OPTIMIZATION OF VEGETABLES PROPORTION IN EMULSION-TYPE CHICKEN SAUSAGE AND STUDY ON ITS STORAGE STABILITY

by

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Approval Letter

This *dissertation* entitled *Optimization of Vegetables Proportion in Emulsion-type Chicken Sausage and Study on its Storage Stability* presented by Tanka Bhattarai has been accepted as the partial fulfillment of the requirement for the M. Tech. degree in Food Technology

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Abstract

This study was conducted with an objective of optimizing vegetables proportion in emulsion-type chicken sausage and evaluation of storage stability. The primary screening process was conducted in the first phase with various percentages (10, 20, 30 and 40) of vegetables (soybean, carrot and mushroom) on the basis of sensory evaluation and physicochemical properties of the prepared sausage. 30% formulation was selected from primary screening and 11 runs were generated from D-optimal mixture design. In second phase, the best percentage formulation was carried out on the basis of sensory evaluation and physicochemical properties which was then further analyzed for proximate composition, carotenoids content and antioxidant properties. The final phase of the study was carried out on storage stability of optimized sausage samples at -2°C in polyester based films (PET). Finally, optimized product was compared on the basis of sensory score with chicken sausage (control) available in Dharan sub-metropolitan.

The optimized product from sensory analysis had a proximate composition, comparing 60.47, 11.89, 17.55, 0.43 and 4.17 moisture, crude fat, crude protein, crude fiber and total ash, respectively. The carotenoid content of the superior product was found to be 30 μg/ 100 g and DPPH radical scavenging activity of sausage was found to be 46.76%. There was a gradual increase in Total Plate Count (TPC) reaching maximum on day 18 (9 log/cfu) while *Salmonella* and coliform were not detected up to day 18. Peroxide value was also found to increase during refrigeration storage of sausage from 3.05 MeqO$_2$/kg at day 0 to 11.46 MeqO$_2$/kg on day 18. Vegetable-incorporated chicken sausages were successfully developed by incorporating vegetables in the formulation without any deteriorating effect on the sensory attributes and acceptability of the product. The results indicate that incorporation of vegetable in chicken based sausage like product can have the added dual benefit of improved nutritional quality and antioxidant.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AOAC	Association of official analytical collaboration
BHA	Butylated hydroxyl lanisole
BHT	Butylated hydroxyl toluene
CDFT	Central Department of Food Technology
CL	Cooking loss
DoE	Design of experiment
DPPH	Diphelyl-1-picrylhydrazyl
GMP	Good manufacturing practice
LDL	Low density lipoprotein
MDCM	Mechanically deboned chicken meat
NaCl	Sodium chloride
PET	Polyethylene terephthalate
PV	Peroxide value
ROS	Reactive oxygen species

RSC	Radical scavenging capacity
SPSS	Statistical package for the social sciences
TPC	Total plate count
WHC	Water holding capacity

Part I

Introduction

1.1 General introduction

Sausages are one of the oldest processed foods known to man. Several hundreds of varieties of sausages are produced worldwide with outstanding social and economic relevance (Carballo, 2021). Sausage making has a rich history in most cultures. Sausage was a way to store meat for extended periods when mechanical refrigeration was unavailable. Today, the combination of meat and seasonings produces a broad type of sausages and generates different categories from fresh, smoked, cured, and cooked sausages. The wide variety of sausages is due to differences in composition, shape, size, and cultural preferences and traditions (Flores, 2016).

Healthier meat-sausage formulations need to contain less saturated fat and/or promote the presence of specific healthy compounds as they affect the quality attributes of cooked meat emulsions (Jiménez-Colmenero *et al.*, 2001). Fibers in the form of vegetables and fruits have been found successful in improving functional value, enhancing cooking yield and improving texture of the products besides reducing the formulation cost (Zargar *et al.*, 2017b). High-fiber foods tend to reduce the risk of colon cancer, obesity, cardiovascular diseases and several other disorders (Bhat and Hina, 2011). Several studies have proven that dietary fibers have the potential to reduce blood low density lipoprotein cholesterol in blood, risk of diabetes Mellitus type 2, coronary heart disease, blood pressure, obesity and colorectal cancer. Thus, an increase in the level of dietary fiber in the daily diet has been recommended by several scientific organizations (Eastwood, 1992). Solubility, water binding, swelling, viscosity, gelation and surfactant properties are important functional properties of soy proteins in meat and dairy based systems.

Comminuted meats (sausage, bologna, luncheon meats) usually contain more fat than normal meat, hence soy proteins are used to enhance and stabilize fat emulsion, improve viscosity, impart texture upon gelation following cooking and improve moisture retention and overall yields (Jideani, 2011). Nitrite can cause the formation of carcinogenic nitrosamines in cured products due to its reaction with secondary amines and amino acids in muscle proteins. Residual nitrite in cured meats may form N-nitrosamines in the gastrointestinal tract. Thus, the meat industry continues to search for alternative methods to produce nitrite-free meats that maintain the color characteristics of nitrite cured meat products. Processed chicken meats are generally manufactured with the addition of various food additives for improvement of shelf life and sensorial properties and for convenience. Sodium chloride is an essential ingredient of processed meats because of its multiple roles (Sebranek, 2009). Generally, processed meats contain 7–39 g/kg sodium chloride. Nevertheless, the high concentration of sodium in processed meats is considered a health risk factor because high intake of dietary sodium increases the risks (Choe *et al.*, 2018).

The winter mushrooms (*Flammulinav elutipes*) are popular edible mushroom and widely cultivated in many Asian countries. Various studies have shown that the winter mushroom has several biological functions such as anticancer and anti-inflammatory effects. Various studies have revealed the water-binding effect of dietary fiber in processed meat. Furthermore, the increase of pH in pork meat batter with the addition of winter mushroom powder was recently reported (Choi *et al.*, 2018). The winter mushroom contains various flavor precursors such as free amino acids and nucleotides, which are the compounds responsible for the sweet and umami flavors of mushrooms.

Lipid oxidation and microbial growth in meat products can be controlled or minimized by using either synthetic or natural food additives. Various synthetic antioxidants, such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), are commonly used to delay the development of rancidity in food products (Martinez-Tome *et al.*, 2001). However, consumers are concerned about the safety of synthetic food additives. This concern has led to arouse a great interest in natural additives. Natural agents possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural. Garlic is one of the most commonly used ingredients as a flavor enhancement for sausage. In addition to flavoring the foods, garlic is appreciated for its medicinal properties.

1.2 Statement of the problem

In this age, not only production of vegetables is being decreased but also loss of this is increased drastically. The reason behind of heavy loss is improper preservation of vegetable. As vegetable is highly prone to spoilage but it can be processed and incorporate to other processed product so that its nutrient value will be retained. Consequently, decrease the loss of vegetables. Many researches have been conducted to explore the feasibility of using non-meat ingredients to promote a healthier meat sausage product, emphasizing the physicochemical properties and sensory characteristics in relation to the addition of new ingredients (Drosinos and Board, 1995; Grasso *et al.*, 2014).

Conventional sausage contains surplus amount of protein and fat but lacks micronutrients like minerals, vitamins, fibers and essential phytochemicals/antioxidant which improves immunity against different diseases. Prevalence of lifestyle diseases like diabetes, hypertension, renal diseases and cancer is increasing in Nepalese population. Consumption of conventional type sausages is hazardous for the population. Conventional sausage is an expensive food product as it contains meat as a sole ingredient and is beyond the reach of general Nepalese population due to high price. Conventional type sausage production cost is high due the use of various food additives like nitrate, filler, antioxidants, binder and phosphates. Suitable alternative for theses additives will reduce health and economic issues (Abdolghafour and Saghir, 2014). At present condition sausage industry is exception for vegetable market. Soybean, mushroom and carrot are rarely used in sausage industry.

During surplus season storage of freshly harvested mushroom and carrot is challenging for farmers as they contain high moisture. General Nepalese farmers and traders lack technology and are economically deprived for storage. These lead to necessary of immediate scope for use of fresh mushroom and carrot in vegetable incorporated meat products.

Recent studies suggested that consumption of fresh vegetables is rapidly declining resulting in mineral and vitamin deficiency among the population which ultimately huddles the sustainable development goals of the government. Besides having many health benefits, fiber improves many technological properties of the products. Fiber is suitable for addition to meat products and has previously been used in cooked meat products to increase the cooking yield due to its water-binding and fat-binding properties and to improve texture (Talukder, 2015).

1.3 Objectives

1.3.1 General objective

The general objective of this study was optimization of vegetables proportion in emulsion type-chicken sausage and study on its storage stability.

1.3.2 Specific objectives

The specific objectives were as follows:

i. To optimize vegetables proportion in emulsion-type chicken sausage.
ii. To analyze the proximate composition and sensory quality of the optimized sausage.
iii. To evaluate the physicochemical properties of prepared sausages.
iv. To study the peroxide value, *Salmonella* count, coliform total plate count of the optimized sausage.
v. To study the antioxidant properties and beta-carotene content of the optimized sausage.

1.4 Significance of the study

There are great challenges to the government to increase the consumption of such nutritionally rich produce among the fragile population like school-going children, adults and geriatric population. Consumption of processed food cannot be completely discouraged in present situation. So, variation in composition of conventional processed food like meat sausage can be a suitable way of encouraging consumption of nutritionally dense vegetables. Incorporation of vegetables will improve nutritionally quality resulting in increase of carbohydrate, minerals and vitamins in new formulation. Fruits and plant byproducts contain a wide variety of phytochemicals, such as polyphenols, carotenoids, and vitamins. (Liu, 2003) mentions there are more than 5000 individual phytochemicals identified in fruits, vegetables, and grains where some of these contribute to the antioxidant potential. They also contain valuable substances such as fibers, pigments, sugars, organic acids, flavors, antibacterial and antioxidants substances (Hernández-Alcántara *et al.*, 2018). According to Yogesh and Ali (2014), the natural antioxidants present in fruits and vegetables could scavenge free radicals and provide oxidative stability to many food items including high fat meat products.

Incorporation of carrot powder did not significantly affect pH value of sausage samples. The cooked buffalo meat sausage prepared by incorporating carrot powder improved the nutritional quality of the product due to presence of antioxidants (carotenoids) and dietary fibers in carrot powder (Khan and Ahmad, 2015).

Despite the technological benefits of nitrite, one of the main challenges in meat products industry is finding ways to reduce nitrites consumption because the reaction between nitrites and second, third and fourth type amino, leads to the formation of nitrosamine which has a high potential to cause cancer of the digestive tract in the consumers (Pegg and Shahidi, 2008). In this study use of nitrite is discouraged which prevent the risk of cancer. Carotenoids from carrot will provide desirable color to the finished product. Proper and careful selection of ingredients and good manufacturing practice (GMP) procedure followed during manufacture of product will inhibit and discourage possible microbial contamination. Natural antimicrobial and antioxidant property from those vegetables will suppress the growth of microorganisms and improve the shelf life stability of the product.

People tend to taste new and unique products day to day. Research and development works are routinely carried out in commercial manufacturing to address changing demand of the consumer. In this study partial replacement of expensive chicken meat by common local vegetables lead to reduction in price and development of novel product in sausage industry. Major problem generally encountered during sausage manufacturing is poor emulsification. To prevent it, conventional sausage manufacturer uses artificial emulsifier which is costly and is questionable for its purity. In this study, incorporation of soybean leads to incidental addition of lecithin naturally present in soybean. Water soluble fiber from vegetables will bind water resulting in reduced amount of artificial binder as additives. Vegetable and spices naturally contain antioxidants and other phenolic compounds so no need of external addition for preservation. Overall, reduced use of additives for technical characteristics of sausage will decrease production cost. Farmers are bound to destroy their agricultural produces due to low availability of market during surplus season. Incorporation of these vegetables will add new market for farmers and help government to achieve commitment for market availability.

Use of fresh vegetable will reduce storage load which requires modern well-equipped cold storage which is economically not feasible for farmers as well as the government.

1.5 Limitations of the study

i. Variation in spices formulation was not performed.

ii. Optimization of vegetable proportion in preliminary study was not carried out.

iii. Natural casing in all formulation sausage was not used.

iv. Complete amino acid profiling was not studied due to lack of facilities.

v. Optimization of packaging materials for shelf life study was not performed.

Part II

Literature review

2.1 Overview of processed meat-based product

Meat, the flesh from animals, has contributed to the welfare of man for centuries, the muscle and associated fatty tissues supplying him with a major portion of his protein and energy needs. Meat which has been modified in order either to improve its taste or to extend its shelf life (Varnam *et al.*, 1995).

Methods of meat processing include salting, curing, fermentation, and smoking. Processed meat is usually composed of pork or beef, but also poultry, while it can also contain offal or meat by products such as blood. Processed meat products include bacon, ham, sausages, salami, corned beef, jerky, canned meat and meat-based sauces. Meat processing includes all the processes that change fresh meat with the exception of simple mechanical processes such as cutting, grinding or mixing (Pearson and Gillett, 2012).

Meat is a product with high nutritional value, containing large quantities of available bio-compounds and consumers have a great passion for its flavorful taste. However, in terms of food security, in the area of production, processing of meat products has more sensitivity compared to other food products (Amani *et al.*, 2017). Despite massive processed meat consumption, it has always been criticized by medical nutrition specialists. Many studies have shown adverse effects on human health included colon, breast, and prostate cancers, growth hormone abuse, cardiovascular disease, preventive antibiotic residues, diabetes and classical swine fever (Verbeke and Ward, 2001). Mentioned diseases can be accorded due to high amount saturated fatty acids (40-50%) in meat fat like myristic and palmitic acids, which play a vital role in increasing blood levels of LDL cholesterol. Furthermore, during meat frying, the oxidation of cholesterol and other fatty acids may occur which can give rise carcinogenic compounds like aldehydes, esters, alcohols and short-chain carboxylic acids (Amani*et al.*, 2017).

In addition, incorporation of nitrite has created several major problems in terms of consumer health. Thus far, different approaches have been utilized in order to solve the problems like eliminating or reducing fat, replacing nitrite by healthier ingredients, using meat alternatives, etc. This review paper evaluates these approaches and provides the available solutions for these challenges. The increased interest of consumers as to the nature and composition of the purchased foods as well as an increase of nutritional awareness make the consumer want to buy and consume foods safe for health, produced with natural ingredients, without artificial additives, and processed as little as possible (Salejda *et al.*, 2017).

Chicken meat and products have grown in popularity due to their nutritional characteristics, while also providing an excellent source of animal protein for consumers in developing countries. Chicken meat provides high protein and low fat, and chicken lipids are characterized by relatively high levels of unsaturated fatty acids, which are positive and healthy by consumers. Chicken emulsion sausage is a popular chicken meat-based product. Chicken meat processors are responding to the marketplace demand by producing reduced fat chicken meat products. The production and consumption of chicken sausages has been increasing globally. These sausages are becoming more popular due to their sensory characteristics and ease of preparation, which reflects the development of more functionality-enhanced chicken emulsion sausages with added dietary fiber (Park *et al.*, 2012).

2.2 Sausage

Sausage is the oldest form of processed meat products known through historical evidence. It is considered one of the most appetizing, nutritious, enjoyable, and convenient meat products. The history of sausage is literally given by its name and many of today's sausages derive their names from the city where they originated, such as Vienna, Frankfurt, Mettwurst, Genoa, Knoblanch, Bologna, Salami, and many other (Kadim and Mahgoub, 2007). Sausage continues to grow in popularity today. Sausage can be made by grinding or emulsifying meat, poultry, or game, mixing in salt and other seasonings, and then stuffing into a container or casing (Busboom and Field, 1996).

2.2.1 Types of sausage

There are four broad categories of sausages: fresh sausages, cooked sausages, semi-dry or dry sausages, and emulsion sausage.

2.2.1.1 Fresh sausage

Sausages are one of the western style meat-based products that have grown in popularity amongst Malaysian consumers. Sausages are usually cylindrical in shape, made from finely minced meat either beef, veal, pork, lamb or chicken. All varieties of fresh sausages are commonly prepared from coarse or finely comminuted pork, beef or veal to which water is added along with a selection of spices that varies with the desired type of sausages to be produced. Fresh sausages have a unique appearance as they do not have any cure (nitrite) added and phosphate. It also has high fat content. In the United States, certain binders and/or extenders are used in the production of fresh sausages such as cereal, vegetable starch, nonfat dry milk and dried whey at levels not exceeding 3.5% by weight. The sausage batter is stuffed into a natural or artificial casing, twisted and cut to form individual sausage link, which is cooled rapidly to preserve freshness and flavor (Kadim and Mahgoub, 2007).

2.2.1.2 Cooked sausage

They are usually cured with nitrite, heated to an internal temperature of 65.55-68.33°C during processing, and frequently smoked. Cooked sausages require refrigeration. They will keep for at least 2 weeks in unopened vacuum sealed packages and for 1 week in non-vacuum packages or after you open vacuum-sealed packages. Freeze for longer storage. These sausages can be eaten without heating, but many are heated before serving to enhance their flavor. Examples of cooked sausages include hotdogs and luncheon meats such as bologna, cotto salami, polish sausage, and braunschweige (Busboom and Field, 1996).

2.2.1.3 Fermented sausage

Fermentation and drying are considered the oldest ways of meat preservation. Fermented sausages are characterized by their relatively longer shelf life, which is due to the production of lactic acid during the fermentation process. They are classified into semi-dry and dry. The production utilizes curing ingredients, spices and relatively 10 large numbers of cultured microorganisms (starter culture) in a fermentation process. The development of the pathogenic bacteria is inhibited by the acid produced by fermentation (Caplice and Fitzgerald, 1999). The low pH and the dry nature of the product are primarily responsible for the long-lasting quality.

