EFFECT OF VEGETABLE INCORPORATION ON THE NUTRITIONAL AND SHELF LIFE QUALITIES OF CHICKEN-BASED SAUSAGE-LIKE PRODUCT

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by

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LIST OF ABBREVIATIONS

a*	Redness
b*	Yellowness
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
C	Carrot
C°	Celcius
CP	Capsicum
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAO	Food and Agriculture Organization
GAE	Gallic acid equivalent
L*	Lightness
OM	Oyster mushroom
PC	Purple cabbage
PV	Peroxide value
RAE	Retinol activity equivalents
RDA	Recommended dietary allowances
S	Spinach
SA%	Scavenging activity
TBHQ	Tert-butylhydroquinone
TBARS	Thiobarbituric acid reactive substances
TCEP-HCl	Tris (2-carboxyethyl) phosphine hydrochloride
USDA	United States Department of Agriculture

KESAN PENAMBAHAN SAYURAN KE ATAS KUALITI PEMAKANAN DAN JANGKA HAYAT PRODUK SEAKAN SOSEJ BERASASKAN AYAM

ABSTRAK

Ciri-ciri kualiti dan kestabilan produk seakan sosej berasaskan ayam yang ditambah sayur-sayuran terpilih; capsicum, lobak merah, bayam, kubis ungu dan cendawan tiram telah dikaji. Pada fasa pertama, proses saringan telah dijalankan dengan menggunakan pelbagai peratusan (30%, 40%, dan 50%) sayur-sayuran dalam formulasi sosej. Fasa kedua melibatkan pemilihan peratusan formulasi terbaik sosej, yang kemudiannya dianalisis dari segi mikrostruktur, komposisi kimia, dan sifat-sifat antioksidan. Fasa terakhir adalah kajian kestabilan penyimpanan sampel sosej pada suhu 4°C selama 21 hari. Sebanyak 16 sampel termasuk kawalan telah disediakan dan dianalisis dalam kajian awal. Hasil kajian menunjukkan bahawa 14 sampel mempunyai julat skor 4.00-5.00 untuk ujian lipatan, menunjukkan kekuatan gel yang baik telah dihasilkan oleh sampel. Jenis dan peratusan sayur-sayuran dimasukkan ke dalam formulasi sosej tidak menjejaskan pH secara signifikan ($P<0.05$) dan memberikan kehilangan memasak yang lebih rendah berbanding sampel kawalan. Sosej didapati menunjukkan peningkatan dalam keupayaan memegang air pada 40% penggabungan sayur-sayuran. Dalam hal kekerasan dan daya ricih, sosej kawalan memberikan nilai tertinggi, dan menunjukkan penurunan secara beransur-ansur dengan peningkatan peratusan sayur-sayuran. Warna sosej dihasilkan menunjukkan kepelbagaian dengan signifikan ($P<0.05$) bergantung kepada warna asal sayur-sayuran yang digunakan. Keputusan penilaian deria dalam kalangan panel menunjukkan bahawa sosej dengan capsicum, lobak merah dan cendawan tiram adalah lebih disukai.

Berdasarkan kajian awal, sosej diformulasi dengan 40% sayur-sayuran telah dipilih sebagai formulasi terbaik. Analisis mikrostruktur menunjukkan bahawa sosej dengan sayur-sayuran mempunyai mikrostruktur yang kurang padat terutamanya disebabkan oleh peningkatan kandungan kelembapan, pengurangan kandungan lemak dan pengurangan kepadatan protein. Penggabungan bayam dalam sosej memberikan nilai yang paling tinggi ke atas zat besi, kalsium, magnesium, tembaga, vitamin A dan K_1 secara signifikan ($P<0.05$) manakala sosej dengan capsicum menunjukkan vitamin C tertinggi (26.37mg/100g). Nilai DPPH dan jumlah kandungan fenolik tertinggi telah ditunjukkan oleh sosej dengan kubis ungu manakala penggabungan sosej dan bayam memberikan jumlah kandungan flavonoid yang tertinggi. Komposisi asid lemak telah diubah dengan penggabungan sayur-sayuran, iaitu peratusan asid lemak tepu diturunkan dengan signifikan ($P<0.05$) berbanding dengan sampel kawalan. Penggabungan cendawan tiram mempamerkan peratusan tertinggi asid lemak politaktepu. Pada hari terakhir penyimpanan, nilai peroksida dan nilai asid thiobarbiturik dalam sosej dengan kubis ungu berjaya melambatkan kesan pengoksidaan lipid berbanding sampel kawalan dengan signifikan ($P<0.05$), iaitu daripada 1.62meq/kg ke 0.58meq/kg dan 6.00mg MDA/kg ke 3.11mg MDA/kg. Aktiviti mikrob telah berkurang dengan penggabungan sayuran ke atas sosej yang kemudiannya dapat membantu melanjutkan hayat penyimpanan sampel. Hasil kajian mencadangkan bahawa sayur-sayuran yang digunakan berpotensi dalam meningkatkan kualiti pemakanan dan menunjukkan ciri-ciri anti pengoksidaan dalam produk seakan sosej berasaskan ayam.

