products Packaging.*

Malaguti, M., Dinelli, G., Leoncini, E., Bregola, V., Bosi, S., Cicero, A. F., and Hrelia, S. 2014. Bioactive peptides in cereals and legumes: agronomical, biochemical and clinical aspects. *Int. J. Mol. Sci.* 15(11): 21120-21135.

Malav, O. P., Talukder, S., Gokulakrishnan, P., and Chand, S. 2015. Meat analog: A review. *Crit. Rev. Food Sci. Nutr*. 55(9): 1241-1245.

Malganji, S., Sohrabvandi, S., Jahadi, M., Nematollahi, A., and Sarmadi, B. 2016. Effect of refrigerated storage on sensory properties and viability of probiotic in grape drink. *Appl. Food Biotechnol.* 3(1): 59-62.

Malik, M. A. and Saini, C. S. 2018. Improvement of functional properties of sunflower protein isolates near isoelectric point: Application of heat treatment. *LWT* Food *Technol.* 98: 411-417.

Man, M. D. and Jones, A. 2000. *Shelf Life Evaluation of Foods*. Aspen Publishers, New York, 264p.

Manfredi, M. J., Brandi, E., Conte, P., Pidutti. F., Gosetti, E., Robotti, E., Marengo, E., and Cecconi. D. 2017. IEF peptide fractionation method combined to shotgun

proteomics enhances the exploration of rice milk proteome. *Anal. Biochem*. 537: 72-77.

Maphosa, Y. and Jideani, V. A. 2017. The role of legumes in human nutrition. In: Hueda, M. C. (ed.), *Functional Food-Improve Health through Adequate Food*. IntechOpen Ltd., London, pp. 103-120.

Maray, A. R. M., Mostafa, K. M., and El-Fakhrany, A. M. A. 2018. Effect of pretreatments and drying methods on physico-chemical, sensory characteristics and nutritional value of oyster mushroom. *J. Food Process. Preserv*. 42(1): e13352.

Marinangeli, C. P., Curran, J., Barr, S. I., Slavin, J., Puri, S., Swaminathan, S., Tapsell, L., and Patterson, C. A. 2017. Enhancing nutrition with pulses: defining a recommended serving size for adults. *Nutr. Rev*. 75(12): 990-1006.

Mashabela, M. N., Ndhlovu, P. T., and Mbeng, W. O. 2022. Herbs and spices antimicrobial properties and possible use in the food sector. In: Ivanisova, E. (ed.), *Herbal and spices-New advances.* Intechopen, London, pp. 96-100.

Maurya, N. K. and Kushwaha, R. 2019. Novel Protein Foods: Alternative Sources of Protein for Human Consumption. In: Verma, D. (ed.), *Research trends in food technology and nutrition*. AkiNik Publications, New Delhi, pp. 129-142.

McClements, D. J., Weiss, J., Kinchla, A. J., Nolden, A. A., and Grossmann, L. 2021. Methods for testing the quality attributes of plant-based foods: Meat-and processed-meat analogs. *Foods* 10(2): 260.

Mishra, K. K., Pal, R. S., Kumar, R. A., Chandrashekara, C., Jain, S. K., and Bhatt, J. C. 2013. Antioxidant properties of different edible mushroom species and

increased bioconversion efficiency of Pleurotuseryngii using locally available casing materials. *Food Chem.* 138: 1557-1563.

Mogensen, L., Heusale, H., Sinkko, T., Poutanen, K., Sozer, N., Hermansen, J. E., and Knudsen, M. T. 2020. Potential to reduce GHG emissions and land use by substituting animal-based proteins by foods containing oat protein concentrate. *J. Clean. Prod.* 274: 122914.

Molu, K. R. 2018. Standardisation and quality evaluation of nutri spreads. M.Sc. (Community Sci.) thesis, Kerala Agricultural University, Thrissur, 102p.

Mustafa, K. D. and Adem, E. 2014. Comparison of autoclave, microwave, IR and UV-stabilization of whole wheat flour branny fractions upon the nutritional properties of whole wheat bread. *J. Food Sci. Technol.* 51(1): 59-66.

Nadarajah, S. and Thevaki, M. 2015. Influence of storage conditions on the quality characteristics of wheat-defatted coconut flour biscuits packed in metalized polypropylene. *Int. J. Eng. Res. Technol.* 4(7): 948-951.

Nansereko, S., Muyonga, J., and Byaruhanga, Y. B. 2022. Optimization of drying conditions for jackfruit pulp using Refractance Window Drying technology. *Food Sci. Nutr.* 10(5): 1333-1343.

Nisha. 2008. Standardisation of green gram based meat analogues. M. Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 115p.

Odoemelam, S. A. 2005. Functional properties of raw and heat processed jackfruit (*Artocarpus heterophyllus*) flour. *Pak. J. Nutr*. 4(6): 366-370.

Onwezen, M. C., Bouwman, E. P., Reinders, M. J., and Dagevos, H. 2021. A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. *Appetite* 159: 105058.

Page, A. L., Miller, R. H., and Keeney, D. R. 1982. *Methods of soil analysis. Part 2. Chemical and Microbiological properties*. American Society of Agronomy, Soil Science Society of America, America, 1159p.

Pal, A., Kamthania, M. C., and Kumar, A. 2014. Bioactive compounds and properties of seaweeds-A Review. *OALib* 1: 1-17.

Pan, A., Sun, Q., Bernstein, A. M., Schulze, M. B., Manson, J. E., Willett, W. C., and Hu, F. B. 2011. Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *Am. J. Clin. Nutr*. 94(4): 1088-1096.