Fermented sausages have relatively higher meat content and take longer time to be prepared due to the series of required drying processes (Abdolghafour and Saghir, 2014). Semi-dry fermented sausages differ from dry fermented sausages because of their pronounced tangy flavor due to forced fermentation, resulting in lactic acid accumulation and a bulk of other products from fermentation breakdown. Semi-dry sausages are often smoked and slightly cooked by heat used in the smokehouse, which occasionally reaches nearly 60°C for a limited time. After smoking, the sausages will be air dried for a relatively short time. These products have higher water activity (>0.90-0.91) compared with dry fermented sausages.

Hence, a lower pH (4.7-5.4) is needed for satisfactory protection against undesired microorganism but still require refrigeration with lactic acid content of 0.5 to 1.3% (Beriain *et al.*, 2018).The examples of sausages are summer sausage or cervelat and Lebanon bologna. Dry fermented sausage has longer ripening and drying process, whereby the biochemical and physical changes occurred strongly influence their stability and safety. The drying times vary between 10 and 90 days, and the moisture loss during drying is 20 to 40% of the weight of the freshly smoked product. The aw of the sausages ranges from 0.85-0.91 and have a final pH ranging between 5.2-5.8 with 0.5-1.0% lactic acid content which exhibits high shelf life stability and can be kept without refrigeration (Toldrá, 2010). Acidification and ammonia content mostly depend on the microbial flora, which varied due to several factors such as the initial contamination and types of technology used. The examples of sausage are salami, pepperoni, and genoa.

2.2.1.4 Emulsion sausage

Emulsion sausages have very fine texture, homogenous, tender interior and relatively mild flavor that is cooked and/or smoked, best known in the form of frankfurters or wieners depending on their presumed origins (Council, 1985).They are made by combining pork, beef or poultry with fat, salt, nitrate, flavoring and water, and mixing together in a large mixer until form a smooth homogenous batter. The fat is evenly dispersed in small droplets surrounded and stabilized by a fragment of the muscle cell and by salt dissolved in muscle protein (McGee, 2007). The temperature during mixing process is critical and controlled to prevent unstable emulsion and leak fat. The batter is then stuffed into a casing and cooked at about 70°C. The meat protein is coagulated due to heat and turns the batter into cohesive, solid mass form that allows the casing to be removed. Emulsified sausages have relatively high-water content, around 50-55% and must be refrigerated (McGee, 2007).

2.3 Vegetable incorporated sausage

Fruits and plant byproducts contain a wide variety of phytochemicals, such as polyphenols, carotenoids, and vitamins. There are more than 5000 individual phytochemicals identified in fruits, vegetables, and grains where some of these contribute to the antioxidant potential (Liu, 2003). They also contain valuable substances such as fibers, pigments, sugars, organic acids, flavors, antibacterial and antioxidants substances (Balestra and Petracci, 2019). As much attention has been paid to develop meat and meat products with functional food to promote healthier food and prevent the risk of diseases, fruit and vegetables are used as an ingredient in sausage formulation. The natural antioxidants present in fruits and vegetables could scavenge free radicals and provide oxidative stability to many food items including high fat meat products (Yogesh and Ali, 2014).

In the sausage production, synthetic antioxidants have been commonly included to encounter the major problems in meat processing due to lipid oxidation followed by cooking and subsequent refrigerated or frozen storage. It affects the quality of the product due to the loss of desirable color, odor, flavor and a reduced shelf life (Coronado *et al.*, 2002). This fact caused the manufacturer to develop sausage with vegetables/fruits added as a natural antioxidant. Furthermore, the presence of fiber in vegetables improves many technological properties of the sausage products. Fiber is suitable for addition to sausage, which helps to increase the cooking yield due to its water-binding and fat-binding properties and to improve texture (Cofrades *et al.*, 2000). Various types of fiber have been studied alone or combined with other ingredients for sausage formulations.

2.3.1 Raw materials

2.3.1.1 Chicken meat

Chicken meat is appropriate for quick and simple preparation, yet it offers a variety of combination with different foodstuffs, thus making itself a usual choice of consumers faced with modern lifestyle. Sausage making is a great way to use less tender, low-value cuts and trim pieces (Baker and Bruce, 1995).

It is important to mention that chicken with skin contains 2–3 times high fat than chicken without skin, so it should be eaten without skin to ensure the intake of high-quality protein without extra calories and fat. When compared to red meat, the main advantage of white chicken meat is in its low caloric value and a low portion of saturated fat, so consumption of white chicken meat is recommended to people who want to reduce the fat intake as well as to people suffering from heart and coronary diseases (Marangoni *et al.*, 2015). Food value of chicken meat is shown in Table 2.1.

Table 2.1 Food value of chicken meat

Nutrients	Chicken meat
Energy (Kcal)	165
Water (g)	65.26
Protein (g)	31.02
Total fat (g)	3.57
Saturated fatty acids (mg)	1.010
Mono unsaturated fatty acids (mg)	1.240
Poly unsaturated fatty acids (mg)	0.770
Cholesterol (mg)	85

Source: Kralik *et al.* (2018)

2.3.1.2 Soybean

Soybean is a widely used, inexpensive and nutritional source of dietary protein. Its protein content (40%) is higher and more economical than that of beef (19%), chicken (20%), fish (18%) and groundnut (23%). Soy protein used in the meat production is increasing because of their unique functional characteristics as meat extenders and functional ingredients.

Meat extenders are defined as proteins which are non-meat proteins but are usually plant proteins. A wide variety of meat extenders are available for use in emulsion type sausages to improve consistency, emulsifying and water holding capacity (Etiosa *et al.*, 2017a).

The increasing use of soy proteins in meat product is due to the availability and low cost of soybean relative to other extenders such as wheat flour, sodium caseinate, egg protein and non-fat dry milk (Lecomte *et al.*, 1993). Health benefits of soy in meat include prevention of heart diseases, cancer, high blood pressure, diabetes-related disease and many others. Soy bean oil is rich in fatty acids and devoid of cholesterol. It is an excellent source of calcium, iron, and vitamins such as niacin, thiamin and riboflavin. Soy contains all essential amino acid apart from methionine and tryptophan. These amino acids closely match those required for humans. Increasing research into soybean production and utilization would ensure a steady avenue for providing the much needed cheap but balanced protein. Table 2.2 shows food value of soybean seed.

Table 2.2 Food value of soybean seed

Parameters	Value
Moisture (%)	8.07
Protein (%)	37.69
Crude fiber (%)	5.44
Crude fat (%)	28.2
Ash (%)	4.29
Carbohydrate (%)	16.31
Energy (Kcal/100 g)	469.80

Source: Ogbemudia Ruth Etiosa *et al.* (2017b)

2.3.1.3 Mushroom

Edible mushrooms have the potential to contribute enormously to food value and diet, especially in the supply of both macro- and micro-nutrients. They are rich in vitamins and mineral elements (Unterseher *et al.*, 2013) and the bio availability of some elements depend on the level of interactions with various nutrients.

Mushrooms are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Choe *et al.*, 2018). They fall between the best vegetables and animal protein source. The protein content of mushrooms has been reported to be twice that of vegetables and four times that of oranges and significantly higher than that of wheat (FAO, 2006). The crude fiber content values reported in several studies suggest that mushrooms are potential sources of dietary fiber (Peter and Tolulope, 2015). Mushrooms generally contain low fat and oil content (Phan and Sabaratnam, 2012). As a result, they are recommended as good source of food supplement for patients with cardiac problems or at risk of lipid induced disorders. Mushrooms are also a source of some minerals, including iron, selenium, potassium and phosphorous (Jayathilake, 2008).

Al-Dalain (2018) assured that mushrooms may be a new "diet food" especially as a substitute for higher calorie and fat staples like meat. He reported that by replacing chicken meat with oyster mushroom into patty formulation, it saved an ingredient cost and enhanced the nutritional composition and sensory qualities. The food value of mushroom is shown in the Table 2.3.

Table 2.3 Food value of mushroom

Nutrients	Value
Moisture (%)	46.38
Crude protein (%)	17.42
Crude fiber (%)	2.05
Crude fat (%)	0.88
Ash (%)	1.55
Sodium (mg)	21.80
Potassium (mg)	13.08
Calcium (mg)	2.05
Phosphorus (mg)	0.56

Source: Sunday *et al.* (2016)

2.3.1.4 Carrot

Carrot (*Daucus carota*) is one of the most popular vegetable consumed in raw and processed form throughout the world. It is widely used for preparation of salads, pickles, soups and halwas. Beta carotene present in carrot is strong anti-carcinogenic and also prevents heart attack, ulcers, gum disease, colitis and stroke. It regulates sugar level and has laxative, antiseptic and vermicidal action(Kaur *et al.*, 2015b). The carrots are the unique roots containing other compounds, such as phenolic compounds and organic acids and have a characteristic flavor due to the presence of terpenoids and polyacetylenes, therefore, it has nutritional property for human health (Ahmad *et al.*, 2020a). It also contains the trace mineral molybdenum, rarely found in many vegetables. Molybdenum aids in metabolism of fats and carbohydrates and is important for absorption of iron. It is also a good source of magnesium and manganese (Guerrera *et al.*, 2009). Nutritive value of fresh carrot is shown in Table 2.4.

In a study conducted by Saleh and Ahmed (1998), incorporation of boiled carrot (100 g/ kg meat) improved the color, texture and nutritive value of beef patties; whereas, carrot powder improved cooking yield, color, texture and vitamin-A content. Also, Prepared sobrassada with 3% added carrot had higher acceptability for various physicochemical and sensory attributes but its textural parameters were significantly affected (Eim *et al.*, 2008).

Table 2.4 Nutritive content of fresh carrot

Nutrients	Value
Moisture (%)	86
Crude protein (%)	0.9
Crude fiber (%)	1.2
Crude fat (%)	0.2
Ash (%)	1.1
Carbohydrate (%)	10.6
Calcium (mg /100 g)	80
Iron (mg /100 g)	2.2

Source: Gopalan *et al.* (1971)

2.3.2 Proximate composition of raw material

2.3.2.1 Moisture

The moisture content of carrot varies from 75-88% (Gopalan *et al.,* 1971). The moisture content of soybean is 8.07% (Bayero *et al.*, 2019). This low moisture content of soya bean is of advantage because it enables the seed to be stored for a very long time. Most of the mushrooms contain high moisture content, ranging from 84.15 to 90.21%. The moisture content of chicken meat varied from 73.7 to 75.8%.

2.3.2.2 Protein

The edible portion of carrots contains protein ranging from 0.8 to 1.1 g/100 g (Sharma *et al.,* 2012). Carrot does not contain much protein but can still be used as protein food supplements in food. As contained in the McGraw-Hill Encyclopedia of Science and Technology (2002) soya beans contain about 40% protein. Soybeans protein content is higher and more economical than that of meat protein Snyder and Kwon (1987). The protein content of mushroom on dry basis was found to be 17.42% (Sunday *et al.,* 2016). The protein content of chicken meat is around 31.02% (Kralik *et al.*, 2018).

2.3.2.3 Crude fat

The high crude fat content of 18.8–40.1% is an indication that soybean may be an excellent source of oil. The observed results support the usefulness of soya bean seed like a good source of edible oils which can be used in cooking industry (Bayero *et al.*, 2019). Likewise, the fat content of carrot is 0.367, as it was known to be a cholesterol-lowering food, and this will be very good for the body and be able to digest easily. All mushrooms contain low fat contents ranging from 0.14%–2.91%.The fat content of chicken meat is around 3.57% (Kralik *et al.*, 2018).

2.3.2.4 Total ash

The ash content indicates the mineral content in the sample. The ash content of carrot is around 1.1%. According to report by Lokuruka (2010) soya beans have an ash content of about 5%, the major minerals in soya beans are potassium, phosphorus, magnesium, calcium, and manganese (Van Eys *et al.*, 2004). Most mushrooms have a low ash content ranging from 0.27 to 1.53% (Gopalan *et al.*, 1971).

2.3.2.5 Fiber

The fiber content of the soybean ranges from 5.44- 6.83% (Etiosa *et al.*, 2017a). Soybean is also of interest because of its cholesterol-lowering abilities in patients with type II hyper lipoproteinemia (Mitchel, 1993). The crude fiber in carrot roots consist of 71.7, 13.0 and 15.2% cellulose, hemicellulose and lignin, respectively. The crude fiber content of cultivated mushrooms is in the range 2.11– 15.32% (Sharma *et al.*, 2012a).

2.4 Other ingredients for sausages production

Other ingredients are commonly known as binders, fillers, and extenders. Many countries have restrictions on type, amount, and quality of ingredients used in sausages production.

2.4.1 Binder and filler

Binders are protein rich agent which changing water binding properties which help binding together different material in sausage product. The binders provide satisfactory control of shrinkage in cooking. It is used to improve the bind of meat and fat when making sausage, improving fat and moisture retention. The use of binder in meat industry is popular to bring about significant improvement in organoleptic properties of product. Binders have a macromolecular structure that have the capacity to form matrices to retain aroma and nutrients along with entrapment of large amount of water released during thermal processing to prevent exudation (Singh *et al.*, 2008).

Carbohydrate products which are used to absorb excessive water and not much affect to emulsification properties. Common fillers are cereal flour, starches derived from corn, rice, potato etc. (Balestra and Petracci, 2019).

2.4.2 Fats and oil

Compared with animal fats, vegetable oils are free of cholesterol and have a higher ratio of unsaturated to saturated fatty acids (Liu *et al.*, 1991). Vegetable oils such as olive oil and canola oil have been used as partial substitutes for pork back fat in low-fat frankfurters and other cooked products (Pappa *et al.*, 2000). Because vegetable oils differ considerably in their physical properties such as color, flavor, free fatty acids and fatty acid composition, different oils could have different effects on the quality characteristics and nutritional value of meat products (Lee *et al.*, 2015).

In addition to nutritional significance, fat has a major effect on the overall sensory properties, binding properties and palatability of final processed meat products. But the cardiovascular diseases associated with high dietary fat have made consumers health conscious. Even the minimum required fat content in ground products include significant amount of saturated fatty acids. Thus, reduction in the amount of fat in processed products without affecting the sensory appeal of product is a major challenge for food industry today. With this concept varieties of low fat meat products are emerging in the market (Ofori and Hsieh, 2012).

2.4.3 Salt

Salt is an essential ingredient of any sausage formulation. Salt is used to preserve the product, enhance the flavor, and or solubilize the meat proteins to improve the binding properties of the formulation. Since the advent of refrigeration, the preservative properties are the least important use of salt, though dry sausages still use salt for preservation. The most important use of salt in a sausage product is its ability to solubilize proteins. This enhances the product texture and improves water and fat binding. Since sodium chloride (NaCl) salt has been linked to hypertension, other non-sodium salts, such as potassium and calcium chlorides, are sometimes substituted for a portion of the sodium chloride (da Silva Dias, 2014).

2.4.4 Sodium tri-phosphate

Phosphates used in meat processing industries are the salts of phosphoric acid and sodium or potassium. Salts have a major effect on ionic strength and could extract myosin from myofibrillar structures in meat. Salts could enhance swelling of the protein structure but they (on their own) do not solubilize much protein (Knight and Parsons, 1988). On the other hand, phosphates on their own hardly activate proteins; they can only remove the link between actin and myosin (Feiner, 2006). Thus, through the addition of salts together with phosphates at the same time to a meat product, the muscular protein becomes soluble and solubilized, or activated; and the solubilized protein can immobilize high levels of added water as well as emulsify a large amount of fat (Bendall, 1954). Phosphates are slightly bacteriostatic as it slows down the growth of some gram-positive bacteria. Phosphates are not considered as direct preservatives. They only can impart some desirable properties when used as acidulants or in combination with other food ingredients (Long *et al.*, 2011).

2.4.5 Spices

Spices and condiments are products of plants, which are mostly used for seasoning, flavoring and thus enhancing the taste of foods, beverages and drugs (Nwinuka *et al.*, 2005). Spices come from the bark (cinnamon), root (ginger, onion, garlic), buds (cloves), seeds (yellow mustard, sesame), berry (black pepper), or the fruit (allspice, paprika) of tropical plants and trees. Spices are the building blocks of flavor in meat products. Their primary functions are to flavor meat products and to provide aroma, texture, and color. Spices also have strong, moderate or slight inhibitory activity against specific bacteria. The aldehydes, sulfur, terpenes and their derivatives, phenols and alcohols found in spices exhibit strong antimicrobial activity. Spices such as cloves, cinnamon, turmeric, black pepper, ginger, garlic and onions exhibit antioxidant properties in different food systems (Younathan *et al.*, 1980). Some spices have antioxidant properties. Antioxidant properties of spices are due the presence of flavanoids, terpenoids, lignans and polyphenolics (Craig, 1999).

The natural occurring phenolic compounds in spices are effective against oxidative rancidity of fats and color deterioration of the processed meat product pigments. During the process of oxidative rancidity, fats are broken down into peroxides (free radicals) with exposure to air or oxygen and finally into aldehydes and alcohols that give a rancid taste. Spices can halt the oxidative process by blocking or scavenging: the free radicals (Namiki, 1990).

2.4.5.1 Black pepper (*Pipernigrum*)

Black pepper is suitable for dishes of meat, seafood and eggs. It is used as multifunctional spice, imparting flavor, taste, color and masking off flavor in foods. Piperine is major pungent component of pepper and volatile oil is responsible for the aroma and flavor (Chaudhary, 2013).