EFFECT OF VEGETABLE INCORPORATION ON THE NUTRITIONAL AND SHELF LIFE QUALITIES OF CHICKEN-BASED SAUSAGE-LIKE PRODUCT

ABSTRACT

Quality characteristics and stability of chicken-based sausages-like product incorporated with selected vegetables; capsicum, carrot, spinach, purple cabbage and oyster mushroom were studied. The screening process was conducted in the first phase with various percentages (30%, 40%, and 50%) of vegetables in the sausage formulations. The second phase involved the selection of the best percentage formulation of sausages, which was further analyzed for the microstructure, chemical composition, and antioxidant properties. The final phase was the storage stability study of sausage samples at 4°C for 21 days. A total of 16 samples included control were prepared and analyzed during preliminary study. The results revealed that 14 samples had the range of 4.00-5.00 score for the folding test, indicated a good gel strength of the samples produced. Both type and percentage of vegetables incorporated into the sausage formulations did affect the pH significantly ($P<0.05$) and gave a lower cooking loss as compared to the control sample. The sausages were found to show an increase in water holding capacity at 40% incorporation of vegetable. With regards to hardness and shear force, the control sausage exhibit the highest value, and the value decrease gradually with the increase in percentage of vegetables added. The colour of sausages produced varied significantly ($P<0.05$) among samples depending on the original colour of vegetables. Sensory evaluation results demonstrated that sausages with capsicum, carrot, and oyster mushroom were more preferable among panelists.

Based on the preliminary study, sausages formulated with 40% vegetables were selected as the best formulation. The analysis of microstructure showed that sausage with vegetables had a less dense microstructure mainly due to the consequent increase in moisture content, decrease in fat content and decrease in protein density. Incorporation of spinach in sausage gave the highest value of iron, calcium, magnesium, copper, vitamin A and K_1 significantly ($P<0.05$) while sausage with capsicum exhibit the highest vitamin C (26.37mg/100g). The highest DPPH value and total phenolic content were demonstrated by the sausages incorporated with purple cabbage while incorporation of spinach gave the highest total flavonoid content. Fatty acid composition was altered with the incorporation of vegetables, where the percentage of saturated fatty acid was lowered significantly ($P<0.05$) compared with the control. Incorporation of oyster mushroom exhibited the highest percentage of polyunsaturated fatty acid. During the end of storage study, the peroxide value and thiobarbituric acid value in sausages incorporated with purple cabbage significantly ($P<0.05$) delayed lipid oxidation compared to the control from 1.62meq/kg to 0.58meq/kg and 6.00mg MDA/kg to 3.11mg MDA/kg. The microbial activity was reduced with the incorporation of vegetables which subsequently would help to extend the shelf-life of the samples. The results suggest that vegetables used are potentially useful in improving the nutritional quality and exhibiting some antioxidative properties in chicken-based sausage-like product.

CHAPTER 1

INTRODUCTION

1.1. Background of study

In recent years, the global growth of fast, convenient, and healthier food products is essentially a result of the changes in lifestyles. This trend has produced a significant influence on the meat industry, thus has commenced extensive research for the purpose of developing reduced fat and incorporating health-enhancing ingredients in meat-based products. The consumption of meat products commonly results in cardiovascular disease (CVD), coronary heart disease (CHD), and certain types of cancer, due to its contents of saturated fat, cholesterol, and salt (Lin & Huang, 2003; Desmond, 2006). The fat content in meat products helps to stabilise meat emulsions, reduce cooking loss and contribute to nutritional, textural, and sensory attributes of meat products (Candogan & Kolsarici, 2003; Yang et al., 2007; Choi et al., 2014; Baer & Dilger, 2014). Therefore, the reduced fat will render the product to become unsatisfactory in terms of the texture, flavour, and appearance (Yılmaz et al., 2002; Serdaroğlu, 2006; Yang et al., 2007). The inclusion of functional ingredients such as stabilized rice bran, non-meat proteins and hydrocolloids are used in the formulation of sausage to limit the detrimental effects of fat reduction as well as to lead it into desirable textural properties and improved the image of sausage products, especially for health conscious consumers (Weiss et al., 2010).