Pandey, S. and Ukkuru, M. 2004. Value added products and by products from jack fruit, Ph. D. (FS&N) thesis, Kerala Agricultural University, Vellayani, Kerala, 308p.

Pandey, K. B. and Rizvi, S. I. 2009. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxid. Med. Cell. Longev.* 2(5): 270-278.

Panghal, A., Khatkr, B. S., and Singh, U. 2001. Cereal proteins and their role in food industry. *Indian. Food. Ind*. 25(5): 58-62.

Peng, Y., Kyriakopoulou, K., Rahmani, A., Venema, P., and van der G. A. J. 2021. Isochoric moisture heating as a tool to control the functionality of soy protein. *LWT Food Technol*. 150: 111979.

Perez-Santaescolastica, C., Munekata, P. E. S., Pateiro, M., Domínguez, R., Misihairabgwi, J. M., and Lorenzo, J. M. 2021. Modern Food Production: Fundaments, Sustainability, and the Role of Technological Advances. *Sustain. Prod. Technol. Food*. 5: 1-22.

Perkin-Elmer. 1982. *Analytical Methods for Atomic Absorption Spectrophotometry.* Perkin-Elmer Corporation, USA, 114p.

Peters, J. P. C. M., Vergeldt, F. J., Boom, R. M., and van der G. A. J. 2017. Water-binding capacity of protein-rich particles and their pellets. *Food Hydrocoll*. 65: 144-156.

Philip, T. E. 1993. *Modern Cookery for Teaching Trade.* Novena Publishers, Chennai, 567p.

Pillai, K. C. 2001. Shelf life of breadfruit (*Artocarpus altilis* (Park) Fosberg.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Thrissur, 85p.

Piskov, S., Timchenko, L., Grimm, W. D., Rzhepakovsky, I., Avanesyan, S., Sizonenko, M., and Kurchenko, V. 2020. Effects of various drying methods on some physico-chemical properties and the antioxidant profile and ACE inhibition activity of oyster mushrooms (*Pleurotus ostreatus*). *Foods* 9(2): 160.

Popkin, B. M., Adair, L. S., and Ng, S. W. 2012. Review: Global nutrition transition and the pandemic of obesity in developing countries. *Nutr. Rev.* 70(1): 3-21.

Popkin, B. M., Adair, L. S., and Nq, S. W. 2012. Global Nutrition and the pandemic of obesity in developing countries. *Nutr. Rev.* 70(3): 21.

Pragya, S. and Rita, S. R. 2012. Finger millet for food and nutritional security. *Afr. J. Food. Sci*. 6(4): 77-84.

Qamar, S., Manrique, Y. J., Parekh, H., and Falconer, J. R. 2020. Nuts, cereals, seeds and legumes proteins derived emulsifiers as a source of plant protein beverages: A review. *Crit. Rev. Food. Sci. Nutr.* 60(16): 2742-2762.

Raes, K., Knockaert, D., Struijs, K., and Van Camp, J. 2014. Role of processing on bioaccessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. *Trends Food Sci Technol*. 37(1): 32-41.

Raghuramulu, N., Nair, K. M., and Kalyanasundaram, S. 2003. *A Manual of Laboratory Techniques*. National institute of Nutrition, Hyderabad, 421p.

Ragone, D. 2014. *Breadfruit Nutritional Value and Versatility*. Breadfruit Institute of the National Tropical Botanical Garden, Hawaii, USA, 2p.

Rahman, A., Rehman, M., Gadahi, J. A., Samo, M. T., Bhutto, B., Ullah, Q., and Khaskeli, M. 2012. Studies on the evaluation of moisture and ash content in Kamori, Pateri and Tapri goat meat. *Int. J. Agro Vet. Med. Sci.* 6(1): 62-68.

Rana, S. S., Pradhan, R. C., and Mishra, S. 2019. Image analysis to quantify the browning in fresh cut tender jackfruit slices. *Food Chem.* 278: 185-189.

Rashidi, A. M. and Yang, T. A. 2016. Nutritional and antioxidant values of oyster mushroom (P. sajor-caju) cultivated on rubber sawdust. *Int. J. Adv. Sci. Eng. Inf. Technol.* 6(2): 161-164.

Riaz, M. N. 2015. Processing, characteristics and uses of extruded plant protein ingredients. *Protein Trends Technol Semin*, Oak Brook, Illinois, USA, 1(7):12–16.

Ritchie, H. and Roser, M. 2020. Environmental impacts of food production [on-line]. Available: https://ourworldindata.org/environmental-impacts-of-food [20 Nov. 2020].

Rohrmann, S., Overvad, K., Bueno-de-Mesquita, H. B., Jakobsen, M. U., Egeberg, R., Tjonneland, A., Nailler, L., Boutron-Ruault, M. C., Clavel-Chapelon, F., Krogh, V., and Palli, D. 2013. Meat consumption and mortality-results from the European prospective investigation into cancer and nutrition. *BMC Med.* 11(1): 1-12.

Roman, L., Reguilon, M. P., and Gomez, M. 2018. Physicochemical characteristics of sauce model systems: Influence of particle size and extruded flour source. *J. Food Eng.* 219: 93-100.

Rousseau, S., Kyomugasho, C., Celus, M., Hendrickx, M. E., and Grauwet, T. 2020. Barriers impairing mineral bioaccessibility and bioavailability in plant-based foods and the perspectives for food processing. *Crit. Rev. Food Sci. Nutr.* 60(5): 826-843.

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods for Agricultural Sciences* (8th Ed.). Wiley Eastern Ltd., New Delhi and Tamil Nadu Agricultural University, Coimbatore, 422p.