2.4.5.2 Capsicum (*Capsicum annuum*)

In Asia word chilly is associated with highly pungent varieties of *C. annuum* and *C. frutescens* whereas a capsicum refers to non-pungent sweet bell peppers. The capsanthin is the pigment responsible for red color and is present in all capsicums (Batiha *et al.*, 2020)

2.4.5.3 Cardamom (*Eletteria cardamomummaton*)

Cardamom is commonly referred as queen of spices. It is commonly used in pickles, meat and canned soups as flavoring agent, but oil of cardamom develops off flavor within few days after contact with atmosphere (Rajathi *et al.*, 2017).

2.4.5.4 Cinnamon (*Cinnamomum verum*)

Cinnamon is used for culinary purposes and is commonly used in meat and fast food seasoning. Cinnamon is used in a ground form in processed meat (Thomas and Kuruvilla, 2012).

2.4.5.5 Clove (*Syzygium aromaticum* L.)

Cloves are strongly aromatic and have a pungent, spicy taste. Whole cloves are a must for pickling meats and studding hams. The flavor is quite strong so use sparingly. Cloves are used in a ground form in processed meat industry (David, 2008).

2.4.5.6 Coriander (*Coriandrum sativum* L.)

Coriander is an annual herb from plants, seeds and leaves are obtained. Principal component is coriandrol (d-linalool). The seeds are aromatically sweet and make a mild and spicy flavor (Krishan Datt Sharma *et al.*, 2012b). Seed and its extracts are used in sausage item (Alassaf, 2003).

2.4.5.7 Cumin (*Cuminum cyminum* L.)

Cumin imparts flavor and acts as preservative It also posses' medicinal properties like stimulant, antispasmodic, carminative and antimicrobial property. Ground cumin is used in lemon based marinates for chicken, turkey, lamb and pork, curries (Gadekar *et al.*, 2006).

2.4.5.6 Nutmeg (*Myristica fragrans* Hoult)

Nutmeg is the dried kernel of seed and mace of the dried aril surrounding the seed, Nutmeg is widely used in processed meats. Nutmeg and its oleoresin are used in the preparation of meat a product, soups, seasoning of meat products. Mace is used in processed meat produces like sausages, soups, pickles and chutneys (Gadekar *et al.*, 2006).

2.4.5.7 Turmeric (*Curcuma domestica*)

Turmeric is a perennial herb. Turmeric is contains coloring pigment, curcumin which imparts yellow color to turmeric and possess potent antioxidant activity (Debjit Bhowmik *et al.*, 2009).

2.4.5.8 Ginger (*Zingiber officinale*)

Ginger is tropical perennial herb. It is indispensable ingredient due to its refreshing pleasant aroma and carminative property. The functionally significant components of ginger are primarily its aroma and secondarily its pungency (Vasala, 2012). The primary flavoring constituents of the oil include cineol, borneol, geraniol, linalool and farnasene (Vasala, 2012).

2.4.5.9 Onion (*Allium cepa*)

Onion is popular spice commonly consumed worldwide. Onion is characterized by its distinct flavor and pungency which is due to sulfur-containing compounds. Beside its culinary uses, onion also possess medicinal properties such as stimulant, diuretic, expectorant, hypoglycemic and reducing cholesterol (Farooqi *et al.*, 2005).

2.5 Processing technology of sausage

Meat processing technology comprises the steps and procedures in the manufacture of processed meat products. Processed meat products, which include various different types and local/regional variations, are food of animal origin, which contribute valuable animal proteins to human diets. Animal tissues, in the first place muscle meat and fat, are the main ingredients, besides occasionally used other tissues such as internal organs, skins and blood or ingredients of plant origin (Musa *et al.*, 2020).

2.5.1 Meat grinding

The grinding of meat and fat ingredients has largely been practiced for many years and is still done today, mainly by small processors, particularly in the manufacture of specialty products. The lean meats are first ground by running them through a 3-6 mm grinder plate while fat trimmings or fatty tissues are reduced through a 6-9 mm grinder plate. Grinding through a coarser plate increases the capacity of the machine and heats the meat plate (Pearson *et al.*, 1977).

The curing salts are then added, and the batch is mixed in a mechanical mixer to ensure that the ingredients are well dispersed. The curing process may take place either overnight in a chiller at 1-4°C or after final chopping in the cutter with other ingredients and stuffing, i.e, prior to or during the smoking (Abdolghafour and Saghir, 2014).

2.5.2 Mincing

After grinding, the meat is minced in to a very fine particle size for easy protein extraction. Proteins have the function of binding the water surrounding fat droplets and keeping them dispersed (Marchello and Garden-Robinson, 2017). Preparation of sausage emulsion is basically in two phases. First, the lean meat, either previously ground or not, is placed on the cutter and chopped. This is done by the simultaneous addition of all the phosphate and/or citrate for the total batch and one-third of the total amount of finely crushed ice or water. Increased salt concentration in the water phase of the mixture will result in a greater extraction of the meat protein and is of paramount importance in forming a stable emulsion (Heinz and Hautzinger, 2007).

After that, fat trimmings and other fat meats, then spices and the remaining two-thirds of the total water are added (Marchello and Garden, 2003). Chopping continued until the batch is thoroughly chopped, however the temperature of the meat emulsion should not be reaches more than 18°C (second phase). With the high salt content and the longest cutting process, more salt soluble proteins are extracted and the binding quality of the finished product is improved (Abdolghafour and Saghir, 2014).

2.5.3 Filling

Before filling into casings, oxygen should be excluded from the mixture (by vacuum-filling devices) and the temperature of the mix should not exceed 2°C. Evacuation of air from the product enhances color stability and the visual effect of the sausages. It also reduces fat oxidation and bacterial action and prevents proteolysis. A longer shelf life of sausages is therefore achieved by vacuum filling (Abdolghafour and Saghir, 2014).

2.5.4 Casings

Natural casing (made from the intestines of slaughter animals) and casings made from modified collagen or cellulose is used. For un-dried products, synthetic casings are also used. Most products made of natural casings come out with a curve after filling and cooking. Artificial casings are now made with collagen, cellulose and plastic materials to suit a wide range of applications. Through a series of mechanical and chemical actions, collagen is extracted from the connective tissue of animals and used for manufacturing casings (Akpan, 2017). Apart from providing the required sausage shape, casings also increase product shelf life by providing high moisture and oxygen resistant properties with a seal strength and density. Casings therefore contribute in minimizing product weight loss during cooking (Abdolghafour and Saghir, 2014).

2.5.5 Cooking

There are many methods of cooking: by immersing in the cooking vat, hot showering that is conducted in a smokehouse equipped with shower nozzles, hot showering in separate hot water spray cabinets to which sausages are moved immediately after smoking, cooking by dry heat by raising the smokehouse temperature and giving only a final brief hot water shower, cooking in tight boxes into which live steam is injected etc (Oshibanjo *et al.*, 2019). However, the cooking schedules vary markedly. This usually requires temperature of 80-82°C for about 15-20 min. The right cooking schedule should be developed by carefully studying the yield and quality of the sausage. After cooking in vats, sausages are hot showered to remove any adhering grease (Abdolghafour and Saghir, 2014).

2.5.6 Cooling and packaging

The sausage should be showered or soaked in water till reach an internal temperature of 38- 40°C, then remove the casing and cooled to 4°C for packaging. The main purpose of packaging is to prevent meat and meat products from microbial contamination, physical and chemical changes (Abdolghafour and Saghir, 2014). Packaging materials for sausages whether primary or secondary should be good enough to offer an acceptable visual and structural presentation of the product to the customer. Vacuum packaging is used on saveloys, frankfurters and cooked sliced sausages to prolong shelf life, and there is an increasing popularity of its use in cooked, chilled and frozen products (Stasiewicz *et al.*, 2014). Packaging meat and meat products with appropriate plastic film and laminates plays significant role in retention of the quality and extension of shelf life during refrigerated storage (Sahoo and Anjaneyulu, 1997).

2.6 Physiochemical attributes of sausage

2.6.1 Cooking loss

Cooking loss measures the ability of system to bind water and fat after protein denaturation and aggregation. Fiber retains water and decreases cooking losses. The use of water and dietary fibers from vegetables for meat replacement improve water binding capacity thereby reduce the cooking loss (Marchetti and Andrés, 2021).

Heating the fiber causes swelling and gelatinization. The number of polysaccharides present in the dietary fiber determines these processes (Sánchez *et al.*, 2006). Several constituents like starch and pectin interact with the protein and lead to a reduced ability for moisture migration during cooking, which results in the reduction of cooking loss. But reducing the fat content of sausages decreased the quality of the products, particularly in terms of cook loss (García and Totosaus, 2008).

2.6.2 Folding test

Folding test is a simple and fast method of predicting the textural quality of gel composite products such as sausages and meatballs. Folding test helps to determine the elasticity of texture or springiness. Non-meat proteins could hold more water and fat contents, which may fill the interstitial spaces within the protein matrix and reduces springiness. Folding ability is directly related to the formation of protein gel-network during cooking process. In meat emulsion, myofibrillar proteins are responsible for creation of this network (Jang *et al.*, 2015). The mixture of plant proteins had lesser ability to form this protein network as compared to myofibrillars and their presence markedly reduced the gel strength and folding ability in final product (Akesowan, 2010).

2.6.3 Moisture retention

Moisture retention value represents the amount of moisture retained in the cooked products. Dietary fiber could improve the moisture retention when it was added into meat products. During cooking, the water released from the meat matrix would retain the dietary fiber of product (Cheng and Sun, 2008). The high absorption capacity of fiber in the vegetable increases the moisture content, cooking yield and moisture retention (Gull *et al.*, 2017).

2.6.4 Fat retention

Fat retention value represents the amount of fat retained in the cooked products. It specifically shows how well the juices are retained in system. Fat retention depends on protein which is hydrophilic in nature. Proteins create an adhesive gel matrix and consequently bring about stabilization for emulsion (Nacak *et al.*, 2021). Also, fat-encapsulating agents supplement myosin and acto-myosin prevents fat separation during cooking (Kamani *et al.*, 2019). Fat separation is considered as undesirable factors in comminuted meat products.

2.6.5 Antioxidant property of vegetable

Antioxidants are substances that neutralize free radicals or their actions. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The use of antioxidant helps to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products (Ahmad *et al.,* 2020b).

Plants produce a very impressive array of antioxidant compounds that includes carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid and tocopherols to prevent oxidation of the susceptible substrate (Dogan *et al.* (2010). These plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species oxidants (Benzie, 2003).

2.6.6 Beta-carotene

Carrots are best known for their rich content of antioxidant like beta carotene. Carotenoids represent a large group of phytochemicals that may contribute to health and disease prevention (Reddy *et al.*, 2020). Beta carotene present in carrot is strong anti- carcinogenic and also prevents heart attack, ulcers, gum disease, colitis and stroke. It regulates sugar level and has laxative, antiseptic and vermicidal action (Zarger *et al.,* 2017). Saleh and Ahmed (1998) conducted studies on ground beef patties formulated with carrot and reported that color, yield, texture, and vitamin A content of beef patties were improved by the addition of boiled carrot. Therefore, fortification of provitamin A in meat products could be a long-term solution for combating vitamin A deficiency in human beings.

2.6.7 Sensory attributes

2.6.7.1 Color

Color is an important quality attribute as consumers are often willing to pay more for poultry products based on color. It is the primary criterion that determines the acceptability and rejection of product (Kennedy *et al.*, 2005). Color reflects the freshness as well as hygienic quality of product (Sofos, 1994). Several factors influence meat color including pH, myoglobin concentration, nitrites, and oxidation state of the heme iron (Mancini, 2013).

2.6.7.2 Flavor

Flavor is a complex sensation comprising mainly of odor and taste. It is sensed collectively by the oral and olfactory senses. It results from the combination of the basic tastes sweet, salt, bitter, sour and umami (Toldrá, 2010). Flavor has profound effect on overall acceptability of the product. The raw meat is characterized by salty, metallic and bloody taste with sweet aroma. During cooking process typical taste and aroma is developed as a result of complex interaction of precursors derived from lean and fat compositions of meat like Maillard reaction, lipid interaction(Khan *et al.*, 2015). Lipids generally have the greatest influence on the production and release of aroma and flavor components (Thu, 2006).

2.6.7.3 Texture

Consumer studies showed that flavor and texture are more important among all the quality attributes. Texture is the attribute of a substance resulting from a combination of physical properties and perceived by the sense of touch, sight and hearing (Thapa, 2016). Though texture can be assessed by objective method too, tenderness as perceived by subjective approach is one of the sensory attributes used to assess the overall texture of meat (Juárez*et al.*, 2012). Texture profiles are affected by many processing factors such as the type and amount of ingredients, additives, heat treatment and equipment used (Yetim, 2000). Hardness is the most important quality parameter when evaluating the textural properties of sausages (Klettner, 1993).

2.6.7.4 Taste

Taste is another important attribute that reflects the sensory quality of meat and meat products. It occupies third importance behind tenderness and flavor Pearson and Dutson (1994). One of the most important factors affecting the juiciness of meat is the cooking procedure. Method which causes greatest retention of meat fluid results the product with enhanced juiciness. As a result moisture content, salt, water holding capacity of meat and marbling or intramuscular fat directly influence the juiciness of product. According to Pearson and Dutson (1994), incorporation of meat by-products like heart, tripe, liver etc. also have been found to have significant effect on juiciness of the product.

2.6.7.5 Overall acceptance

Overall acceptance is one of the important factors in sensory evaluation. It is an overall judgment of properties such as color, flavor, surface structure, shape, appearance and any defects present. For processed product like sausage, the ingredients used play a major role.

2.6.8 Shelf life of sausage

2.6.8.1 Peroxide value of sausage

The peroxide value (PV) is used as an indicator of the primary oxidation in sausage samples. Lipid oxidation is responsible for rancid flavors and sensory defects in meat products and has negative effects on other quality characteristics such as color, texture, and nutritive values in meat products. Oxidation is affected by the availability of oxygen, free ionic iron (Kanner, 1994), the proportion and degree of unsaturation of the fatty acids in the lipids (Ahn *et al.*, 1996), additives, and the presence and amounts of antioxidants in meat. The fatty acid composition of the phospholipids of muscle cell membranes is especially important in determining the oxidative stability of meat, since the oxidative changes in meat are initiated mainly from the membrane components of muscle (Ahn *et al.*, 1996).

2.6.9 Microbiology

Meat and meat products are considered as a major vehicle of most reported food poisoning outbreaks. Therefore, it is important to use the microbiological criteria as it gives guidance on the acceptability of meat products and their manufacturing, handling and distribution processes. The increasing number and severity of food poisoning outbreaks worldwide has considerably increased public awareness about food especially meat and meat products which are one of the most important sources of human infections with a variety of food borne pathogen (Osman *et al.*, 2016).

In open pan evaporation microbial contamination is more susceptible due to longer time of exposure to air during cooking process. Microbial count for the meat extract can be found in table 2.5. The total viable count and coliform count of ready to eat meat products should not be $\geq 10^4$ cfu/g (FSSAI) and ≥ 100 cfu/g (ICMSF), respectively. Average microbial counts for meat extract are shown in Table 2.5.

Table 2.5 Average microbial counts for meat extract

Meat extractive	Microbial counts
Total plate count	<1000/g
Coliform	<10/g
Salmonella	Negative/g

Source: Ockerman and Pellegrino (1988)

There may be variation in microbiological level due to sanitation, processing, storage, moisture and temperature conditions. The large number of thermophilic bacteria remaining in the product after processing would not be expected to increase during storage of the 16% moisture product, but may become inoculums when mixed with other foods of higher moisture content (Ockerman and Pellegrino, 1988).

2.7 Previous study and research gap

Many researches have been conducted to explore the feasibility of using non-meat ingredients to promote a healthier meat sausage product, emphasizing the physicochemical properties and sensory characteristics in relation to the addition of new ingredients and the substitution of animal fat. But no research has been conducted in the chicken meat incorporated with carrot, mushroom and soybean. Healthier meat-sausage formulations need to contain less saturated fat and/or promote the presence of specific healthy compounds as they affect the quality attributes of cooked meat emulsions. Incorporation of carrot, soybean and mushroom has been shown to lower the fat content, improve emulsion stability and increase the amount of fiber. Moreover, many researchers have also carried out studies on the addition of dietary fiber in lower fat meat product as a fat substitute to restore products rheological properties and cooking yield due to its water-binding and fat-binding properties. Carrot and mushroom can provide several health benefits in daily diet. Carrot contains high amounts of beta-carotene, which account for about half of the pro-vitamin A carotenoid found in the food supply. Carrot also contains other compounds, such as phenolic compounds and organic acids, which contribute not only to the sensory qualities but as an additional nutritional property for human health (Syuhairah *et al.*, 2016).

Part III

Materials and methods

3.1 Raw materials and source

Mechanically Deboned Chicken Meat (MDCM) and three types of vegetables (soybeans, carrot and mushroom) were purchased from the local market, Dharan sub-metropolitan city, Sunsari, Nepal.

3.2 Instruments and equipment

Instruments and equipment were provided by Central Department of Food Technology (CDFT) and all the process were carried out in pilot plant and laboratory of the department.

3.3 Methodology

3.3.1 Preparation of raw materials

3.3.1.1 Raw meat

Raw material during preparation was fresh and frozen. Chicken were brought from Ganga farm house, sub-metropolitan city. The lean meat was well trimmed. The trimmed lean meat thus, being practically free from sinews and gristle and entirely free from ligament, bone and cartilage particles and finally chopped to 4 mm opening size using mincer and bowl chopper for 12 min.

3.3.1.2 Soybean (*Glycine max.*)

Fresh soybean seeds were used in the sausage preparation and the preparation of soybean flour was done as described by Odiase *et al.*, (2013) shown in the Fig. 3.1.

3.3.1.3 Mushroom (*Pleurolus ostreatus*)

Fresh good quality mushroom was prepared before adding to the sausage as method described by Jayathilake (2008) with slight modification as shown in Fig. 3.2.