As one of the oldest forms of processed meat products, sausage is regularly consumed around the world and made from a variety of sources, namely beef, veal, pork, mutton, and poultry (Savic, 1985; Huda et al., 2010). Sausage is classified as oil-in-water emulsions where the continuous phase is water and soluble compound while the disperse phase is oil and protein which acts as an emulsifier (Pereira et al., 2000).

The products differ depending on the shape, length, ingredients, additives, and curing techniques such as fresh, fermented, cooked or emulsion type sausage (Essien, 2003; Wan Rosli et al., 2015). Food additives are often used to achieve desirable functions during the production such as colouring, antimicrobial, antioxidant, preservative, improved nutrition, increased emulsification, and altered flavour (Essien, 2003; Quasem et al., 2009).

Lipid oxidation is one of the most critical quality defects of meat product during the storage (Liu et al., 2009; Das et al., 2012). It was highlighted that the oxidative rancidity of fats during the storage can develop objectionable odour and flavour, deteriorate the texture, and contribute to deleterious nutritional effects, such as destroying the essential fatty acids and losing the vitamins (Ali et al., 2010; Das et al., 2012). Hence, the use of antioxidants is currently receiving more attention upon the effort to minimise rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase the shelf life of meat-based product (Das et al., 2012; Pil-Nam et al., 2015). However, the safety of synthetic antioxidants used in the food industry has been questioned (Rižnar et al., 2006; Liu et al., 2009). This has led to the research of natural antioxidants which covers many species of fruits, vegetables, herbs, spices, and cereals (Das et al., 2012) that contain significant amount of bioactive components such as flavonoids, isoflavones, soluble dietary fibers, carotene, and isothiocyanates, which may be able to provide useful health benefits beyond the basic nutrition (Kris-Etherton et al., 2002).

According to Jiménez-Colmenero et al. (2001) and Ayo et al. (2008), healthier meat-sausage formulations need to contain less saturated fat and/or promote the presence of specific healthy compounds because they might affect the quality attributes of cooked meat emulsions. The vegetable incorporated in sausage formulation can produce a fascinating combination of protein intake, value-added nutrition, and unique natural colour that helps to promote healthier sausages compared to the traditional type of sausage available in the market. In addition, regular intake of fruits and vegetables could reduce the risks of cancer, cardiovascular disease, stroke, Alzheimer disease, cataracts, and some other functional declines associated with aging (Liu et al., 2000; Liu, 2003; Hung et al., 2004).

In this study, vegetables; capsicum, carrot, spinach, purple cabbage, and oyster mushroom are selected as non-meat ingredients in the production of sausage, due to their potential antioxidant effect, bioactive composition, and natural colouring properties. Capsicum is a good source of vitamins C and E as well as provitamin A and carotenoids (Palevitch & Craker, 1995; Matsufuji et al., 2007). As for carrot, it contains a high amount of α and β-carotene, which accounts for about half of provitamin A carotenoid found in the food supply (Van Vliet et al., 1996). Carrot also contains phenolic compounds and organic acids which contribute to the nutritional and sensory properties (Sharma et al., 2012). Spinach contains an abundance of phenolic compounds and ranked high among other vegetables due to its antioxidant capacity, which advocates that consuming spinach may allow protection against oxidative stress mitigated by free-radical species (Pandjaitan et al., 2005). Purple cabbage is a rich source of anthocyanins, minerals, vitamins, oligosaccharides, and bioactive substances (Wiczkowski et al., 2013). Apart from some nutritional benefits, purple cabbage is also valued by the consumers for its taste and colour, which increases the esthetic value of the food (Wiczkowski et al., 2013). Oyster mushrooms contain unique flavour and texture, and perceived as an important source of biologically active compounds of medicinal value (Breene, 1990). They are also treasured health food because they are an excellent source of vitamins, minerals, proteins, unsaturated fatty acids as well as they contain a high amount of fibers which is often regarded as an ideal and healthy food for people (Manzi et al., 1999; Yim et al., 2010).