Samard, S. and Ryu, G. H. 2019. Physicochemical and functional characteristics of plant protein-based meat analogs. *J. Food Process. Preserv.* 43(10): 1-11.

Sampels, S. 2014. Towards a more sustainable production of fish as an important source for human nutrition. *J. Fish. Livest. Prod.* 2(2): 119.

Samtiya, M., Aluko, R. E., and Dhewa, T. 2020. Plant food anti-nutritional factors and their reduction strategies: an overview. *Food. Prod. Process. Nutr.* 2: 6.

Sanchez, A. and Vazquez, A. 2017. Bioactive peptides: A review. *Food Qual. Saf.* 1: 29-46.

Sandberg, A. S. 2002. Bioavailability of minerals in legumes. *Br. J. Nutr.* 88(3): 281-285.

Sasimaporn, S. and Gi-Hyung, R. 2019. Physicochemical and functional characteristics of plant protein-based meat analogs. *J. Food Process. Pres.* 10: 43-45.

Satterlee, L. D., Marshall, H. F., and Tennyson, J. M. 1979. Measuring protein quality. *J. Am. Soc. Cereal Chemist*. 56(3): 103-109.

Savage, G. P. and Martensson, L. 2010. Comparison of the estimates of the oxalate content of taro leaves and corms and a selection of Indian vegetables following hot water, hot acid and *in vitro* extraction methods. *J. Food Compos. Anal.* 23(1): 113-117.

Sebastian, K., Joseph, M., Gomez, P. S., and Sheela, K. B. 2021. Meat analogue: The vegetable meat-A review. *Indian Food. Ind.* 3(6): 52-60.

Shaghaghian, S., McClements, D. J., Khalesi, M., Garcia-Vaquero, M., and Mirzapour-Kouhdasht, A. 2022. Digestibility and bioavailability of plant-based proteins intended for use in meat analogues: A review. *Trends Food Sci. Technol.* 129: 646-656.

Shah, W. H. 2001. Tannin contents and protein digestibility of black grams (*Vigna mungo*) after soaking and cooking. *Plant Foods Hum. Nutr*. 56(3): 265-273.

Shurtleff, W. and Aoyagi, A. 2014. *History of meat alternatives (965 CE to 2014): Extensively annotated bibliography and sourcebook*. Soyinfo Center, USA, 1437p.

Singh, M., Trivedi, N., Enamala, M. K., Kuppam, C., Parikh, P., Nikolova, M. P., and Chavali, M. 2021. Plant-based meat analogue (PBMA) as a sustainable food: A concise review. *Eur. Food Res. Technol*. 247(10): 2499-2526.

Singh, V. P., Mendiritta, S. K., Agharwal, Sanyan, M. K., and Dube, P. C. 2009. Storage stability of aerobically packaged chicken snacks at ambient temperature. *J. Vet. Public Health* 7: 45-51.

Singhal, A., Karaca, A. C., Tyler, R., and Nickerson, M. 2016. Pulse proteins: from processing to structure-function relationships. In: Goyal, A. K. (ed.), *Grain legumes*. Intech publishers, Croatia, pp. 55-78.

Sirangelo, T. M. 2019. Sensory descriptive evaluation of food products: a review. *J. Food Sci. Nutr. Res*. 2(4): 354-363.

Soifoini, T., Donno, D., Jeannoda, V., Rakoto, D. D., Msahazi, A., Farhat, S. M. M., Oulam, M. Z., and Beccaro, G. L. 2021. Phytochemical composition, antibacterial activity, and antioxidant properties of the *Artocarpus altilis* fruits to promote their consumption in the comoros islands as potential health-promoting food or a source of bioactive molecules for the food industry. *Foods* 10(9): 2136.

Springmann, M., Godfray, H. C. J., Rayner, M., and Scarborough, P. 2016. Analysis and valuation of the health and climate change co-benefits of dietary change. *Proc. Natl. Acad. Sci*. 113(15): 4146-4151.

Srivastava, R. and Singh, A. J. 2020. Biggest fruit with high nutritional and pharmacological values: A review. *Int. J. Curr. Microbiol. App. Sci*. 9(8): 764-774.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., and de Haan, C. 2006. *Livestock's Long Shadow: Environmental issues and options*. Food and Agriculture Organisation, Rome. 377p.

Stiefel, G., Anagnostou, K., and Boyle, R. J. 2017. BSACI guideline for the diagnosis and management of peanut and tree nut allergy. *Clin. Exp. Allergy* 47: 719-739.

Sulieman, A. M. E., Ali, R. A. M., and Razig, K. A. A. 2012. Production a nd effect of storage in the chemical composition of Mozzarella cheese. *Int. J. Food Sci. Nutr. Eng.* 2(3): 21-26.

Sultana, A., Parvin, R., Alam, M. K., Akter, F., and Alim, M. A., 2015. Physico-chemical, functional properties and storage characteristics of jackfruit seed flour. *Bangladesh J. Vet. Anim. Sci.* 3(1): 20-25.

Sun, C., Ge, J., He, J., Gan, R., and Fang, Y. 2021. Processing, quality, safety, and acceptance of meat analogue products. *Eng.* 7(5): 674-678.

Swami B. S., Thakor N. J., Haldankar P. M., and Kalse S. B. 2012. Jackfruit and its many functional components as related to human health: A review. *Compr. Rev. Food Sci. Food Saf.* 11(6): 565-576.

Tan, M., Nawaz, M. A., and Buckow, R. 2021. Functional and food application of plant proteins-a review. *Food. Rev. Int.* 1-29.