3.3.1.4 Carrot (*Daucus carota*)

Fresh carrot was prepared as described by Zarger *et al.*, (2017) and some modification will be carried out during carrot preparation as shown in Fig. 3.3.

3.3.2 Preparation of soybean flour

Sorting of soybean seeds

↓

Soak in sodium bi-carbonate for 12 h

↓

Boil for 45 min.

↓

Sun drying

↓

Milling and sieving

↓

Packaging

↓

Store

Fig. 3.1 Preparation of soy flour

Source: Odiase *et al.* (2013)

3.3.3 Preparation of mushroom strips

Fresh mushroom

↓

Cleaning, grading, sorting

↓

Slicing

↓

Blanched in boiled water for 3 min

↓

Crushing and grinding

↓

Freezing

Fig. 3.2 Preparation of Mushroom

Source: Jayathilake (2008)

3.3.4 Preparation of carrot

Fresh Carrot

↓

Washing and cleaning

↓

Mechanical peeling

↓

Slicing (2-4 mm)

↓

Water blanching (80°C for 10 min)

↓

Coarse grinding

↓

Fine mincing

Fig. 3.3 Preparation of carrot

Source: Zarger *et al.* (2017)

3.3.5 Other ingredients

3.3.5.1 Binders and fillers

According to literature, corn starch which is relatively cheap and readily available for vast production was used as both binding and filling agent (Zarger *et al.*, 2017).

3.3.5.2 Spices

Pepper, chilly, onion, garlic, turmeric and clove were used in powder foam and others cinnamon, cardamom and nutmeg were cleaned and ground to very fine past without remaining of fiber parts in paste.

3.3.6 Sausage preparation

During preliminary screening, number of sausage formulations were prepared using different percentage levels of vegetables (10, 20, 30 and 40) in equal proportion of vegetables in all level and control (100% chicken). From the selected percent level (30), the numbers of combinations were obtained from the mixture; D-optimal design with 11 numbers of runs.

Other ingredients added in the sausage processing were spices, water, fat, salt, additives and preservatives. Recipe used during preliminary screening for acceptability test of different vegetables incorporated sausage is shown in Table 3.1. Typical flow diagram of vegetable incorporated chicken sausage is given in Fig. 3.4.

Table 3.1 Preliminary screening for acceptability test of different vegetables incorporated sausage

Ingredients	E (Control)	A (10%)	B (20%)	C (30%)	D(40%)
Meat (%)	100	90	80	70	60
Vegetable (%)	0	10	20	30	40
Fat (%)	20	20	20	20	20
Water (%)	10	10	10	10	10
Cornstarch (%)	8	8	8	8	8
Salt (%)	2.1	2.1	2.1	2.1	2.1
Sodium tri-phosphate (%)	0.5	0.5	0.5	0.5	0.5
Chili powder (%)	1.2	1.2	1.2	1.2	1.2
Pepper powder (%)	0.8	0.8	0.8	0.8	1.5
Turmeric powder (%)	0.2	0.2	0.2	0.2	0.2
Garlic (%)	0.9	0.9	0.9	0.9	0.9
Onion (%)	0.2	0.2	0.2	0.2	0.2
Clove (%)	0.5	0.5	0.5	0.5	0.5
Cardamom (%)	0.15	0.15	0.15	0.15	0.15
Cinnamon (%)	0.15	0.15	0.15	0.15	0.15
Nutmeg (%)	0.1	0.1	0.1	0.1	0.1

Source: Jayathilake (2008)

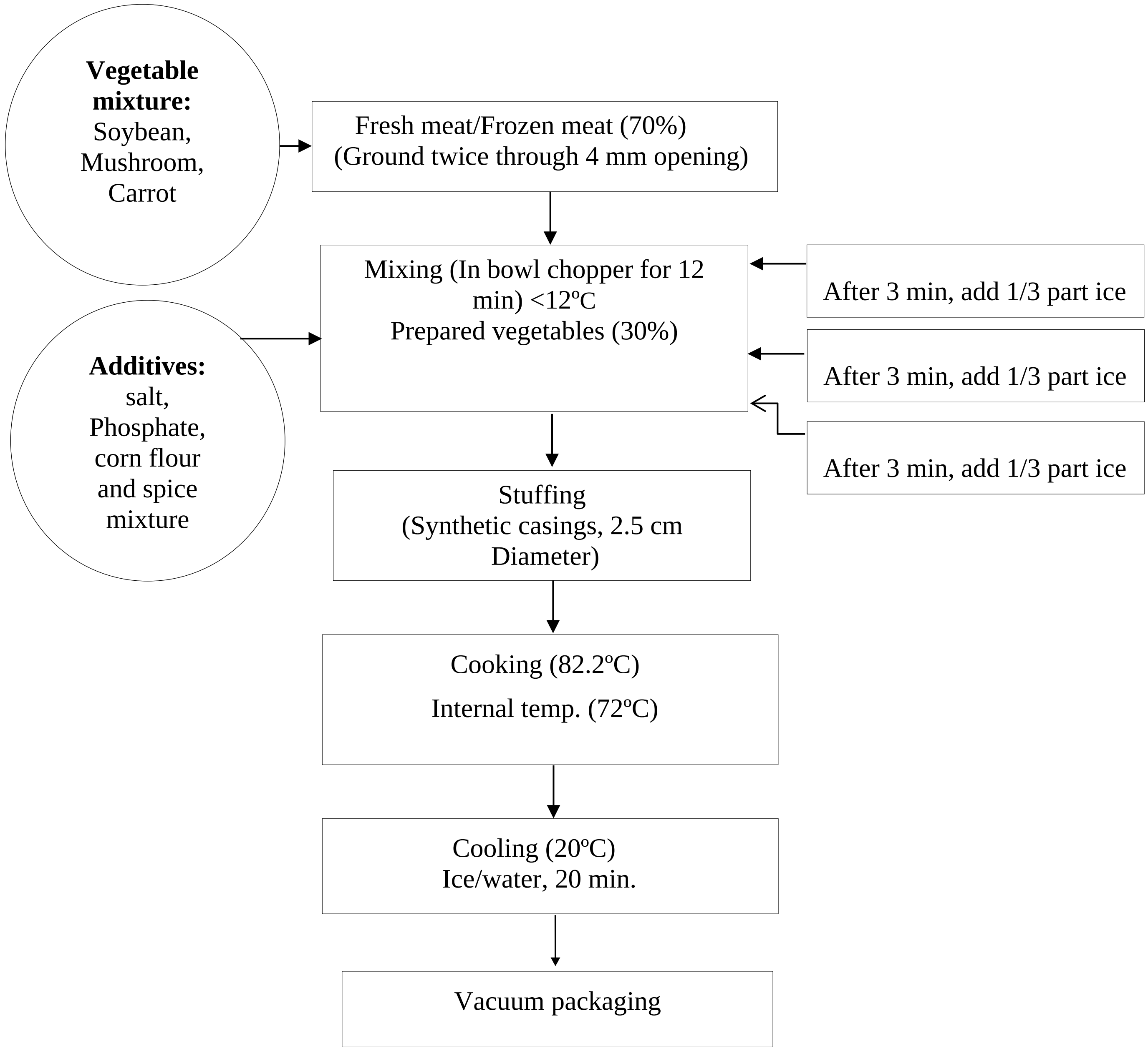

Fig. 3.4 Typical flow diagram of vegetable incorporated chicken sausage

3.3.7 Experimental design

The experiment was arranged as a design of experiment (DoE) in Design- Expert software, version 10. The screening process was conducted in the first step with various percentages (10, 20, 30 and 40%) of vegetables in the sausage formulations. The proportions of all the vegetables in all percent level were made equal. Based on sensory attributes and physicochemical characteristics (cooking loss, folding test, moisture retention and fat retention), optimized percent level of the vegetable incorporation was selected. From the selected percent level, the numbers of combinations were obtained from the mixture; D-optimal design with 11 numbers of runs to optimize the formulation of the product and desirability was calculated based on the effect of different responses. Formulation of sausage with 30% combination of vegetables is shown in Table 3.2.

Table 3.2 Formulation of sausage with 30% combination of vegetables

S.N	Meat (70%)	Combination of vegetables (30%)		
		Mushroom	Soybean	Carrot
1	70	0	15	15
2	70	22	0	8
3	70	15	15	0
4	70	5	5	20
5	70	0	15	15
6	70	10	10	10
7	70	15	0	15
8	70	5	20	5
9	70	15	15	0
10	70	22	8	0
11	70	15	0	15

3.3.8 Physiochemical analysis

3.3.8.1 Cooking loss

The cooking loss of the sample was determined according to the method of Choi *et al.* (2009) with slight modification. 30 g emulsion was stuffed into screw top test tubes and was heated in a steam bath at 70ºC for 30 min. The cooked samples were quickly immersed in cool water for 10 min. Cooking losses were determined by weighing individual sample before and after cooking and difference expressed as a percentage of the original weight.

3.3.8.2 Folding test

The folding test was performed using a five-point grade system according to the method of Cardoso *et al.* (2008). Sausages were sliced into 3 mm thick pieces. The slices were folded slowly in half to observe the way in which they broke. They were graded as follows: (1) breaks by finger pressure, (2) cracks immediately when folded in half, (3) cracks gradually when folded in half, 4) no cracks showing after folding in half, and (5) no cracks showing after folding twice.

3.3.8.3 Moisture and fat retention

The moisture and fat retained was determined according to Karami *et al.* (2009). The values in the cooked product per 100 g of the sample was determined. The values were calculated according to the following equations:

$$\text{Moisture retention }(\%) = \frac{\text{Yield }(\%) \times \text{Moisture of cooked sausage }(\%)}{100}$$

$$\text{Fat retention }(\%) = \frac{\text{Cooked weight} \times \text{Fat of cooked sausage }(\%)}{\text{Raw weight} \times \text{Fat of raw sausage }(\%)} \times 100$$

3.3.8.4 Antioxidant property

Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity was determined by the DPPH radical scavenging method as described by Bhattarai *et al.*, (2019).

The DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay is based on the capability of stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with H-donors. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine. The degree of stable DPPH de colorization to DPPH-H (reduced form of DPPH) which is yellow indicated the scavenging efficiency. DPPH solution (0.004% w/v) was prepared in 95% methanol. The samples were mixed with 95% methanol in 1: 9 ratio so as to make final volume 10 ml, thus the extract was prepared. Equal volume of extract and freshly prepared DPPH (0.004% w/v) were mixed and the tubes were incubated at room temperature in dark for 10 min. the absorbance was taken at 517 nm using a UV-V is spectrophotometer. 95% methanol was used as blank. The scavenging activity of the extract against the stable DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = (A - B) / A \times 100$$

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

3.3.8.5 Beta-carotene

Beta-Carotene was estimated by method as suggested by Durrani *et al.* (2011). 5 g of sample was grinded with few crystals of anhydrous sodium sulphate and homogenized with 10 ml acetone. It was decanted and then supernatant was collected in a beaker. The process was repeated twice and transferred the combined supernatant to a separating funnel. 10 ml of petroleum ether was added and mixed thoroughly. Two layers were separated out on standing. Discarded the lower layer and collected the upper layer in 100 ml volumetric flask, volume was made up to 100 ml with petroleum ether and optical density was recorded at 452 nm. Petroleum ether was used as blank. The β-carotene was then calculated using the following expression:

$$\beta - \text{carotene}\left(\mu g / 100\ \text{g}\right) = \frac{\text{O. D. of sample} \times 13.9 \times 104 \times 100}{\text{Weight of sample} \times 560 \times 1000}$$

3.3.8.6 Moisture content

Moisture was determined by hot air oven method, AOAC official method 925.10 as described in AOAC (2005). The product was finely grounded before weighing. The sample was dried in hot air oven until the constant weight was obtained.

3.3.8.7 Fat

Crude fat content of the samples was determined by Soxhlet extraction method as described in AOAC (2005). 10 g of sample was taken and packed in a thimble. Then the fat was extracted using petroleum ether solvent.

3.3.8.8 Protein

Crude fat content of the samples was determined by Soxhlet extraction method as described in AOAC (2005). 10 g of sample was taken and packed in a thimble. Then the fat was extracted using petroleum ether solvent.

3.3.8.9 Ash

Ash content was determined by dry ashing as described in AOAC (2005) following AOAC official method 923.03. The grounded sample was ash in muffle furnace at 550±10ºC until constant weight was obtained.

3.3.8.10 Crude fiber

Crude fiber of the sample was determined gravimetrically by acid and alkali treatment following AOAC official method 962.09 as described in AOAC (2005)

3.3.9 Shelf life study

The finished product was packed in 2 layer polypropylene by single vacuum packaging machine at -2^{o}C. The net weight of finished product was 500 g. Shelf life of vacuum-packed finished product was conducted in 3 day interval till to the acceptance level of the product.

3.3.9.1 Microbiological analysis

Microbial analysis was performed for both raw materials and finished product prepared aseptically and analyzed for this purpose.

3.3.9.1.1 Total Plate Count

Determination of total plate count was performed according to the method prescribed by Harrigan and McCane (1979) using plate count agar and distilled water as diluent.

i. 1 ml of sample from 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilution was withdrawn with the help of sterile pipette and will be transferred to each sterile petri-plate.

ii. To each plate, 10 ml of molten Plate Count Agar (PCA) at 45°C was added and the medium and inoculums was mixed immediately by a combination of to and fro shaking and circular movements lasting 5-10 s.

iii. The plates were allowed to set and were incubated at 37ºC for 24-48 h.

3.3.9.1.2 Total Coliform Count

Total coliform count was done by pour plate technique as given by Drosinos and Board (1995) using Violet Red Bile Salt Agar and distilled water for dilution.

3.3.9.1.3 Salmonella

Salmonella detection was done by pour plate technique as given by Ekawati *et al.* (2017) using Salmonella-Shigella Agar (SS Agar) and distilled water for dilution.

3.3.9.2 Peroxide value

Measurement of peroxide value (PV) was determined according to the Sallam *et al.*, (2004). The PV was calculated and expressed as milli-equivalent peroxide per kg of sample:

$$\text{PV (Meq } O_2 \text{ / kg} = \frac{S \times N \times 100}{W}$$

Where, S = Volume of titration (ml) N = Normality of sodium thiosulfate solution W = Weight of sample (kg).

3.3.10 Sensory evaluation

Sensory evaluation of the product was conducted using 9 point hedonic rating scale and 5-point intensity rating scale (Ranganna, 2007). The samples were cut into a uniform size and served warm (~50ºC) to the panelists. All the samples were coded with a random three- digit number and presented to the panelists in a random order. Sensory analysis was carried out for all formulations. The panelist for sensory analysis was students and teachers of Central Department of Food Technology and Central Campus of Technology, Dharan, Hattisar. Each panelist was distributed with score card and sample. The panelist was asked to rank the sample products based on its quality attributes like color, flavor, texture, taste and over all acceptability.

3.4 Statistical analysis

The results were analyzed using two-way statistical analysis of variance (ANOVA), using SPSS package (SPSS 21.0 for Windows, SPSS Inc, Chicago, Illinois, U.S.A). All data was analyzed using two-way ANOVA to detect a significant difference between mean values of treatments with factors: Different vegetables at same percentage and different percentage of the same vegetable. Statistical significance was indicted at 95% confidence level.

Part IV

Results and discussion

The present study was conducted to prepare vegetable incorporated chicken sausage. Three vegetables (mushroom, carrot and soybean) were used to replace 30% of meat in sausage preparation. Effect on different physiochemical analysis (cooking loss, folding test, moisture retention and fat retention) and sensory evaluation of the prepared sausage were studied. Sausage prepared without addition of vegetable was used as control for comparative analysis. Finally, the optimized formulation was obtained. Further, proximate analysis and sensory evaluation of the optimized formulation was carried out (Table 4.1). Overall result and discussion are discussed in following headings.

4.1 Proximate composition

4.1.1 Proximate composition of vegetables

Table 4.1 Proximate composition of incorporated vegetables

Parameters	Soybean (mean±sd)	Mushroom (mean±sd)	Carrot (mean±sd)
Moisture content (%)	9.26±0.01	89.89±1.88	89.89±1.88
Crude fat (%)	18.50±0.10	0.5±0.01	0.18±0.03
Protein (%)	27.0±0.05	9.92±0.01	0.38±0.09
Total ash (%)	4.60±0.02	1.20±0.03	3.38±0.01
Crude fiber (%)	0.43±0.02	0.57±0.10	0.38±0.38

*Values are the means of triplicate with standard deviation

4.1.1.1 Moisture content

The moisture content of soybean, mushroom and carrot were found to be 9.26±0.01, 89.89±1.88, and 89.89±1.88% respectively as shown in Table 4.1. The difference in moisture content of soybean was found to be 1.19 as reported by Sanni *et al.* (2008). The difference in value may be due to the processing methods. The moisture content in mushroom is in the range of 85-89%. Sausage prepared with mushroom will have high moisture content as the moisture content of mushroom is high (Valverde *et al.*, 2015).

The moisture content of carrot varies from 86-89% as reported by Sharma *et al.* (2012a). High Moisture content of carrot increases the moisture content of the carrot incorporated products. According to Choe *et al.* (2018), addition of dietary fiber increases the moisture content of meat emulsion systems, providing higher water retention and improves emulsion stability.