Some studies have evaluated the influence of vegetables and fruits such as tomato pomace (Savadkoohi et al., 2014), avocado (Valenzuela-Melendres et al., 2014), oyster mushroom (Wan Rosli & Maihiza, 2015), shiitake powder (Pil-Nam et al., 2015), chestnut (Choi et al., 2010a), and pumpkin (Choi et al., 2012) substituting the relatively textural and nutritional characteristics of sausage. However, only a little information exists on the application of capsicum, carrot, spinach, purple cabbage, oyster mushroom and their influence on nutritional value, physico-chemical characteristics, sensory qualities, and storage stability in producing sausage. The present work aims to investigate the right percentage to which they should be incorporated, without compromising the quality of the sausage produced. Therefore, the objectives of this study are described in detail as the following.

1.2 Objectives of the study

The general objective of this study is to explore the quality characteristic and storage stability of sausages incorporated with selected vegetables. The specific objectives are as follows:

i. To evaluate the physical properties and sensory evaluations of sausages incorporated with different types of vegetables at various percentages.

ii. To analyse the microstructure, chemical compositions, and antioxidant activity of sausage according to the selected formulation.

iii. To study the storage stability of selected sausages based on the lipid oxidation and microbial activity during the refrigerated storage (4°C).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of processed meat based products

Consumption of meat has a limitation due to its short shelf life. However, the circumstance changed when consumers started to overcome the problems and found ways to preserve the meat efficiently by using salt (Young, 2008). Therefore, meat based products evolved in the market and increased popularity globally. Nevertheless, modified from their natural state for convenience and safety reasons, processed meat based products nowadays face society’s skepticism; food processing can improve a consumer’s life by preserving and extending shelf life, enhance flavour and improve the consistency of food products. Nonetheless, processed meat products are usually high in fats, salt and preservatives which are often indicated as unhealthy and cause of illnesses (Miguel et al., 2000; Desmond, 2006).

Thus, the meat food industry has been evolving rapidly and creating solutions that can fit the consumers’ demand. This includes the rise of functional products and reduced fat and salt products (Jiménez-Colmenero et al., 2001; Desmond, 2006). Specifically, the intake of saturated fatty acids is relates to cardiovascular risk. The fat content in traditional frankfurter is between 20-30%, nugget is 20-25%, salami is 30-50%, and beef patty is 20-30%, which contributes to the negative image of processed meat products among consumers (Colmenero, 2003; Desmond, 2006). The term ‘functional food’ in processed meat products refers to foods containing ingredients that are nutritious and can aid specific body functions; provide health benefits as well as nutrients. This includes the addition of dietary fibers, the use of sugar alcohol, lactic acid bacteria, unsaturated fatty acids, amino acids and vitamins (Jiménez-Colmenero et al., 2001). Dietary fibers from oat, sugar beet, soy, apple, pea, pumpkin, are included in the formulation of several meat products such as patties and sausages (Cofrades et al., 2000; Jiménez-Colmenero et al., 2001; Besbes et al., 2008; Choi et al., 2012).

According to Guerrero-Legarreta (2010), meat based products can be classified in various ways, depending on the specific objective of the food items; degree of comminuting (mechanically deboned meat, coarsely ground, finely ground), processing characteristics (cured, emulsified, battered, marinated, smoked, roasted), shelf life expected (fresh, dried, semi dried, fermented) and particular specifications (low fat, low salt, special diets, halal, kosher). In particular, a wide range of food grade chemicals is added during meat food manufacture to extend shelf life by stabilizing chemical change or by inhibiting the microbial growth such as triphosphates, polyphosphates, and sodium nitrate (Romans et al., 1985). Table 2.1 shows the categories of processes of meat products available around the world and the following subtitle discusses more on sausage products.

On the other hand, poultry meat and products are widely accepted and consumed in all parts of the world, as they are cheaper than other meats such as beef and pork (Guerrero-Legarreta, 2010). Malaysia has one of the highest per capita consumption rates in the world for chicken. Chicken meat is the most popular and cheapest source of meat protein among Malaysians whereby there is no dietary prohibitions or religious restrictions against consumption of chicken meat. Therefore, processed chicken products such hamburgers, nuggets, chicken frankfurters and cocktail sausages are the most popular items among consumers, which resulted in the growth of quick-service restaurants based on processed chicken products (Guerrero-Legarreta, 2010).