Tasic, A., Kureljusic, J., Nesic, K., Rokvic, N., Vicentijevic, M., Radovic, M., and Pisinov, B. 2017. Determination of calcium content in mechanically separated meat. *IOP Conf. Ser. Earth Environ. Sci*. 85(1): 012056.

Teagasc Agriculture and Food Development Authority. 2016. *Teagasc Technology Foresight: Technology Transforming Irish Agri-Food and Bio-Economy*; Teagasc Agriculture and Food Development Authority: Carlow, Ireland.

Terriente-Palacios, C. and Castellari, M. 2022. Levels of taurine, hypotaurine and homotaurine, and amino acids profiles in selected commercial seaweeds, microalgae, and algae-enriched food products. *Food Chem.* 368: 130770.

Tolera, K. D. and Abera, S. 2017. Nutritional quality of Oyster Mushroom (*Pleurotus ostreatus*) as affected by osmotic pretreatments and drying methods. *Food Sci. Nutr.* 5(5): 989-996.

Tome, D. 2013. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements-functional aspects. *Food Nutr. Bull.* 34(2): 272-274.

Torres, J., Rutherfurd, S. M., Munoz, L. S., Peters, M., and Montoya, C. A. 2016. The impact of heating and soaking on the *in vitro* enzymatic hydrolysis of protein varies in different species of tropical legumes. *Food Chem.* 194: 377-382.

Toth, A. J., Dunay, A., Battay, M., Illes, C. B., Bittsanszky, A., and Suth, M. 2021. Microbial spoilage of plant-based meat analogues. *Appl. Sci.* 11(18): 8309-8319.

Vagadia, B. H., Vanga, S. K., and Raghavan, V. 2017. Inactivation methods of soybean trypsin inhibitor-A review. *Trends Food Sci. Technol.* 64: 115-125.

van Kernebeek, H. R., Oosting, S. J., van Ittersum, M. K., Bikker, P., and de Boer, I. J. 2016. Saving land to feed a growing population: consequences for consumption of crop and livestock products. *Int. J. Life Cycle Assess*. 21(5): 677-687.

Van Kernebeek, M. J., Oosting, S. J., Van Ittersum, M. K., Bikker, P., and De Boer, I. J. M. 2016. Saving land to feed a growing population: Consequences for consumption of crop and livestock products. *Int. J. Life Cycle Assess.* 21: 677-687.

Veldhorst, M., Smeets, A. J. P. G., Soenen, S., Hochstenbach-Waelen, A., Hursel, R., Diepvens, K., Lejeune, M., Luscombe-Marsh, N., and Westerterp-Plantenga, M. 2008. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol. Behav.* 94(2): 300-307.

Veldhuizen, L. J., Giller, K. E., Oosterveer, P., Brouwer, I. D., Janssen, S., van Zanten, H. H., and Slingerland, M. A. 2020. The missing middle: Connected action on agriculture and nutrition across global, national and local levels to achieve sustainable development goal 2. *Glob. Food Sec.* 24: 100336.

Wallace, T. C., Murray, R., and Zelman, K. M. 2016. The nutritional value and health benefits of chickpeas and hummus. *Nutr.* 8(12): 766.

Walther, B. and Sieber, R. 2011. Bioactive proteins and peptides in foods. *Int. J. Vitam. Nutr. Res.* 81(2): 181-196.

Wang, S., Li, C., Copeland, L., Niu, Q., and Wang, S. 2005. Starch retrogradation: A comprehensive review. *Compr. Rev. Food Sci. Food Saf.* 14: 568-585.

Wang, X. M., Zhang, J., Wu, L. H., Zhao, Y. L., Li, T., Li, J. Q., Wang, Y. Z., and Liu, H. G. 2014. A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. *Food Chem.* 151: 279-285.

Wang, Y., Tuccillo, F., Lampi, A. M., Knaapila, A., Pulkkinen, M., Kariluoto, S., Coda, R., Edelmann, M., Jouppila, K., Sandell, M., and Piironen, V. 2022. Flavor challenges in extruded plant-based meat alternatives: A review. *Compr. Rev. Food Sci. Food Saf.* 21(3): 2898-2929.

Warnakulasuriya, S. N. and Nickerson, M. T. 2018. Review on plant protein-polysaccharide complex coacervation, and the functionality and applicability of formed complexes. *J. Sci. Food Agric.* 98: 5559-5571.

Williams, P. 2007. Nutritional composition of red meat. *Nutr. Diet.* 64(4): S113-S119.

Wolk, A. 2017. Potential health hazards of eating red meat. *J. Intern Med.* 281(2): 106-122.

Wouters, A. G. B., Rombouts, I., Fierens, E., Brijs, K., and Delcour, J. A. 2016. Relevance of the Functional Properties of Enzymatic Plant Protein Hydrolysates in Food Systems. *Compr. Rev. Food Sci. Food Saf.* 15: 786-800.

WRAP Food Futures: From Business as Usual to Business Unusual. [(accessed on 12 March 2017)]; Available online: http://www.wrap.org.uk/content/food-futures

Wu, G. 2016. Dietary protein intake and human health. *Food Funct.* 7(3): 1251-1265.

Xing, Q., de Wit, M., Kyriakopoulou, K., Boom, R. M., and Schutyser, M. A. I. 2018. Protein enrichment of defatted soybean flour by fine milling and electrostatic separation. *Innov. Food Sci. Emerg. Technol.* 50: 42-49.

Yusnita, H., Revathi, S., Nor, H. I., and Suhana, M. H., 2019. Effects of cross linking on breadfruit starch and its batter properties. *Asian J. Agric. Biol.* 7(2): 234-243.