4.1.1.2 Crude fat

The crude fat content of the soybean, mushroom and carrot were found to be 18.50±0.10, 0.5±0.01 and 0.18±0.03% respectively as shown in Table 4.1. In meat products, soy proteins promote absorption and retention of fat. The addition of soy protein as non-meat ingredients in processed meat products may be the possible solution to the recent consumer demands for low fat and low cholesterol meat products (Yadav *et al.*, 2013). High crude fat content of 18.8-40.1% is an indication that soybean may be an excellent source of oil (Banaszkiewicz, 2000). The observed results support the usefulness of soya bean seed like a good source of edible oils which can be used in cooking purposes (Bayero *et al.*, 2019).

The fat content of carrot was similar to the value as described by Gopalan *et al.* (1971) which is 0.2%. As the fat content of carrot is low, gradual decline in fat content in the incorporated product may be observed. Low fat content will be very good for the body and be able to digest easily (Gopalan *et al.*, 1971).

All mushrooms contain low fat contents ranging from 0.14 to 2.91% (Maimulyanti and Prihadi, 2016). The reduction in the fat content of sausages formulated with fresh oyster mushroom might be used to the lower fat content of oyster mushroom when compared to fat content of chicken.

4.1.1.3 Protein

The protein content of soybean, mushroom and carrot were found to be 27.0±0.05, 9.92±0.01 and 0.38±0.09% respectively as shown in Table 4.1. As contained in the Manion (2002) soya beans contain about 40% protein. This difference may be due to difference in variety. Soybeans protein content is higher and more economical than that of meat protein (Snyder and Kwon, 1987). High protein content of soybean could be used in improving the palatability of foods in which they are incorporated (Etiosa *et al.*, 2017c). The edible portion of carrots contains protein ranging from 0.8 to 1.1 g/100 g (Krishan Datt Sharma *et al.*, 2012b).The difference in results may be due to variation in species i.e., the species used during the present study may be different. Carrot does not contain much protein but can still be used as protein food supplements in food.

According to Sunday *et al.* (2016), the protein content of mushroom was found to be 10.42%. The incorporation of mushroom will influenced the crude protein of sausages. The crude protein content of sausages may decrease with increase in the levels of mushroom. This declining pattern was in agreement with the previous study done by Wan Rosli *et al.* (2015) on the inclusion of oyster mushroom powder into the chicken frankfurters.

4.1.1.4 Total ash

The total ash content of the soybean, mushroom and carrot were found to be 4.60±0.02, 1.20±0.03 and 3.38±0.01% respectively as shown in table 4.1. The ash content indicates the mineral content in the sample. But the value reported by Etiosa *et al.* (2017b) was 1.01-1.6% higher than the result of the study. The difference in value may be due to the different areas of cultivation. Addition of soybean in the sausage may increase the ash content of the product. This may be because of the addition of soy fiber (Fedulova *et al.*, 2018).

The ash content of carrot was found to be 1.1%. In carrot incorporated turkey meat sausages, significant decline in as percentage was observed. Similar findings were reported by Kaur *et al.* (2015b) who recorded non-significant decrease in ash content in chicken nuggets incorporated with carrot. Most mushrooms have a low ash content ranging from 0.27% to 1.53%. The ash content of mushroom incorporated sausage may decrease with the increasing level of mushroom added into the sausage batter. This trend was in line with the study done by on the increment level of mushroom powder in raw chicken patties. According to González *et al.* (2012), the protein content could negatively influence the ash content.

4.1.1.5 Crude fiber

The crude fiber content of the soybean, mushroom and carrot were found to be 0.43±0.02, 0.57±0.10 and 0.38±0.38% respectively as shown in Table 4.1. The 0.43% crude fiber of soybean was lower than the values 2.97, 2.98 and 3.01% found in the literature (Kaur *et al.*, 2015a). The fiber content in the product will be similar to the fiber content of the product incorporated.

The crude fiber in carrot roots consist of 71.7, 13.0 and 15.2% cellulose, hemicellulose (Rashidi and Yang, 2016) and lignin, respectively Surbhi *et al.* (2018). The crude fiber content of cultivated mushrooms is in the range 2.11-15.32%. A crude fiber content of fresh mushroom was considered higher as compared to the crude fiber content of the broiler chicken breast. This difference may be due to different processing method.

4.2 Preliminary screening of vegetable proportion

The product formulation of sausage with addition of equal vegetable proportion in 10%, 20, 30 and 40% was done and physicochemical tests (Table 4.2) and sensory evaluation of the formulated products were done.

4.2.1 Physicochemical analysis of sausage prepared during preliminary screening

Table 4.2 Physicochemical analysis of different formulated sausage

Sample	Folding test	Cooking loss (%)	Moisture retention (%)	Fat retention (%)
A (10%)	4	1.92	72.77	40.25
B (20%)	3	2.4	69.42	47.55
C (30%)	3	8.7	55.50	70.20
D (40%)	3	9.25	59.63	68.12
E (Control)	4	1.52	88.56	51.89

*Values are the means of triplicate

4.2.1.1 Folding test

The folding test scores in this study ranged from 4 to 3 when the sausages were prepared by conventional heating methods respectively. Higher folding test scores resulted from the sausage formulated with 10 and 20% vegetable mix. Higher incorporation of vegetable proportion had lower folding score whereas lower proportion had better folding score.

There was no difference between controls, 10 and 20% vegetable incorporated sausage sample. Folding ability is directly related to the formation of protein gel-network during cooking process. In meat emulsion, myofibrillar proteins are responsible for creation of this network. The mixture of plant proteins had lesser ability to form this protein network as compared to myofibrillar and their presence markedly reduced the gel strength and folding ability in final product (Kamani *et al.*, 2019). Syuhairah *et al.* (2016) reported that the folding test score range of commercial chicken sausage was 4.20 to 5.00. A report by Kamani *et al.* (2019) showed that incorporation of flour with fish mince decreased the folding test value in fish sausage. Overall, the folding test results revealed that replacement of meat by plant proteins reduce the gel formation/elasticity in the cooked emulsion.

4.2.1.2 Cooking loss

Vegetable proportion with 10, 20, 30 and 40% incorporation had 1.92, 2.4, 8.7 and 9.25% drain weight respectively. Similarly, the minimum cooking loss was observed in control product with 1.52% as drain weight. This result indicates that increasing the vegetable proportion in sausage increases the cooking loss whereas control product showed minimal cooking loss. Sausage with 30% vegetable incorporation showed acceptable cooking loss. The present findings are in consistent with the study, which reported positive effects of plant ingredients on reduction of cooking loss in emulsified meat batters (Kamani *et al.*, 2019).

Cooking loss measures the ability of system to bind water and fat after protein denaturation and aggregation. The cooking loss can be dependent on the various factors such as temperature, time and method of cooking, additives used in emulsion, type and content of fat in formulation and casing material (Choi *et al.*, 2009).

Zarger *et al.* (2017) observed an increasing trend in the cooking loss of the chicken sausages with increasing levels of carrot. This could be due to formation of comparatively less stable emulsion in the formulations containing carrot. Low emulsion stability may have resulted in more loss of moisture during cooking. Similar increase in cooking loss was also reported by Verma *et al.* (2013) in sheep meat nuggets incorporated with guava powder. Increase in cooking loss was noticed up to 15% carrot incorporated turkey meat sausages as compared to control (Reddy *et al.*, 2020).

4.2.1.3 Moisture retention

Sausage with 40% vegetable showed the lowest value of 59.63% as compared to other samples for moisture retention. The highest value for moisture retention 72.77% was observed in 10% formulation. Moisture retention of control was found to be 88.56%.

The results are in contrast to the study by Mousavi *et al.* (2019), where increase in the amount of tofu incorporation in sausage increased the moisture retention. Similar results were reported by Zaini *et al.* (2020) where the addition of 5% albedo orange to sausage resulted in a rise of moisture retention up to 75.68%, whereas the control sausage had moisture retention of 73.61%.

Research shows that soy is commonly used as meat binders due to their various functionalities such as water retention, binding and emulsifying characteristics which results in improved binding and texture of the sausage (Abdolghafour and Saghir, 2014). Incorporation of 2% soy protein isolate in meatballs showed that level enhanced of moisture content (Odiase *et al.*, 2013).

4.2.1.4 Fat retention

Sausage with 10% vegetable showed with fat retention value of 40.25%. In other hand, the value of fat retention in 40% formulation was found to be 68.12%. Fat retention in control sample was found to be 51.89%.

This result is also supported by the findings of Serdaroglu and Ozsumer (2003), who reported improved moisture and fat retention in cooked beef sausages using soy, gluten and whey proteins. According to study by Mousavi *et al.* (2019), increase in the amount of tofu incorporation in sausage decreased the fat retention. This might due to fat reduced through cooking caused by weak binding properties between water and fat. Other reason could be due to the unsuitable type of fat used in the formulation.

4.2.2 Sensory evaluation of different formulated sausage

During preliminary screening the formulation of the product was designed based on the equal proportion of all vegetables incorporated in the sausage. Sensory evaluation of vegetable incorporated sample A, B, C, D and E (control) was carried out for color, flavor, taste, texture and overall acceptance by 10 semi trained panelists using 9 point hedonic rating (1= dislike extremely, 9= like extremely) as described by (Ranganna, 2007).

The 10 semi trained panelists (students of Central Department Food Technology, CDFT) were provided with 5 samples. Before the sensory evaluation a brief instruction regarding the color, flavor, taste, texture and overall acceptance was given to the panelists. The mean scores of sausage samples based on hedonic rating are shown in Fig. 4.1

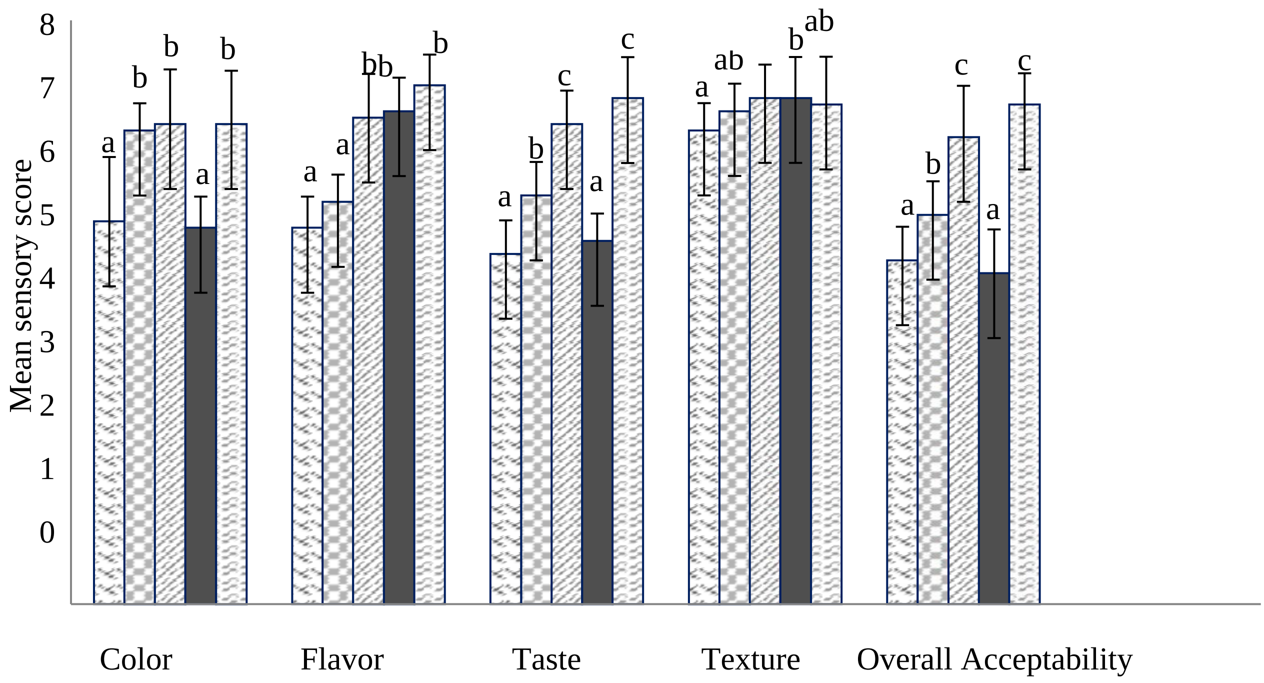

Fig. 4.1 Mean scores of sensory attributes

4.2.2.1 Color

From two-way ANOVA, it was found that there was no significant difference in color between samples A and D (p>0.05). Similarly, samples B, C and E show no significant difference (p>0.05) while they differ from A and D.

The mean score for color of the samples A, B, C, D and E was found to be 5.9, 7.3, 7.4, 5.8 and 7.4 with standard deviation 0.99, 0.48, 0.51, 0.42 and 0.51 respectively. Comparing them, sample C and E were found to be most preferred than other samples. Likewise, the least mean score was obtained for the sample A.

Carrot enhanced the color and appearance score in sausages. This might be due to addition of carrot as it contains color pigments; carotenoids which impart color to the product which is in accordance with the findings of Bhosale *et al.* (2011). Fiber may influence the color of meat products due to their diversified colors and presence of pigments. For meat batter and sausage, the lightness may be reduced since the fiber of the vegetables incorporated was transferred to the samples, thereby causing a change in the sausage and the batter. According to study by Bastos *et al*, (2011), it was reported that precooked browning might be due to non-enzymatic reaction among the amino acids in meat and the carbohydrates in vegetables incorporated.

4.2.2.2 Flavor

From two-way ANOVA, it was found that A and C sample were significantly different ($p>0.05$) from other formulation while B, D and E sample were significantly similar ($p<0.05$) in sensory score of the flavor attribute.

The mean score for flavor of the samples A, B, C, D and E was found to be 5.8, 6.2,7.5, 7.6 and 8.0 with standard deviation 0.42, 0.42, 0.51, 0.42 and 0.51 respectively. Comparing them, sample E was found to be most superior in terms of flavor than other samples. Likewise, the least mean score was obtained for the sample A that may be due to high fiber incorporation in the sausage. The flavor of the sausage may be affected by the beany flavor of soybean incorporated. Dilution of meaty flavor at higher levels of fiber incorporation resulted in a decrease in flavor scores of fiber enriched sausages. According to Yadav *et al.* (2018) a significant decline in flavor scores was noticed at 9% level in Wheat bran and 6% level in dried carrot pomace incorporated sausages. Also, addition of carrot dietary fiber at a level above 3% resulted in a decrease in sensory acceptability of dry fermented sausage (Eim *et al.*, 2008).

4.2.2.3 Taste

The statistical analysis two-way ANOVA showed that among 5 sample, A and D and C and E were not significantly different ($p>0.05$) while B was found to be significantly different than the four.

The mean score for taste of samples A, B, C, D and E were found to be 5.4, 6.3, 7.4, 5.6 and 7.8 with standard deviation 0.84, 0.67, 0.51, 0.51 and 0.78 respectively. Samples C and E had higher scoring than other samples. Results of the present study agree with those of Ozturk *et al.* (2014) who reported a decrease in taste and overall acceptability as the Wheat Sprout Powder concentration was increased in beef patties.

4.2.2.4 Texture

From two-way ANOVA statistical analysis, sample C and D and B and E had no significant difference ($p>0.05$) in their texture attribute while A was found to be significantly different ($p<0.05$). The mean score for texture of the samples A, B, C, D and E was found to be 7.3, 7.6, 7.8, 7.8 and 7.7 respectively with standard deviation of 0.48, 0.51, 0.42, 0.63 and 0.67 respectively. From the data, it was clear that samples C and D were not significantly different. Similarly, samples B and E shows no significant difference while sample A is significantly different from other samples.

Good texture score of vegetable incorporated sausage in comparison to control may be due to soybean which improved the texture. Also, addition of corn flour during the preparation may have increase the texture of the sausage. Varied results have been obtained on textural properties of meat products depending on amount and type of fiber. Insoluble fiber can increase the consistency of meat products by forming an insoluble three-dimensional network Cofrades *et al.* (2000) which can influence the rheological properties of the continuous phase of emulsions. Grossi *et al.* (2012) reported that incorporation of carrot dietary fiber in a comminuted meat emulsion, result in a high order of network organization leading to a hard texture and high water binding capacity. Similarly, Kim *et al.* (2011) noticed an increase in hardness value in rice bran added pork meatballs. However, García and Totosaus (2008) had reported that addition of peach, apple and orange fiber resulted in decreased hardness and increased springiness of low fat dry fermented sausages.

4.2.2.5 Overall acceptance

The statistical analysis two-way ANOVA showed that the sample A and D and sample C and E were not significantly different ($p>0.05$) while sample B was found to be significantly different ($p<0.05$) than the other samples in terms of overall acceptance.

The mean score for Overall acceptance of the samples A, B, C, D and E was found to be 5.3, 6.0, 7.2, 5.1, and 7.7 respectively with standard deviation 0.82, 0.47, 0.63, 0.73 and 0.48 respectively. Here sample E got highest score as it was more preferred whereas A got lowest score. Mehta *et al.* (2013) also reported a decrease in overall acceptance scores of chicken rolls and patties with increasing levels of rice.

The order of superiority can be summarized as:

Color: Sample C>Sample E>Sample B > Sample A> Sample D

Flavor: Sample E>Sample D > Sample C>Sample B > Sample A

Taste: Sample E>Sample C > Sample B>Sample D > Sample A

Texture: Sample C, Sample D > Sample E>Sample B > Sample A

Overall acceptance: Sample E>Sample C > Sample B >Sample A > Sample D

From above statistical scores of all sensory attributes, it was found that E was the most preferred one in terms of the attributes (color, flavor, taste, texture and overall acceptance) followed by C, B and A and least preferred being D sample.

4.3 Optimization of vegetable mixture formulation

Optimization of vegetable mixture in vegetable incorporated sausage was formulated by D-optimal mixture design as given by design of expert shown in Table 3.2. The amount of vegetable incorporated in the mixture varies from 5-30%. The responses include cooking loss, folding test, moisture retention and fat retention. Numerical optimization was done with the help of D-optimal mixture design to find out the best solution with the given factor.