Table 2.1: Categories of processed meat products

Type	Definition	Example
Fresh processed meat products	Comprised of comminuted muscle meat with varying quantities of animal fat. Products are only salted, curing is not practiced. Non-meat ingredients are added in smaller quantities for improvement of flavour and binding. All meat and non-meat ingredients are added fresh (raw). Heat treatment (frying, cooking) is applied immediately prior consumption.	Kebab, raw beef patties, fresh sausage, hamburgers, fresh, chicken nuggets
Dried meat	Products are the result of simple dehydration or drying of lean meat in natural conditions or in an artificially created environment.	Meat floss, beef jerkey, biltong
Cured meat pieces	Cured meat cuts are made of entire pieces of muscle meat and can be sub-divided into two groups, cured-raw meats and cured-cooked meats. The curing for both cured-raw and cured-cooked is in similar principle: The meat pieces are treated with small amounts of nitrite, either as dry salt or as a salt solution in water.	Raw cured beef, raw ham, cooked beef, cooked ham, bacon, reconstituted products
Raw-cooked products	The product components of muscle meat, fat and non-meat ingredients which are processed raw, i.e. uncooked by comminuting and mixing. The result of viscous mix/batter is portioned in sausages submitted to heat treatment, i.e. “cooked”. The heat treatment induces protein coagulation which results in a typical firm-elastic texture for raw-cooked products.	Frankfurter, mortadella, lyoner, meat loaf
Precooked-cooked product	There are two heat treatment procedures involved in the manufacture of precooked-cooked products. The first heat treatment is the precooking of raw meat materials and the second heat treatment is the cooking of the finished product mix at the end of the processing stage.	Liver sausage, blood sausage, corned beef

Table 2.1: (Continued)

Raw (dry)-fermented sausages	Uncooked meat products and consist of more or less coarse mixtures of lean meats and fatty tissues combined with salts, nitrite (curing agent), sugars and spices and other non-meat ingredients filled into casings. They receive their characteristic properties (flavour, firm texture, red curing colour) through fermentation processes. Shorter or longer ripening phases combined with moisture reduction ("drying") are necessary to build-up the typical flavour and texture of the final product. The products are not subjected to any heat treatment.	Salami, Nham

Source: FAO (2007)

2.2 Sausage

Sausages are one of the western style meat-based products that has grown in popularity amongst Malaysian consumers. Sausages are usually cylindrical in shape, made from finely minced meat either beef, veal, pork, lamb or chicken (Huda et al., 2010; Wan Rosli et al., 2015). Manufacturers use various ingredients such as herbs, spices, salt, preservatives, fillers and other ingredients to make the sausage become more appealing and meet consumer satisfaction as well as maintaining cost effectiveness. Different manufacturers use different ingredients in formulations. All the ingredients are stuffed into the tube-shaped casing made from either animal intestine or synthetic casing. The types of sausage can be classified depending on their shape, length, ingredients, additives and curing techniques (Wan et al., 2015).

Sausages are emulsions of oil-in-water type; the continuous phase is water and soluble compound, the disperse phase is oil and protein as an emulsifier (Pereira et al., 2000). Based on the Malaysian Food Act and Regulation, the meat product whether in cuts or the form of sausages should contain not less than 65% of meat. It also shall not contain less than 1.7% of nitrogen in organic combination and shall not contain more than 30% of fat. Commercial sausages produced in Malaysia are commonly in frozen form, familiar as one of the ready-to-eat breakfast menus among school children and eaten as a combination with buns. In earlier days, chicken sausage production originated from small family-based enterprise. However, increasing demand for chicken sausage products in recent years has changed chicken sausage manufacturing into large-scale production (Huda et al., 2010).

The development of sausage as a processed meat product and a food component has been identified with different and diverse cultures around the world. Sausage manufacture is a simple process of allowing meat to undergo series of controlled structural and chemical changes. The uses of sausages as a meal and a sandwich component have been well explored by manufacturers. Grocery shop shelves are full not just of sausages of different types and forms, but also ready meals made of cooked sausages from various flavours, types, and dimensions. These sausages are made according to the quality range of premium, middle range, cheaper/ economy or depending on the manufacturer and demands. They could be steamed, fried, grilled or browned for eating and served with a range of accompaniments such as salad, mashed potato, chips, hot dog rolls, ketchup and mustard spread (Essien, 2003).