Zahra, S. M., Hussain, S., Mahmood, S., Kausar, T., Din, G. M. U., Tufail, T., Altaf, N., Yousaf, M., Quddoos, A. R., and Shahid, M. Z., 2020. Shelf stable iron fortified fruit bar's development, proximate estimation and organoleptic characterization. *Int. J. Biosci*. 16(4): 111-135.

Zhang, J., Liu, L., Liu, H., Yoon, A., Rizvi, S. S. H., and Wang, Q. 2019. Changes in conformation and quality of vegetable protein during texturization process by extrusion. *Crit. Rev. Food Sci. Nutr*. 59: 3267-3280.

Zhang, J., Kang, D., Zhang, W., and Lorenzo, J. M. 2021a. Recent advantage of interactions of protein-flavor in foods: perspective of theoretical models, protein properties and extrinsic factors. *Trends Food Sci. Technol.* 111: 405-425.

Zhang, X., Chen, Y., Zhang, R., Zhong, Y., Luo, Y., Xu, S., Liu, J., Xue, J., and Guo, D. 2016. Effects of extrusion treatment on physicochemical properties and *in vitro* digestion of pregelatinized high amylose maize flour. *J. Cereal Sci.* 68: 108-115.

Zhang, X., Zhao, Y., Zhang, T., Zhang, Y., Jiang, L., and Sui, X. 2022. High moisture extrusion of soy protein and wheat gluten blend: An underlying mechanism for the formation of fibrous structures. *LWT Food. Sci. Technol*. 163: 113561.

Zhu, L., Yin, P., Xie, T., Liu, X., Yang, L., Wang, S., Li, J., and Liu, H. 2020. Interaction between soyasaponin and soy β-conglycinin or glycinin: Air-water interfacial behavior and foaming property of their mixtures. *Colloids Surf. Biointerfaces* 186: 110707.

STANDARDISATION OF JACKFRUIT AND BREADFRUIT INCORPORATED MEAT ANALOGUES

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ABSTRACT OF THE THESIS

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ABSTRACT

In the study entitled "Standardisation of jackfruit and breadfruit incorporated meat analogues", meat analogues using cowpea (CWP), chickpea (CP), tender jackfruit (TJ), breadfruit (BF), wheat gluten (WG), defatted soy flour (DSF) and oyster mushroom flour (OMF) were formulated, developed and standardised.

Tender jackfruit and breadfruit incorporated cowpea and chickpea meat analogues were developed in varied combinations of pulse, tender jackfruit or breadfruit and wheat gluten. Defatted soy flour (DSF) and oyster mushroom flour (OMF) were added (5 %) to all the treatments except in controls (T_0 - 100 % CWP and T_6 - 100 % CP).

The developed fresh meat analogue cubes were soft, springy with distinctive texture which in cut surface showed defined interconnected inner framework of its ingredients.

Organoleptic evaluation was conducted in which sensory attributes of tender jackfruit and breadfruit incorporated cowpea and chickpea meat analogues were evaluated. From both tender jackfruit and breadfruit incorporated cowpea and chickpea meat analogues, the treatments T_5 (40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF) and T_{10} (50 % CP + 20 % TJ + 20 % WG + 5 % DSF + 5 % OMF); T_4 (50 % CWP + 20 % BF + 20 % WG + 5 % DSF + 5 % OMF) and T_{11} (40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF)were selected respectively as best treatments based on their organoleptic evaluation scores.

The selected treatments along with their controls were evaluated for their quality attributes such as nutritional studies, *in vitro* investigations and shelf life studies. The range of moisture content in the meat analogues was from 9.25 to 10.62 per cent. Meat analogues of this study were observed to contain total carbohydrate,

protein, total fat, total ash and fibre content in the range of 32.46 to 53.29, 20.79 to 38.03, 1.06 to 1.92, 2.92 to 5.55 and 2.23 to 7.30 g 100^{-1} respectively.

Proximate analysis also showed that meat analogues were abundant in minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc whose contents ranged from 80.25 to 94.67, 255.62 to 325.46, 23.52 to 74.43, 510.49 to 631.50, 103.64 to 181.69, 4.17 to 5.73 and 3.1 to 3.96 mg $100g^{-1}$ respectively.

The protein *in vitro* digestibility of meat analogues was in the range of 62.12 to 80.30 per cent. High *in vitro* protein and mineral availability in most of the treatments was observed in tender jackfruit and breadfruit incorporated meat analogues compared to the controls. The *in vitro* availability of minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc of the selected treatments and the controls was in the range of 34.43 to 87.62, 47.62 to 71.43, 57.66 to 77.20, 62.54 to 82.85, 54.40 to 63.73, 52.40 to 73.32, 55.89 to 64.28 per cent respectively.

The selected meat analogues and the controls were packed in food grade HDPE covers (250 gauge) and were stored at both ambient and refrigerated temperature for a period of three months. Organoleptic evaluation of meat analogues at monthly intervals showed that meat analogues stored under refrigerated condition showed better sensory qualities than their ambient stored counterparts throughout storage.

The meat analogues stored at ambient and refrigerated storage were analysed for their nutritional and *in vitro* aspects. In both the storage conditions, an increase in the moisture content was observed on storage. In both the storage conditions, a general change in the total carbohydrate content, protein, total fat, total ash, fibre and minerals was observed. However, this change was less in most of the treatments under refrigerated storage when compared to ambient storage.

Protein *in vitro* digestibility and *in vitro* availability of minerals such as calcium, phosphorous, sodium, potassium, magnesium, iron and zinc showed a general change in all the treatments. Meat analogues stored in refrigerated condition also showed a change in their protein *in vitro* digestibility and *in vitro* mineral availability after storage but this change difference was not significant in most of the meat analogues.