During setting up the goal in D-optimal mixture design, incorporation of vegetables in different proportion results 11 different formulations which is shown in the Table 4.4. Formulations were analyzed for physicochemical properties. Eight formulations were rejected after physicochemical analysis as the responses were below the acceptance level. Also, formulation including all three vegetable was only accepted. Sensory evaluation of four accepted formulations was done.

Table 4.3 Effect of responses with different formulation in experimental plan

Run	Comp 1 A: Mushroom	Comp 2 B: Soybean	Comp 3 C: Carrot	R1 Cooking loss	R2 Folding test	R3 Moisture retention	R4 Fat retention
1	0	15	15	10.2	2	59.2	70.1
2	22	0	8	9.7	3	9.6	75.9
3	15	15	0	7.2	2	57.5	71.6
4	5	5	20	13.4	3	50.1	63.2
5	0	15	15	9.4	3	59	73.4
6	10	10	10	9.9	2	60.1	73.2
7	15	0	15	8.9	2	49.7	72.1
8	5	20	5	7.7	3	56.5	71.6
9	15	15	0	7.1	2	58.7	71.3
10	22	8	0	7.4	4	57.4	70.4
11	15	0	15	10.2	2	51.2	69.4

*Values are the means of triplicate

* Responses were measured in terms of cooking loss, moisture retention and fat retention in percent (%) and folding test in terms of scale that ranges from 1 to 4.

4.4 Physicochemical properties

From the preliminary study, results of physicochemical and sensory analysis of sausage with different formulation suggest that sausage with 30% vegetable incorporation was found technologically accepted with acceptable sensory attributes. Thus, for 30% vegetables incorporation different proportion of mushroom, carrot and soybean were obtained from D-optimal mixture design which was used to study physicochemical properties of sausage as responses.

4.4.1 Cooking loss

Cooking loss measures the ability of system to bind water and fat after protein denaturation and aggregation. An increase in cooking loss has a large financial impact in meat industry. For example, sausages have significant amounts of high protein quality and are good sources of several essential minerals, including iron and zinc as well as B vitamins. The increased loss of such nutrients deteriorates the meat nutritional quality and lowers its purchase (Jama *et al.*, 2008). The cooking loss can be depending on the various factors such as temperature, time and method of cooking, additives used in emulsion, type and content of fat in formulation and casing material (Choi *et al.*, 2009). ANOVA for cooking loss for mixture cubic and extra term model is shown in Table 4.5.

Table 4.4 ANOVA for Response cooking loss for mixture cubic and extra term model

Source	Sum of Square	df	Mean Square	F-value	P-value	
Model	26.35	2	13.18	13.09	0.0030	Significant
Linear Mixture	26.35	2	16.18	13.09	0.0030	
Residual	8.06	8	1.01			
Lack of Fit	6.89	5	1.38	3.53	0.1640	Not significant
Pure Error	1.17	3	0.3900			
Cor Total	34.41	10				

The "Model F-value" of 1309 implies the model is significant. There is a only 0.30% chance that model F-value this large could occur due to noise. The lack of fit F-value of 3.53 implies the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good; we want the model to fit.

Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, B and C are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms, model reduction may improve the model.

Final equation in terms of actual components:

Cooking loss (%) = + 0.248011×A +0.222476×B +0.460501×C

Where, A= Mushroom, B= Soybean, C= Carrot

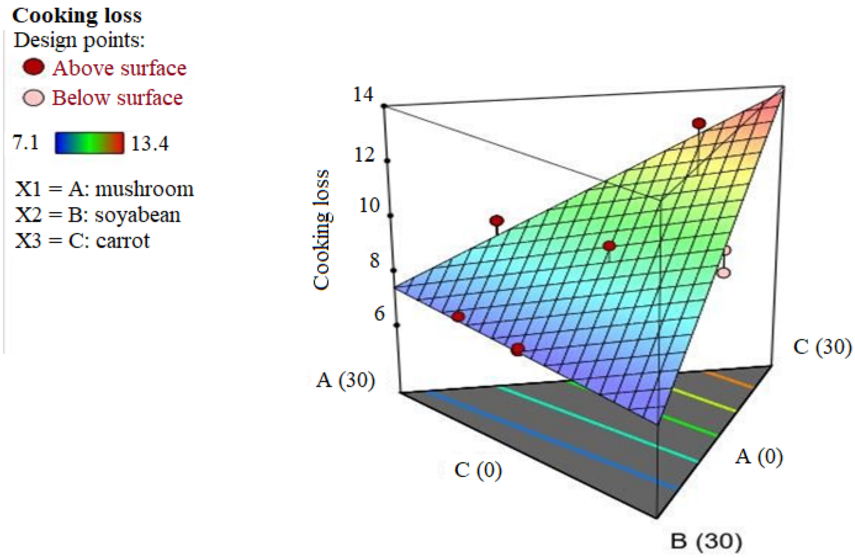

Fig. 4.2 Cooking loss

Fig. 4.2 shows the relationship between the different proportion of mushroom, soybean and carrot in the mixture with cooking loss in the sausage. The cooking loss values were found to be higher in sample with higher carrot percent. This could be due to formation of comparatively less stable emulsion in the formulations containing carrot Zargar *et al.* (2017a) also observed an increasing trend in the cooking loss of the chicken sausages with increasing levels of carrot. Increase in cooking loss was noticed up to 15% carrot incorporated turkey meat sausages as compared to control (Reddy *et al.*, 2020).

Fig. 4.2 indicates that cooking loss increases with increase in proportion of carrot. The product with higher amount of soybean has lower cooking loss which is favorable. The cooking loss of the desired product was 8.37% and the mushroom: soybean: carrot ratios were 20:5:5.

4.4.2 Folding test

Folding test is a simple and fast method of predicting the textural quality of gel composite products such as sausages and meatballs. Higher the proportion of carrot, lower was the score of folding tests. The folding test results revealed that replacement of meat by vegetable mixture containing higher amount of soybean was more preferred. ANOVA for folding test in mixture quadratic and extra term model is shown in Table 4.6.

Table 4.5 ANOVA for folding test in mixture quadratic and extra term model

Source	Sum of square	df	Mean Square	F-value	P-value	
Model	3.90	6	0.6508	3.160	0.0020	Significant
Linear Mixture	0.1561	2	0.0780	0.3796	0.7064	
AB	2.22	1	2.22	10.79	0.0304	
AC	2.90	1	2.90	14.08	0.0199	
BC	1.23	1	1.23	5.96	0.0710	
ABC	1.58	1	1.58	7.69	0.0501	
Residual	0.8225	4	0.2056			
Lack of Fit	0.3225	1	0.3225	1.93	0.2584	Not significant
Pure Error	0.5000	3	0.1667			
Cor Total	4.73	10				

The Model F – value of 3.16 implies the model is significant. Values of Prob> F less than 0.0500 indicates model term are significant. In this case A, B, C, AB, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model as shown in the Fig. 4.3.

The Lack of Fit F-value of 1.93 implies the Lack of Fit is not significant relative to the pure error. Non – significant lack of fit is good; we want the model to fit.

Final equation in terms of L Pseudo-component

Folding test = +8.42×A +7.10×B +8.11×C -22.45×AB-25.65×AC-20.42×BC+46.90×ABC

Where, A= Mushroom, B= Soybean, C= Carrot

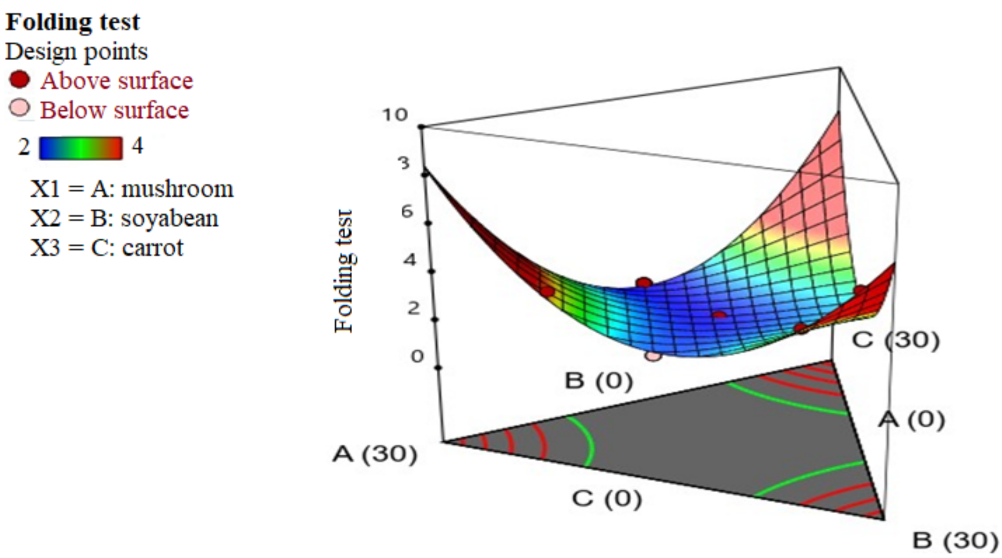

Fig. 4.3 Folding test

The 3-D plot shows the relationship between the different proportions of mushroom, soybean and carrot with folding test of the prepared sausage. The folding test score increases with increase in the proportion of soybean and mushroom while increase of carrot proportion decreases the score.

4.4.3 Fat retention

Retention of moisture and fat specifically shows how well the juices are retained in system. The lowest and highest value observed for fat retention was 63.2 and 75.9% respectively. Increase in the amount of mushroom increase fat retention while increase in amount of carrot decreased fat retention.

It has also been reported that soy protein acts as fat-encapsulating agent by supplementing myosin and acto-myosin and prevents fat separation during cooking (Das *et al*., 2008). This result is also supported by the findings of Serdaroglu and Ozsumer (2003), who reported improved moisture and fat retention in cooked beef sausages using soy, gluten and whey proteins. It indicates that the non-meat ingredients (SPI, gluten) actively involved with meat proteins to hold fat and water. ANOVA for fat retention in mixture quadratic and extra term model is shown in Table 4.6.

Table 4.6 ANOVA for fat retention in mixture quadratic and extra term model

Source	Sum of square	df	Mean square	F-value	P-value	
Model	82.78	5	16.56	4.53	0.0040	Significant
Linear Mixture	15.79	2	7.89	2.16	.2110	
AB	0.1002	1	0.1002	0.0274	0.8750	
AC	21.78	1	21.78	5.96	0.0586	
BC	19.49	1	19.49	5.33	0.0690	
Residual	18.29	5	3.66			
Lack of Fit	9.15	2	4.58	1.50	0.3530	Not significant
Pure Error	9.14	3	3.05			
Cor Total	101.07	10				

The Model F–value of 4.53 implies that there is a 6.15% chance that a Model F-value this large could occur due to noise.

Values of Prob> F less than 0.0500 indicates model term are significant. In this case A, B, Care significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

The Lack of Fit F-value of 1.50 implies the Lack of Fit is not significant relative to the pure error. Non – significant lack of fit is good; we want the model to fit.

Final equation in terms of L Pseudo-components:

Fat retention (%) = +74.04×A +68.44×B +40.90×C -3.54×AB+52.25×AC+65.66×BC

Where, A= Mushroom, B= Soybean, C= Carrot

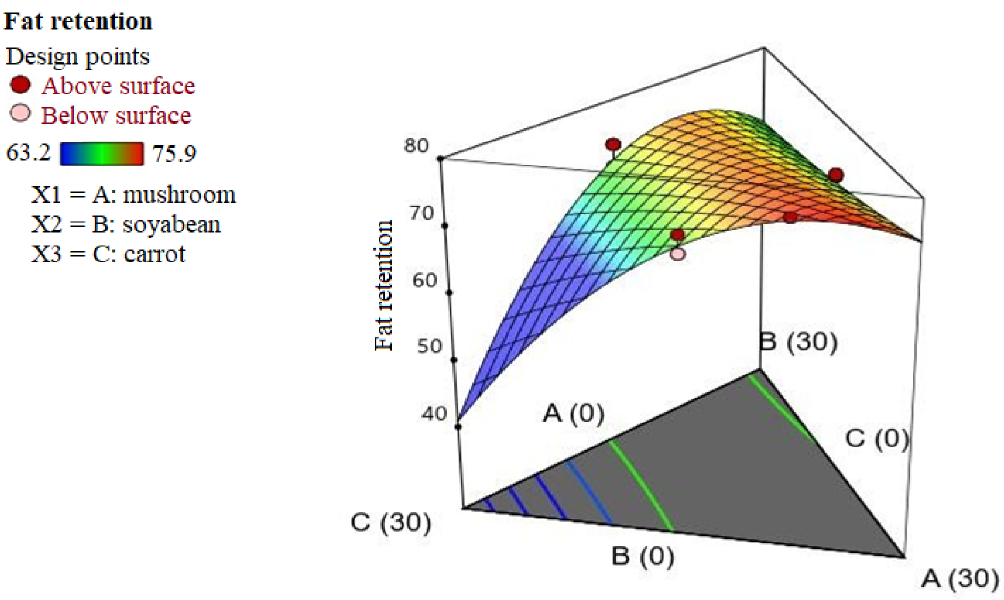

Fig. 4.4 Fat retention

The 3-D plot shows the relationship between the different proportions of mushroom, soybean and carrot with fat retention of the prepared sausage. The fat retention increases with increase in the proportion of mushroom while increase of carrot proportion decreases the retention.

4.4.4 Moisture retention

Moisture retention value represents the amount of moisture in the cooked product. The lowest and highest value observed for moisture retention was 49.6% and 60.1% respectively. The addition of carrot fiber improved water binding capacity of pork sausage (Møller *et al.*, 2011). Verma *et al.*, (2013) have reported that fiber retains water and improves moisture retention. Soy proteins are hydrophilic which hold moisture and fat by creation of an adhesive gel matrix and consequently bring about stabilization for emulsion (Serdaroglu and Ozsumer, 2003). Similar results were reported by Zaini *et al.,* (2020) where the addition of 5% albedo orange to sausage resulted in a rise of moisture retention up to 75.68%, whereas the control sausage had moisture retention of 73.61%. ANOVA for moisture retention for mixture quadratic and extra term model is shown in Table 4.7.

Table 4.7 ANOVA for moisture retention for mixture quadratic and extra term model

Source	Sum of square	df	Mean Square	F-value	P-value	
Model	171.26	5	34.25	16.17	0.0042	Significant
Linear Mixture	125.25	2	62.63	29.57	0.0017	
AB	27.66	1	27.66	13.06	0.00153	
AC	13.39	1	13.39	6.32	0.0535	
BC	40.70	1	40.70	19.22	0.0071	
Residual	10.59	5	2.12			
Lack of Fit	8.72	2	4.36	7.02	0.0739	Not significant
Pure Error	1.87	3	0.6217			
Cor Total	181.85	10				

The Model F–value of 16.17 implies the model is significant. There is only a 0.42% chance that a Model F-value this large could occur due to noise.

Values of Prob>F less than 0.0500 indicates model term are significant. In this case A, B, C, AB, BC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

The Lack of Fit F-value of 7.02 implies the Lack of Fit is not significant relative to the pure error. There is a 7.39% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad—we want the model to fit. This relatively low probability (<10%) is troubling.

Final equation in terms of L Pseudo-components:

$$\text{Moisture retention (\%)} = +\,47.61\times A + 38.90\times B + 30.65\times C + 58.87\times AB + 40.96\times AC + 94.87\times BC$$

Where, A= Mushroom, B= Soybean, C= Carrot.

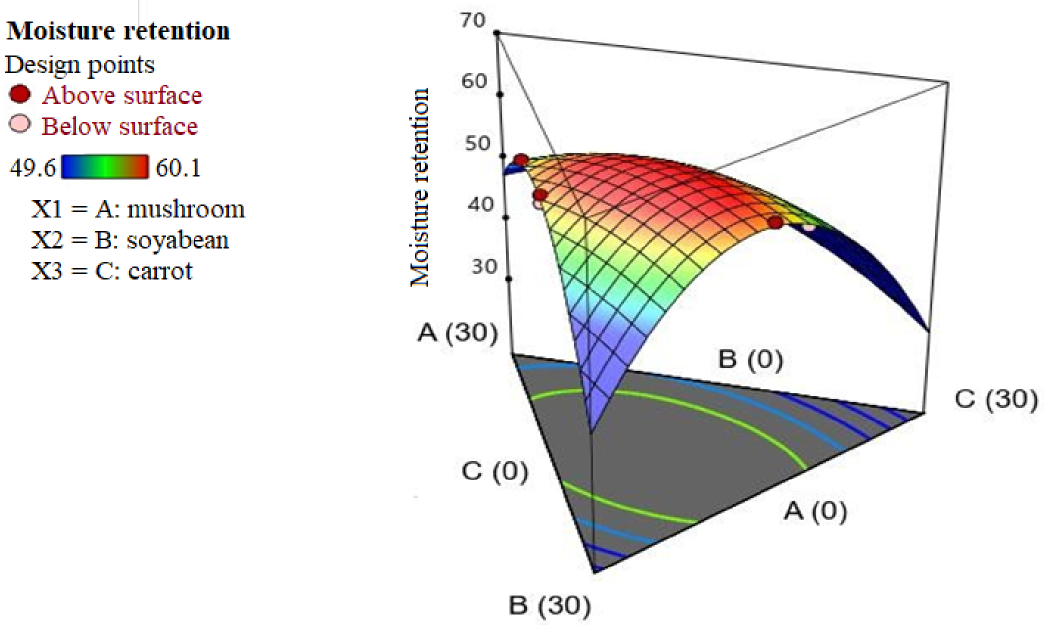

Fig. 4.5 Moisture retention

The 3-D plot shows the relationship between the different proportions of mushroom, soybean and carrot with moisture retention of the prepared sausage.