A gradual increase in microbial count was detected on storage but the increase was very meager. However, the respective viable counts were lower in treatments of meat analogues stored under refrigerated condition against ambient condition. During storage of three months, insect infestation was not observed in any of the different treatments of meat analogues stored in both ambient and refrigerated condition.

In the present study, among the organoleptically selected the treatments T_5 (40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF) and T_{11} (40 % CP + 25 % BF + 25 %WG + 5 % DSF +5 % OMF) were nutritionally superior with higher protein and fibre content and therefore are considered the best treatments from tender jackfruit and breadfruit incorporated meat analogues respectively.

The cost of production of the selected meat analogues and their controls ranged from Rs. 34.00 to 60.00/ 100g in the selected treatments of meat analogues.

The current research found that meat analogues with improved organoleptic properties, nutritional profiles, and shelf stability could be developed using indigenous plant foods such as tender jackfruit and breadfruit, which could be a better, healthier, more cost effective and versatile alternative to other commercial meat analogues and meat.

സംക്ഷിപ്തം

ചക്കയും കടച്ചക്കയും ഉൾപ്പെടുത്തിയ മീറ്റ് അനലോഗിന്റെ ഗുണനിലവാരം വിലയിരുത്തൽ എന്ന പഠനത്തിൽ വൻപയർ, വെള്ളക്കടല, ഇടിച്ചക്ക, കടച്ചക്ക, ഗോതമ്പ് ഗ്ലൂട്ടൻ, കൊഴുപ്പ് നീക്കം ചെയ്ത സോയ പൊടി, ചിപ്പി കൂൺ പൊടി എന്നിവ ഉൾപ്പെടുത്തിയുള്ള മീറ്റ് അനലോഗ് വികസിപ്പിച്ചെടുക്കുകയും അവയുടെ ഗുണനിലവാരം വിലയിരുത്തുകയും ചെയ്തു.

വൻപയറും വെള്ളക്കടലയും സംയോജിപ്പിച്ചു ഇടിച്ചക്ക മീറ്റ് അനലോഗും, കടച്ചക്ക മീറ്റ് അനലോഗും വികസിപ്പിച്ചെടുത്തു. അവയിൽ വ്യത്യസ്ത അനുപാതത്തിൽ പയർ, ഇടിച്ചക്ക, കടച്ചക്ക, ഗോതമ്പ് ഗ്ലൂട്ടൻ, സംയോജിപ്പിച്ചു (5 %) വിവിധ കോമ്പിനേഷനുകൾ ഉണ്ടാക്കിയെടുത്തു. കണ്ട്രോൾ (T_0 – 100% CWP, T_6 – 100% CP) ഒഴികെയുള്ള എല്ലാ കോമ്പിനേഷനുകളിലും കൊഴുപ്പ് നീക്കം ചെയ്ത സോയ പൊടി (DSF), ചിപ്പി കൂൺ പൊടി (OMF) എന്നിവ ചേർത്തു.

വികസിപ്പിച്ചെടുത്ത മീറ്റ് അനലോഗ് സമചതുരാകൃതിയിലുള്ളതും മൃദുവായതും മാംസത്തിന്റേതായ രൂപഘടനയോടു കൂടിയതുമായിരുന്നു. കൂടാതെ മുറിച്ച പ്രതലം അതിന്റെ ചേരുവകളാൽ പരസ്പരബന്ധിതമാണ്

രുചിഗുണസവിശേഷതകൾ വിലയിരുത്തി .പ്രധാനമായും ഇടി ചക്കയും കട ചക്കയും സംയോജിപ്പിച്ചുള്ള വൻപയർ മീറ്റ് അനലോഗും വെള്ള കടല മീറ്റ് അനലോഗിന്റെയും രുചിഗുണങ്ങൾ ആണ് വിലയിരുത്തിയത് . ഈ രണ്ടു മീറ്റ് അനലോഗിൽ നിന്നും കോമ്പിനേഷനുകളായ T_5 (40 % CWP + 25 % TJ + 25 % WG + 5 % DSF + 5 % OMF), T_{10} (50 % CP + 20 % TJ + 5 % DSF + 5 % OMF); T_4 (50 % CWP + 20 % BF+ 20 % WG + 5 % DSF + 5 % OMF), T_{11} (40 % CP + 25 % BF + 25 % WG + 5 % DSF + 5 % OMF) എന്നിവ യഥാക്രമം മികച്ച അനലോഗുകളായി തിരഞ്ഞെടുത്തു.

പ്രധാനമായും അവയുടെ രുചി ഗുണങ്ങൾ അടിസ്ഥാനമാക്കിയാണ് തിരഞ്ഞെടുത്തത്.

തിരഞ്ഞെടുത്ത അനലോഗുകളെ പോഷക പഠനങ്ങൾക്കും സംഭരണ കാലാവധി പഠനങ്ങൾക്കും വിധേയമാക്കി. മീറ്റ് അനലോഗിലെ ഈർപ്പത്തിന്റെ അളവ് 9.25 മുതൽ 10.62 ശതമാനം വരെ ആയിരുന്നു. കൂടാതെ കാർബോഹൈഡ്രേറ്റ്, മാംസ്യം , കൊഴുപ്പ് ആഷ്, നാരുകൾ എന്നി ഘടകങ്ങൾ 32.46 മുതൽ 53.29 വരെ, 20.79 മുതൽ 38.03 വരെ, 1.06 മുതൽ 1.92 വരെ, 2.92 മുതൽ 5.55 വരെയും 2.23 മുതൽ 7.30 ഗ്രാം/100 ഗ്രാം വരെയുമാണ്.