The moisture retention increases with increase in the proportion of mushroom while increase of carrot proportion decreases the retention.

From the solution of desirability table obtained from Design Expert® the sample with high proportion of mushroom had higher desirability. The ratio of mushroom: soybean: carrot in the selected sample was 20:5:5 and the desirability were found to be 0.732.

From the solution of desirability table obtained from Design Expert® the sample with high proportion of mushroom had higher desirability. The ratio of mushroom: soybean: carrot in the selected sample was 20:5:5 and the desirability were found to be 0.732. Selected treatment based on the desirability with desire and expected response is shown in Table 4.8.

Table 4.8 Selected treatment based on the desirability with desire and expected response

Run	Mushroom	soybean	carrot	Cooking loss	Folding test	Moisture retention	Fat retention	Desirability	
1	20	5	5	8.37	3.10	57.05	74.81	0.732	Selected
2	5	20	5	7.99	2.88	57.19	73.13	0.680	
3	5	5	20	11.56	3.12	51.57	64.01	0.211	

But the sensory evaluation showed the sample B was the most preferred in terms of the sensory attributes (color, flavor, taste, texture and overall acceptance). The ratio of mushroom: soybean: carrot in the sample B was 5:20:5 and the desirability was found to be 0.68. So, the sample with second desirability score was accepted as best formulation. The cooking loss, folding test, moisture retention and fat retention of the accepted sample was found to be 7.99, 2.8, 57.19 and 73.13% respectively.

4.5 Sensory evaluation of 30% vegetable incorporated sausage

From preliminary sensory evaluation and physiochemical analysis of sausage incorporated with 10, 20, 30 and 40% vegetable mixture, the best formulation was found to be 30%. Sausage with 30% incorporated vegetables had high mean score in odor, texture, flavor and overall acceptability.

Further, the sensory evaluation of 100% chicken sausage and 30% vegetable incorporated sausage as designed by D-optimal mixture design were performed. From the design 11 runs were developed, among which only three were selected for sensory evaluation based on physicochemical analysis (cooking loss, folding test, moisture retention and fat retention) and remaining eight formulations were rejected.

Fig. 4.6 below shows the mean score of sensory acceptability of 100% chicken sausage and sausage incorporated with 30% vegetable mixture. The addition of vegetables into sausages seems to be acceptable by the panelists since all sausage formulations obtained mean scores above 6.00 in all evaluated sensory attributes. Sample C (100% chicken sausage) was taken as control.

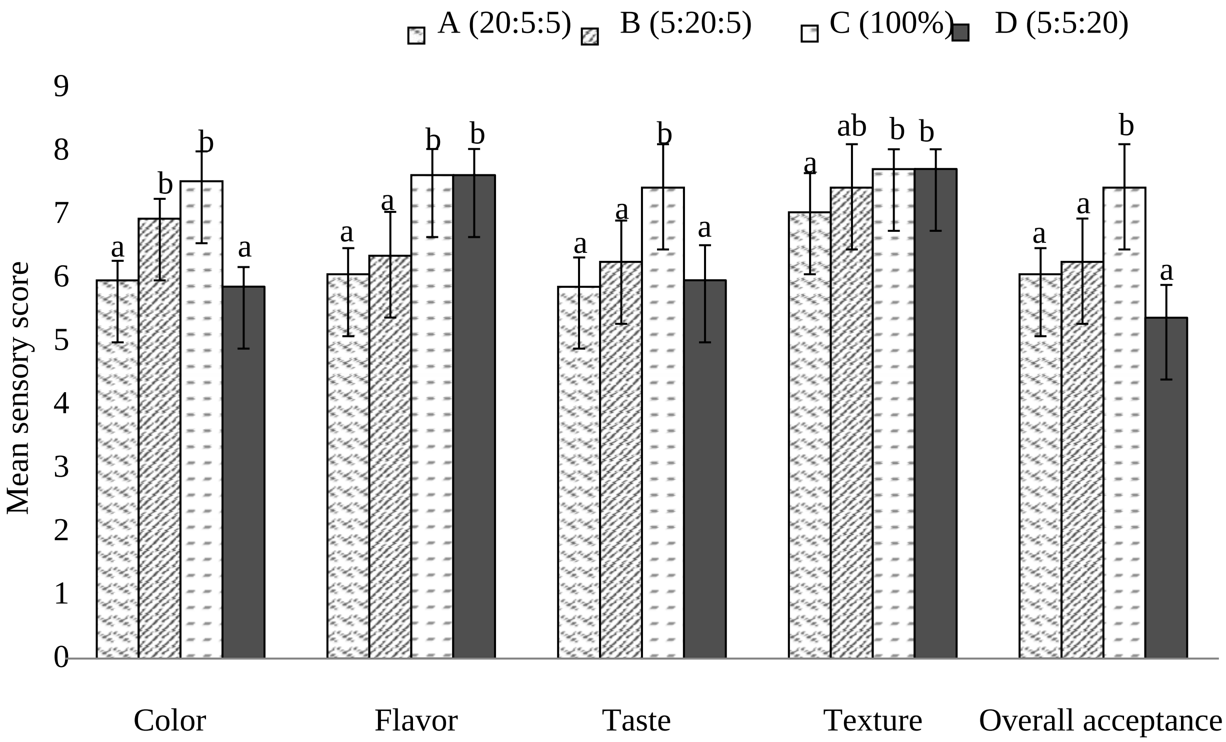

Fig. 4.6 Mean scores of sensory attributes

4.5.1 Color

From two-way ANOVA, it was found that there was no significant difference (p>0.05) in color between samples A and D. Similarly, samples B and C show no significant difference (p>0.05) while they differ from A and D.

The mean score for color of the samples A, B, C and D was found to be 6.1, 7.1, 7.7 and 6.0 with standard deviation 0.99, 0.48, 0.51, and 0.51 respectively. Comparing the 30% vegetable incorporated sausage sample with control, sample C (control) was more preferred. After sample C, second best score was of sample B followed by A and D.

In processing, color has been identified as the single most important factor of meat products that influences consumer buying decision and affects their perception of the freshness of the product (Evivie *et al.*, 2015).

Choi *et al.* (2010) found that the addition of isolated soy protein to sausages resulted increase in lightness because of the brightness of isolated soy protein as compared to meat protein. Also, Yasarlar *et al.* (2007) reported an increase in yellowness after incorporation of corn, oat and rye bran which was attributed to the presence of carotenoid pigments.

4.5.2 Flavor

From two-way ANOVA, it was found that A and B ($p<0.05$) and C and D ($p<0.05$) were significantly similar in sensory score of the flavor attribute.

The mean score for flavor of the samples A, B, C and D was found to be 6.2, 6.5, 7.8 and 7.8 with standard deviation 0.42, 0.42, 0.51 and 0.51 respectively. Comparing them, sample C and D were found to be most superior in terms of flavor than other samples. Likewise, the least mean score was obtained for the sample A.

Para (2014) observed increase in the appearance and color and flavor scores of Chinese style sausages incorporated with carrot and onion. According to Akesowan (2008), the quality of light pork sausage containing konjac gel was improved by incorporating 2% SPI levels, which produced a juicy and appreciable flavor.

4.5.3 Taste

The statistical analysis two-way ANOVA showed that among 4 sample A and B were significantly similar ($p<0.05$). On the other hand D and C were also significantly similar ($p<0.05$).

The mean score for taste of samples A, B, C and D were found to be 6.0, 6.4, 7.6 and 6.1 with standard deviation 0.84, 0.67, 0.51 and 0.78 respectively. Sample C had better score than other samples. Among the 30% vegetable incorporated sausages, sample B had better score.

The juiciness may increase significantly with increasing levels of incorporation of carrot. The scores were highest for samples containing 18% level of carrot. Higher juiciness scores in the products with increase in the level of carrot could be possibly due to high moisture content of carrot. An increase in moisture levels has been reported to increase juiciness in beef patties (Serdaroglu, 2006). Also, the water holding capacity of oyster mushroom is responsible for retaining juiciness of sausages (Cengiz and Gokoglu, 2007).

4.5.4 Texture

From two-way ANOVA statistical analysis, samples C and D shows no significant difference ($p>0.05$) in their texture attribute, while samples A and B were significantly different ($p<0.05$).

The mean score for mouth feels after taste of the samples A, B, C and D was found to be 7.2, 7.6, 7.9 and 7.9 respectively with standard deviation of 0.48, 0.51, 0.42 and 0.67 respectively. From the data, it was clear that samples C and D were not significantly different. Comparing them, sample C and D were found to be most superior in terms of flavor than other samples followed by sample B.

The addition of vegetables influences the hardness, springiness, cohesiveness, gumminess, and chewiness of sausages. Non meat protein presence leads to a better protein network, which has a better resistance against compression (Youssef and Barbut, 2011).

Based on the texture attributes, the sausages added with fresh oyster mushroom have significantly improved the acceptability towards the texture of sausages. This might be influenced by the biting texture of oyster mushroom (Buigut, 2001).

This result was also supported by Syuhairah *et al.*, (2016) on the inclusion of oyster mushroom powder in chicken sausages. The hardness of control sample might due to the protein content of chicken meat. The presence of myofibrillar protein in meat products might contribute the formation of gel (Sun and Holley, 2011). The higher protein content provides the harder gel, thereby producing the highest hardness value to the sausages without addition of fresh mushroom and other vegetables.

4.5.5 Overall acceptance

The statistical analysis two-way ANOVA showed that the samples A, B and D were not significantly different ($p>0.05$) while sample C was found to be significantly different ($p<0.05$) than the other samples in terms of overall acceptance.

The mean score for Overall acceptance of the samples A, B, C and D was found to be 6.2, 6.4, 7.6 and 5.5 respectively with standard deviation 0.82, 0.47, 0.63 and 0.48 respectively. Here sample C got highest score as it was more preferred whereas B got second best score. Among 3 vegetable incorporated samples, sample B was more preferred.

Several studies have shown the sensory properties of meat products are negatively impacted as the concentration of vegetable additives increases. For example, the sensory parameters of beef patties have been shown to decrease with increased flaxseed content (Bilek and Turhan, 2009). Furthermore, Turhan *et al.* (2005) indicated that beef burgers with increasing hazelnut pellicle content results in decreased overall acceptability. Ammar

(2012), who concluded that the incorporation of mustard flour into beef burger patties had no negative effect on sensory properties of beef burger. Shokry (2016) also reported that the beef burger containing quinoa flour exhibited an excellent sensory acceptance especially the texture, tenderness and juiciness which appeared to be reinforcement by adding quinoa flour to the beef burger.

The order of superiority can be summarized as;

Color: Sample C >Sample B > Sample A> Sample D

Flavor: Sample C> Sample D > Sample B >Sample A

Taste: Sample C> Sample B>Sample D > Sample A

Texture: Sample C> Sample D > Sample B > Sample A

Overall acceptance: Sample C > Sample B >Sample A > Sample D

From above statistical scores of all sensory attributes, it was found that sample B was the most preferred vegetable incorporated sausage in terms of the attributes (color, flavor, taste, texture and overall acceptance) followed by A. Thus, sample B was further analyzed for proximate and storage stability.

4.6 Proximate composition

Values of proximate composition of optimized sausage are shown in Table 4.10.

Table 4.9 Proximate composition of optimized sausage

Parameters	Value*(mean±sd)
Moisture content (%)	60.47±0.80
Crude fat (%)	11.89±0.17
Protein (%)	17.55±0.01
Total ash (%)	4.17±0.01
Crude fiber (%)	0.43±0.02

*Values are the means of triplicate with standard deviation

4.6.1 Moisture content

The moisture content of 30% vegetable incorporated sausage was found to be 60.47±0.80%. The moisture content of chicken sausage ranges from 55 to 62%. Based on the results, the moisture content of the final product was within range as standard chicken sausage. This is most probably due to the incorporation of vegetables in sausage formulation, which provided a significant amount of fiber content in the products. According to Choi *et al.* (2010), addition of dietary fiber increases the moisture content of meat emulsion systems, providing higher water retention and improves emulsion stability. These results agree with those reported by Choi *et al.* (2012), who found a significant increase of moisture content in chicken frankfurter when added with pumpkin fiber.

4.6.2 Crude fat

The crude fat content of the sausage was found to be 11.89±0.17%. In a research conducted by Ahmad *et al.* (2020a), the fat content of control was 14.15% while fat content of vegetable incorporated sausage ranged from 5.84 to 7.11%. The added vegetables may have affected fat content, as moisture content relatively increased (Choi *et al*., 2012). These findings reported that replacing pork loin with hydrated oatmeal and tofu at 15% significantly lowers the amount of fat content.

Moreover, higher fat content may be due to high fat content of soybean (Yang *et al*., 2007).

4.6.3 Protein

The protein content of the product was found to be 17.55±0.01%. The main reason for low protein content in comparison to standard chicken sausage is due to the substitution of 30% chicken meat with vegetable, which lead to significant loss of protein and fat in the samples. Although the protein content of soybean is high, incorporation of carrot may have reduced the protein content of the product. Troutt *et al.* (1992) reported a similar trend in low fat beef patties containing poly dextrose and oat flour as texture modifying ingredients. The replacement of lean meat by apple pomace in mutton nugget further supported the result (Huda *et al.*, 2014).

4.6.4 Total ash

Ash content of the sausage was found to be 4.17±0.01%. This value was found to be higher than the standard chicken sausage which is due to increase in fiber content from the added vegetables. Study by Fernández *et al.* (2004) observed that the ash content increases significantly with the addition of dietary fiber such as incorporation of dietary fiber from lemon albedo in low-fat sausage. Similar results were obtained by Choi *et al.* (2012) who studied the physicochemical properties of reduced fat frankfurters by partially substituting pork back fat with less fiber.

4.6.5 Fiber

The fiber content of 30% vegetable incorporated sausage was found to be 0.43±0.02%. According to Ahmad *et al.* (2020a), the crude fiber content of vegetable incorporated sausage ranged from 1.21-2.92%, while the control contained 0.80%. The result of our study is within the range. Increase of fiber content is most probably due to the incorporation of vegetables in sausage formulation, which provided a significant amount of fiber content in the products.

4.7 Beta-carotene

Carotenoids represent a large group of phytochemicals that may contribute to health and disease prevention (Otles and Cagindi, 2007). Beta carotene present in carrot is strong anti-carcinogenic and also prevents heart attack, ulcers, gum disease, colitis and stroke (Kaur *et al.*, 2015a).

The carotenoid content of the product was found to be 30 μg/100 g. According to Kaur *et al.* (2015a), carotenoid content of sausage incorporate with Carrot pomace powder in different proportion ranged from 107- 342 μg/100 g, while the carotenoid content of control was 32.17 μg/100 g. This value may be affected by the type of carrot used. The carotenoid content of fresh carrot is 39.6 mg/100 g while dried carrot powder contains 23.9 mg/100 g (Krishan Datt Sharma *et al.*, 2012b). Also, the difference in the values may be due addition of mushroom and soybean in our product. Saleh and Ahmed (1998) conducted studies on ground beef patties formulated with carrot and reported that incorporation of boiled carrot (100 g/kg meat) improved the color, texture and nutritive value of beef patties; whereas, carrot powder improved cooking yield, color, texture and vitamin-A.

4.8 Antioxidant activity

The use of antioxidant helps to minimize rancidity, retard the formation of toxic oxidation products, maintain nutritional quality and increase the shelf life of food products (Fukumoto and Mazza, 2000). Incorporation of carrot, soybean and mushroom would help to deliver the benefits of high antioxidant properties of these vegetables into sausage samples. In this study, DPPH radical scavenging activity of sausage was analyzed. Based on the result, the observed DPPH value was found to be 46.76%. The high DPPH value in samples indicates the higher ability of antioxidant compounds in these samples to lose hydrogen and possibly acting as a primary antioxidant. The samples also possibly have better reaction with free radicals, particularly of the peroxy radicals, which are the major propagator of the auto oxidation chain of fat, thereby, abort the chain reaction. Susiloningsih *et al.* (2016) reported that antioxidant activity of carrot soyghurt increased for concentration of added carrot juice, also fermentation time increased in carrot soyghurt.

In study done by Ahmad *et al.* (2020a), the antioxidant activity of the vegetable (capsicum, carrot, spinach, purple cabbage and mushroom) incorporated sausage was higher than control. The antioxidant activity of control was 46% while the antioxidant activityofvegetableincorporatedproductrangedfrom45-86%.Pizzocaro *et al.* (1998) reported that addition of 2% carrot and 10% spinach improved oxidative stability of poultry hamburger. The antioxidant in vegetables varies considerably due to several factors; genetics, cultivation practices, environmental, growing conditions, maturation, storage, and processing.

4.9 Microbial analysis

The quality attributes of sausages could deteriorate due to microbial growth. This can lead to major public health hazards and economic loss in terms of food poisoning and meat spoilage. Hence, the incorporation of vegetables into sausage formulation anticipated to serve both functions; antioxidant and antimicrobial properties useful for preserving meat quality, extending shelf-life and preventing economic loss (Sallam *et al.*, 2004).

The initial salmonella and Coliform count of 30% vegetable incorporated sausage on 0 day was nil. The total plate count of sausage was 1 log cfu/g on the 1st day. On 12 th day, the total plate count increased to 6 log cfu/g, which is below the typical spoilage level of ~7.0 log cfu/g (Osburn and Keeton, 1994). All the microbial counts were within acceptability limits up to 12th day of storage. TPC crossed acceptability limit after 12th day of storage. Increase in total plate count after 12th day could be due to less use of nitrite as preservative. Also, cross contamination during preparation of the product may have occurred.