പ്രോക്സിമേറ്റ് വിശകലന പ്രകാരം കാൽസ്യം, ഫോസ്ഫറസ്, സോഡിയം, പൊട്ടാസ്യം, മഗ്നീഷ്യം, ഇരുമ്പ്, സിങ്ക് തുടങ്ങിയ ധാതുക്കൾ മീറ്റ് അനലോഗിൽ ധാരാളം അടങ്ങിയിട്ടുണ്ട്. ഇതിന്റെ ഉള്ളടക്കം യഥാക്രമം 80.25 മുതൽ 94.67 വരെയും 255.62 മുതൽ 325.46 വരെയും 23.52 മുതൽ 74.43 വരെയും 510.49 മുതൽ 631.50 വരെയും 103.64 മുതൽ 181.69 വരെയും 4.17 മുതൽ 5.73 വരെയും 3.1 മുതൽ 3.1 വരെയും 3.96 മില്ലിഗ്രാം/100 ഗ്രാം ആണ്.

മീറ്റ് അനലോഗുകളുടെ പ്രോട്ടീൻ ഇൻ വിട്രോ ദഹനക്ഷമത 62.12 മുതൽ 80.30 ശതമാനം വരെയാണ്. മിക്ക കോമ്പിനേഷനുകളിലും ഉയർന്ന ഇൻ വിട്രോ പ്രോട്ടീനും ധാതുക്കളുടെ ലഭ്യതയും സാധാരണ അനലോഗുകളെ താരതമ്യപ്പെടുത്തുമ്പോൾ ഇടിച്ചക്ക, കടച്ചക്ക ഉൾപ്പെടുത്തിയുള്ള മീറ്റ് അനലോഗുകളിൽ ആണെന്ന് കണ്ടെത്തി. തിരഞ്ഞെടുത്ത അനലോഗുകളിൽ കാൽസ്യം, ഫോസ്ഫറസ്, സോഡിയം, പൊട്ടാസ്യം, മഗ്നീഷ്യം, ഇരുമ്പ്, സിങ്ക് തുടങ്ങിയ ധാതുക്കളുടെ ഇൻ വിട്രോ ലഭ്യത യഥാക്രമം 34.43 മുതൽ 87.62 വരെ, 47.62 മുതൽ 71.43 വരെ, 57.66 മുതൽ 77.20 വരെ, 62.54 മുതൽ 82.85 വരെ, 54.40 മുതൽ 63.73 വരെ, 52.40 മുതൽ 73.32 വരെ, 55.89 64.28 ശതമാനം വരെയാണെന്ന് കണ്ടെത്തി

തിരഞ്ഞെടുത്ത മീറ്റ് അനലോഗുകളും മറ്റു അനലോഗുകളും ഫുഡ് ഗ്രേഡ് എച്ച്ഡിപിഇ കവറുകളിൽ (250 ഗേജ്) പായ്ക്ക് ചെയ്തു, മൂന്ന് മാസക്കാലം സാധാരണ താപനിലയിലും, റഫ്രിജറേറ്റഡ് താപനിലയി സൂക്ഷിക്കുകയും ചെയ്തു. പ്രതിമാസ ഇടവേളകളിൽ മീറ്റ് അനലോഗുകളുടെ രുചിഗുണങ്ങൾ വിലയിരുത്തുകയും ചെയ്തു . റഫ്രിജറേറ്റഡ് അവസ്ഥയിൽ സംഭരിച്ചിരിക്കുന്ന മീറ്റ് അനലോഗുകൾ സംഭരണകാലാവധിലുടനീളം സാധാരണ താപനിലയിൽ വച്ചതിനേക്കാൾ മികച്ച രുചിഗുണങ്ങൾ ഉള്ളതായി കാണിച്ചു.

സാധാരണ താപനിലയിലും റഫ്രിജറേറ്റഡ് താപനിലയിലും സംഭരിച്ചിരിക്കുന്ന സൂക്ഷിച്ചിരിക്കുന്ന മീറ്റ് അനലോഗുകളുടെ പോഷകഗുണങ്ങളും ഇൻ വിട്രോ ലഭ്യതകളും വിശകലനം ചെയ്തു. സംഭരണ സാഹചര്യങ്ങളിൽ ഈർപ്പത്തിന്റെ അളവ് വർദ്ധിച്ചു. കൂടാതെ മൊത്തം കാർബോഹൈഡ്രേറ്റ്, മാംസ്യം കൊഴുപ്പ്, ആഷ് , നാരുകൾ, ധാതുക്കൾ എന്നിവയിൽ പൊതുവായ മാറ്റം നിരീക്ഷിക്കപ്പെട്ടു. എന്നിരുന്നാലും, സാധാരണ താപനിലയുമായി താരതമ്യപ്പെടുത്തുമ്പോൾ ശീതീകരിച്ച സംഭരണത്തിന് കീഴിലുള്ള മിക്ക അനലോഗുകളിലും ഈ മാറ്റം കുറവായിരുന്നു.