Fayaz Ahmed Zargar *et al.* (2014) observed that chicken patties prepared by replacing spent hen meat with 5% sorghum flour, 10% barley flour and 5% pressed rice flour recorded higher total plate count and psychrophilic count, which increased significantly during storage up to 35 days of storage. Mushrooms contain some bioactive compounds such as rutin, gallic acid and catechin, which contain a high antimicrobial effect. The trend was similar with the TPC result obtained where incorporation of vegetables in sausage lowered the yeast and mold count. The inhibition of yeast and mold growth was also probably due to the growth of lactic acid-producing bacteria under anaerobic packaging conditions during refrigerated storage (Bradford *et al.*, 1993). Change in total plate count during storage of optimized sausage is shown in the Fig. 4.7.

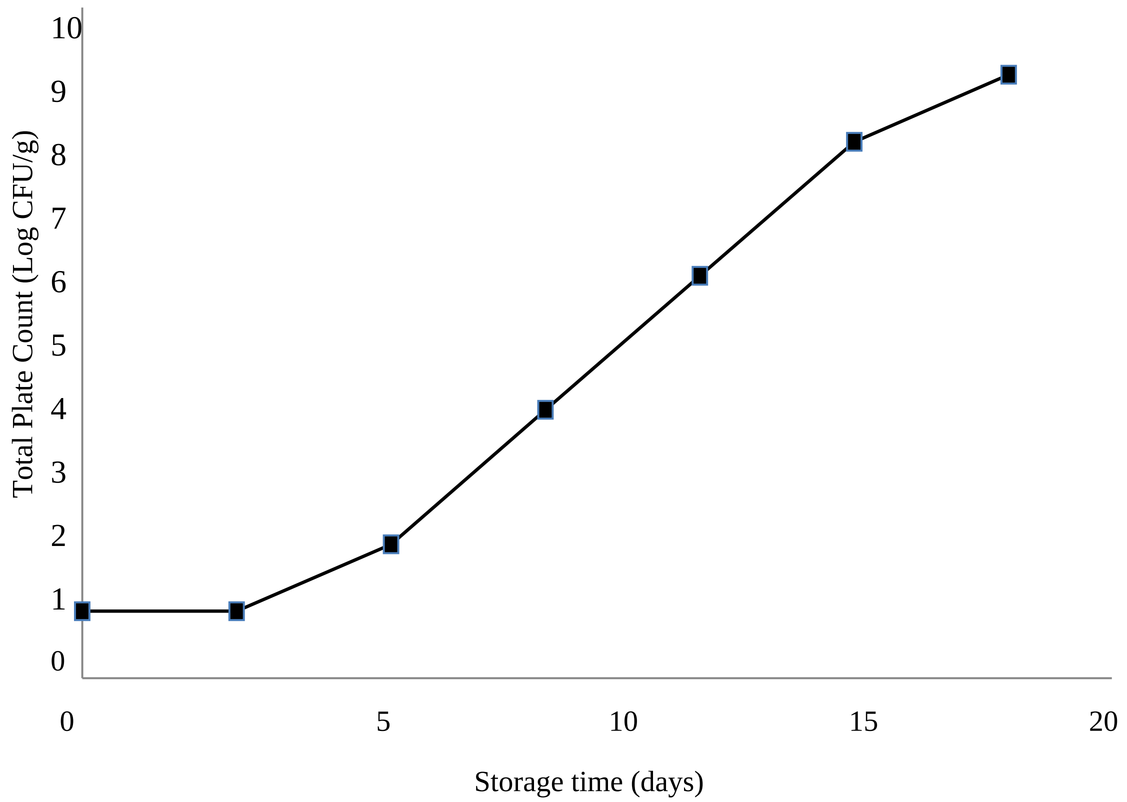

Fig. 4.7 Change in total plate count during storage

4.10 Peroxide value

The peroxide value (PV) is used as an indicator of the primary oxidation in sausage samples. A gradual increase in PV was observed for all samples throughout the storage from day 0 until day 18. As the storage time increased, the PV also increased from 3.05 at day 0 to 11.46 at day 18, indicating that lipid oxidation was increasing in the sample. PV lower than 25 $MeqO_2$/kg, is the limit of acceptability for fatty foods (Abdulhameed *et al.*, 2014).

Sausages incorporated with vegetables will have a lower PV comparative to the chicken sausage probably due to free radical scavenging antioxidants interfere with the initiation or propagation steps of lipid oxidation reactions by scavenging lipid radicals and forming low-energy anti-oxidant radicals that do oxidation of unsaturated fatty acids (Maqsood and Benjakul, 2010). Devatkal and Naveena (2010) observed that the peroxide value increased during the refrigerated storage in cooked goat meat patties added with different plant extract. Disha *et al.* (2020) found a significant increase in peroxide value of control and carrot fiber enriched sausage with an increase in storage period. The results indicate that dried carrot pomace was effective in controlling the lipid oxidation in chicken sausages during refrigerated storage. Presence of bioactive compounds in carrot pomace which exert an anti-oxidant effect may have caused less increase of peroxide value in carrot incorporated sausages (Disha *et al.*, 2020).

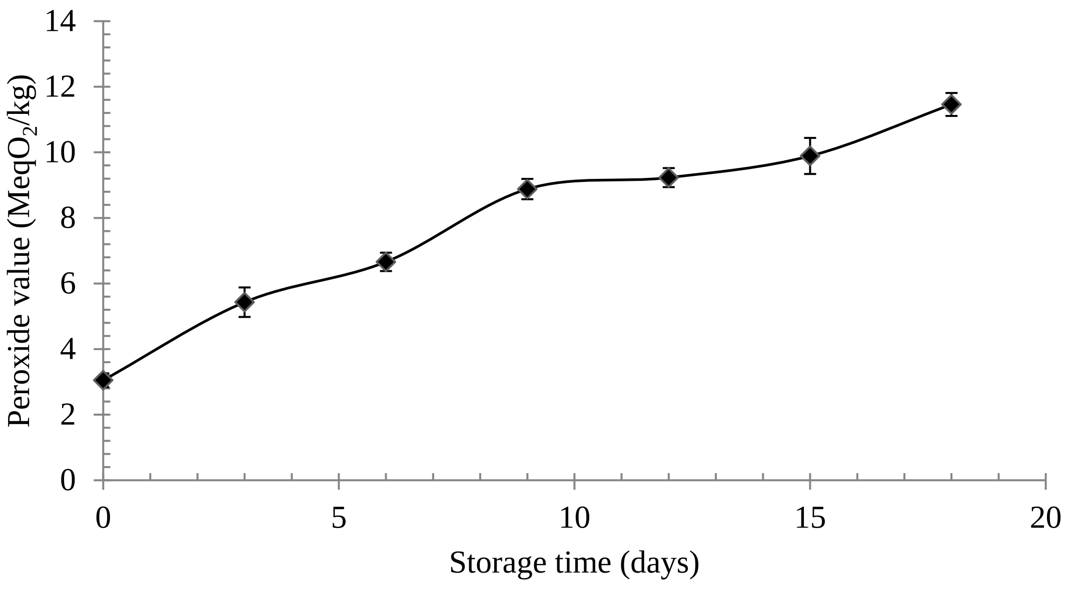

Fig. 4.8 Change in peroxide value during storage period

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the results of the present study, the following conclusions are drawn:

1. Increasing the different vegetable in the sausage changes the technological and sensory properties (cooking loss, fat retention, moisture retention and folding test) of the sausage.

2. 30% vegetable incorporation in sausage can be successfully done with satisfactory technological and sensory attributes.

3. Based on physicochemical properties, optimal mixture of vegetables (mushroom: soybean: carrot) in 30% substitution in sausage was found to be 20:5:5 percent respectively.

4. Sausage with vegetable proportion (mushroom: soybean: carrot) 5:20:5 was most preferred by the sensory panelists.

5. The optimized product from sensory analysis has a proximate composition of 60.47, 11.89, 17.55, 0.43 and 4.17% moisture, crude fat, crude protein, crude fiber and total ash respectively.

6. The carotenoid content of the optimized product was found to be 30 μg/100 g and DPPH radical scavenging activity of sausage was found to be 46.76%.

7. There was a gradual increase in Total plate count (TPC) reaching maximum in day 18 (9) while salmonella and coliform were not detected up to day18.

8. Peroxide value was also found to be increase during refrigeration storage of sausage from 3.05 MeqO_2/kg at day 0 and 11.46 MeqO_2/kg at day18.

9. Vegetable incorporation in sausage showed significant effect on response parameters, the observed models accurately predicted the response parameters (cooking loss, folding test, moisture retention and fat retention) indicated by higher R^2 value.

10. Optimized vegetable incorporated sausage had low shelf-life than market product because sodium nitrate was not used during the preparation of the product.

5.2 Recommendations

1. Mushroom: soybean: carrot in 5:20:5 proportions is recommended to prepare 30% vegetable substituted sausage.

2. Similar study with different vegetable combination in sausage can be carried out.

3. Effect on different technological, nutritional and functional properties, storage stability in different packaging materials and storage condition can be studied.

Summary

Vegetable incorporated chicken sausage was prepared in the lab of CCT College. Primary screening of different vegetables (mushroom, soybean and carrot) proportion was done with equal proportion of all vegetables in all percentage level (10, 20, 30, and 40).

From sensory evaluation and physicochemical properties, the superior percentage was found to be 30%. The moisture retention, fat retention cooking loss and folding test of 30% vegetable incorporated sausage was found to be 55.5%, 70.2%, 8.7% and 3 respectively. Formulation of 30% vegetable was designed by D-optimal mixture by which 11 runs were formulated. Among 11 runs, only 3 formulations containing all vegetables were selected for sensory and physicochemical analysis.

Sensory evaluation of three selected runs was compared with 100% chicken sausage as control product. From the solution of desirability table obtained from Design Expert® the sample with high proportion of mushroom showed higher desirability. The ratio of mushroom: soybean: carrot in the selected sample was 20:5:5 and the desirability was 0.732. But the sensory evaluation showed the ratio of mushroom: soybean: carrot in most preferred product was 5:20:5 with desirability 0.68. Hence, the sample with second desirability score was accepted as best formulation. The cooking loss, folding test, moisture retention and fat retention of the accepted sample was found to be 7.99%, 2.8, 57.19% and 73.13% respectively.

Also, proximate analysis was performed for superior final product. The superior product had a proximate composition of 60.47, 11.89, 17.55, 0.43 and 4.17% moisture, crude fat, crude protein, crude fiber and total ash respectively. The carotenoid content of the superior product was found to be 30 μg/100 g and DPPH radical scavenging activity of sausage was found to be 46.76%. There was a gradual increase in Total plate count (TPC) reaching maximum in day 18 while *Salmonella* and coliform were not detected up to day 18. Peroxide value was also found to be increase during refrigeration storage of sausage from 3.05 $MeqO_2$/kg in day 0 and 11.46 $MeqO_2$/kg in day.

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Appendices

Appendix A

A.1 Chemicals required

- DPPH
- Other basic laboratory chemicals
- Portable water

A.2 Apparatus required

- Chopping board/table
- Cutting knife
- Bowl chopper
- Mincer
- Synthetic filling casing (2.5 cm diameter)
- Filler machine
- Refrigerator
- Cooking vessel
- Water bath
- Thermometer
- Glassware
- Packaging materials
- Sealing machine
- pH meter

Appendix B

Sensory evaluation of 30% vegetable incorporated and 100% chicken sausage

Attributes	A	B	C	D	P-value
Color	6.10 ^{a}	7.10 ^{b}	7.70 ^{b}	6.00 ^{a}	0.135
Flavor	6.20 ^{a}	6.50 ^{a}	7.80 ^{b}	7.80 ^{b}	0.524
Taste	6.00 ^{a}	6.40 ^{a}	7.60 ^{b}	6.10 ^{b}	0.687
Texture	7.20 ^{a}	7.60 ^{ab}	7.90 ^{b}	7.90 ^{b}	0.428
Overall Acceptance	6.20 ^{a}	6.40 ^{a}	7.60 ^{b}	5.50 ^{a}	0.059

Changes in total plate count and peroxide value during storage

Parameters	0 day	3rdday	6thday	9thday	12thday	15thday	18thday
Salmonella count(log cfu/g)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Coliform count (log cfu/g)	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Total plate count (log cfu/g)	1	1	2	4	6	8	9
Peroxide value (MeqO2/kg)	3.05	5.43	6.66	8.88	9.23	9.89	11.46

Proximate composition of raw materials

Parameters	Carrot	Mushroom	Soybean	Meat
Moisture (%)	89.89	89.89	9.26	65.26
Fat (%)	0.18	0.5	16.50	3.57
Protein (%)	0.38	9.92	27.0	31.02
Total Ash (%)	3.38	1.20	4.60	-
Crude Fiber (%)	0.38	0.57	0.43	-

Preliminary sensory

Variate: Color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	27.7200	6.9300	17.97	<.001
Panelists	9	3.5200	0.3911	1.01	0.447
Residual	36	13.8800	0.3856		
Total	49	45.1200			

Variate: Flavor

Source of variation	d.f.	s.s.	m.s.	v.r.F pr.
Samples	4	36.8800	9.2200	23.18<.001
Panelists	9	5.7800	0.6422	1.610.148
Residual	36	14.3200	0.3978	
Total	49	56.9800		

Variate: OA

Source of variation	d.f.	s.s.	m.s.	v.r.F pr.
Samples	4	52.9200	13.2300	38.79<.001
Panelists	9	6.4200	0.7133	2.090.057
Residual	36	12.2800	0.3411	
Total	49	71.6200		

Variate: Taste

Source of variation	d.f.	s.s.	m.s.	v.r.F pr.
Samples	4	45.6000	11.4000	29.31<.001
Panelists	9	6.9000	0.7667	1.970.072
Residual	36	14.0000	0.3889	
Total	49	66.5000		

Variate: Texture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	4	1.7200	0.4300	1.51	0.221
Panelists	9	3.5200	0.3911	1.37	0.238
Residual	36	10.2800	0.2856		
Total	49	15.5200			

Final sensory evaluation

Variate: Color

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	20.0750	6.6917	14.25	<.001
Panelists	9	7.2250	0.8028	1.71	0.135
Residual	27	12.6750	0.4694		
Total	39	39.9750			

Variate: Flavor

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	3	21.4750	7.1583	13.08	<.001
Panelists	9	4.5250	0.5028	0.92	0.524
Residual	27	14.7750	0.5472		
Total	39	40.7750			

Variate: OA

Source of variation	d.f.	s.s.	m.s.	v.r.F pr.
Samples	322.875		7.625	6.560.002
Panelists	97.525		0.836	0.720.687
Residual	2731.375		1.162	
Total	3961.775			

Variate: Taste

Source of variation	d.f.	s.s.	m.s.	v.r.F pr.
Samples	316.2750		5.4250	6.240.002
Panelists	98.2250		0.9139	1.050.428
Residual	2723.4750		0.8694	
Total	3947.9750			

Variate: Texture

Source of variation	d.f.	s.s.	m.s.	v.r.F pr.
Samples	33.3000		1.1000	5.210.006
Panelists	94.1000		0.4556	2.160.059
Residual	275.7000		0.2111	
Total	3913.1000			

Appendix C

List of Plate

Plate 1 Preparation of carrot slice

Plate 2 Sensory evaluation by the Panelists

Plate 3 Frying of final sausage product

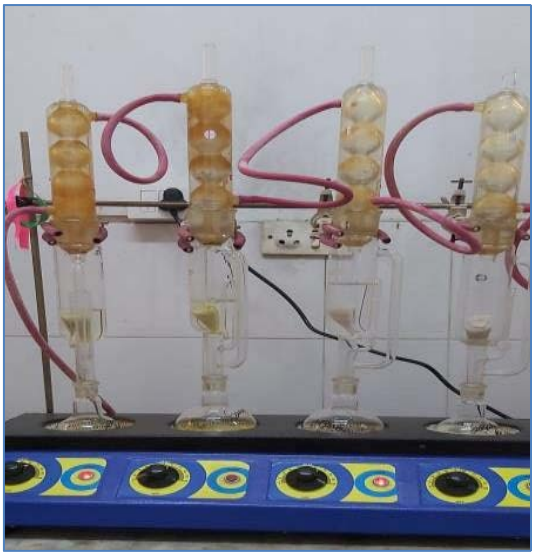

Plate 4 Extraction of crude fat from the product

Plate 5 Analysis of cooking loss

Plate 6 Preparation of meat

Plate 7 Mincing of meat

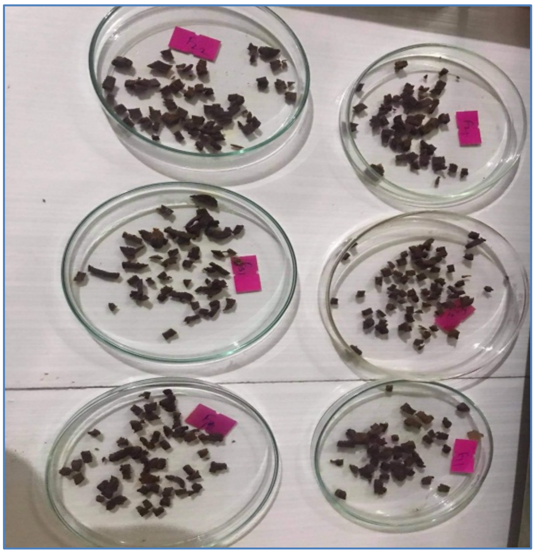

Plate 8 Moisture analysis of sausage

Plate 9 Microbial analysis of sausage

Plate 10 Vegetable incorporated sausage

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