പ്രോട്ടീൻ ഇൻ വിട്രോ ദഹനക്ഷമതയും , കാൽസ്യം, ഫോസ്ഫറസ്, സോഡിയം, പൊട്ടാസ്യം, മഗ്നീഷ്യം, ഇരുമ്പ്, സിങ്ക് തുടങ്ങിയ ധാതുക്കളുടെ ഇൻ വിട്രോ ലഭ്യതയും എല്ലാ അനലോഗുകളിലും പൊതുവായ മാറ്റം കാണിക്കുന്നു. ശീതീകരിച്ച അവസ്ഥയിൽ സംഭരിച്ചിരിക്കുന്ന മീറ്റ് അനലോഗുകളിൽ അവയുടെ പ്രോട്ടീനിലെ ഇൻ വിട്രോ ദഹന സംഭരണത്തിന് ശേഷം ഇൻ വിട്രോ ധാതു ലഭ്യതയിലും മാറ്റം കാണിച്ചു, എന്നാൽ മിക്ക മീറ്റ് അനലോഗുകളിലും ഈ മാറ്റം കാര്യമായിരുന്നില്ല.

സംഭരണകാലാവധിയിൽ സൂക്ഷ്മജീവികളുടെ എണ്ണത്തിൽ ക്രമാനുഗതമായ വർദ്ധനവ് കണ്ടെത്തി, പക്ഷേ വർദ്ധനവ് വളരെ തുച്ഛമായിരുന്നു. എന്നിരുന്നാലും, സാധാരണ താപനിലയിൽ സൂക്ഷിച്ച

അനലോഗുകളെ സംബന്ധിച്ചിടത്തോളം ശീതീകരണ അവസ്ഥയിൽ സൂക്ഷിച്ചിരിക്കുന്ന മീറ്റ് അനലോഗുകളിൽ സൂക്ഷ്മജീവികളുടെ എണ്ണം വളരെ കുറവായിരുന്നു . മൂന്ന് മാസത്തെ സംഭരണ സമയത്ത്, സാധാരണ താപനിലയിലും ശീതീകരണ അവസ്ഥയിലും സംഭരിച്ചിരിക്കുന്ന മീറ്റ് അനലോഗുകളിലൊന്നിലും പ്രാണികളുടെ സാന്നിധ്യം പ്രകടമായില്ല

ഇപ്പോഴത്തെ പഠനത്തിൽ, രുചിഗുണ സവിശേഷതകളാൽ തിരഞ്ഞെടുത്ത അനലോഗുകളിൽ T5 (40% CWP + 25% TJ + 25% WG + 5% DSF + 5% OMF), T11 (40% CP + 25% BF + 25% WG + 5% DSF + 5% OMF) എന്നിവയിൽ ഉയർന്ന മാംസ്യം , നാരുകൾ കൂടുതലായി കണ്ടെത്തി. ആയതുകൊണ്ട് തന്നെ ഇടിച്ചക്ക, കടച്ചക്ക എന്നിവ സംയോജിപ്പിച്ചു ഉണ്ടാക്കിയെടുത്ത മീറ്റ് അനലോഗുകൾ മറ്റുള്ള അനലോഗുകളെ താരതമ്യം ചെയ്യുമ്പോൾ മികച്ചതാണെന്ന് കണ്ടെത്തി.

തിരഞ്ഞെടുത്ത മീറ്റ് അനലോഗ്കളുടെയും അവയുടെ കണ്ട്രോളിന്റെയും ഉൽപാദനച്ചെലവ് 34.00 മുതൽ 60.00/ 100 ഗ്രാം വരെയാണ്.

മെച്ചപ്പെട്ട രുചിഗുണങ്ങൾ, പോഷക മൂല്യങ്ങൾ, സംഭരണ സ്ഥിരത എന്നിവയുള്ള ഈ മീറ്റ് അനലോഗുകൾ ഇടിച്ചക്ക , കടച്ചക്ക പോലുള്ള തദ്ദേശീയ സസ്യ ഫലാദികൾ ഉപയോഗിച്ച് വികസിപ്പിക്കാമെന്ന് നിലവിലെ ഗവേഷണതത്വങ്ങൾ പ്രകാരം കണ്ടെത്തി , ഇത് മറ്റ് വാണിജ്യ മീറ്റ് അനലോഗുകളെ താരതമ്യം ചെയ്യുമ്പോൾ മികച്ചതും ആരോഗ്യകരവും കൂടുതൽ ചെലവ് കുറഞ്ഞതും വൈവിധ്യമാർന്നതുമായ ബദല് തന്നെയാണ്

Appendices

APPENDIX I

Score card for the organoleptic evaluation of tender jackfruit or breadfruit incorporated meat analogues

Name :

Date :

Signature :

Parameter	Treatments											
	T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}
Appearance												
Colour												
Flavor												
Texture												
Taste												
Overall acceptability												

Nine point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

APPENDIX II

Meat analogues roast was prepared by the standard procedure as suggested by Philip (1993).

Meat analogues roast

Ingredients

Meat analogues cubes (replacing meat)	: 250gm
Coriander powder	: 10gm
Chilli powder	: 5gm
Turmeric powder	: 2.5gm
Big onions	: 3 no's
Green gram	: 4 no's
Ginger	: 4 cm piece
Garlic	: 1 pod
Aniseed	: 5gm
Tomato	: 2 no's
Garam masala powder	: 5gm
Coriander leaves	: 20gm
Oil	: 50gm
Sugar	: 5gm
Salt	: As required
Lime	: ½ lime

Procedure

Shallow fry the meat analogues cubes in half the quantity of oil and kept aside. Green chillies, ginger, garlic, coriander powder and aniseed were ground to a fine paste. Sautéed sliced onion for three minutes and added the ground ingredients, chilli powder, *garam masala* powder and turmeric powder and fried for two minutes. Sliced tomatoes

were added and sautéed for five minutes. To that fried meat analogues, salt and 100 ml of water was added and cooked in an open pan till the moisture was completely. Finally added, sugar, lime juice and coriander leaves.