2.3 Types of sausage

2.3.1 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 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. The low pH and the dry nature of the product are primarily responsible for the long-lasting quality. Fermented sausages have a relatively higher meat content and take longer time to be prepared due to the series of required drying processes (Essien, 2003).

Semi-dry fermented sausages differ from dry fermented sausages because of their pronounced tangy flavour 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 a_w (>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 a lactic acid content of 0.5% to 1.3% (Toldrá, 2010). The examples of sausages are summer sausage or cervelat and Lebanon bologna.

According to Vieira (2013), 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 a_w 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 example of sausages are salami, pepperoni, and genoa (Vieira, 2013).

2.3.2 Cooked sausage

Cooked sausages are a type of sausage, which require heating process as part of their production. The sausages are usually bought and consumed without further cooking for several days or longer. However, they are usually recooked just before eating. These sausages are made from the usual mixture of meat and fat, and a number of other ingredients (McGee, 2007). As for cooked sausages, the cooking process is carefully controlled to ensure that the product reaches a specific internal temperature (70°C) for a defined period to achieve a consistent quality. Thermocouples are used to monitor the core of product temperature. The example of cooked sausages are Fresh white sausage (*boudin blanc*) and liver sausage (Essien, 2003).

2.3.3 Fresh sausage

Fresh pork sausage, fresh Italian, fresh bratwurst, Thuringer, bockwurst are examples of fresh sausages manufactured in this category (Essien, 2003). 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. According to Hui (2012), 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 flavour. Unlike cooked and fermented sausages, fresh sausages have a short shelf life and must be constantly refrigerated to prevent the growth of spoilage microorganisms including lactic acid bacteria and micrococci (Ryser and Marth, 2007).

2.3.4 Emulsion sausage

Emulsion sausages have very fine texture, homogenous, tender interior and relatively mild flavour that is cooked and/or smoked, best known in the form of frankfurters or wieners depending on their presumed origins (Essien, 2003; McGee, 2007). They are made by combining pork, beef or poultry with fat, salt, nitrate, flavouring 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).

2.4 Sausage processing technology

2.4.1 Production of sausage

Sausages nowadays can be found in a variety of shapes and sizes to meet consumers' demands. Typically packed in a casing, a traditional sausage making uses casing made of animal intestine, however, now synthetic casing are often used, and may be cured, smoked or cooked. Food additives are used to obtain desirable functions during the production of sausage such as colour, antimicrobial, antioxidant, preservation, improved nutrition, increased emulsification and altered flavour (Essien, 2000; Quasem et al., 2009). The steps involved in preparation of sausage are shown in Figure 2.1.

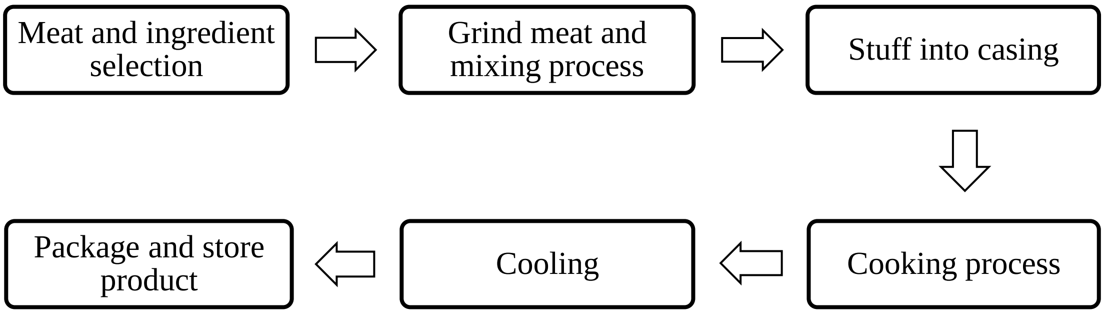

Figure 2.1 Steps involve in sausage processing

The utmost important step in sausage making is the selection of meat and ingredients. The meat ingredients used must be fresh and derived from veterinary-inspected animals. Savic (1985) stated that the lean meat should be well trimmed to a level of less than 10 percent of nontrimmable fat and connective tissue, free from sinews and gristle, entirely free from ligament, bone and cartilage particles and have an excellent binding capacity. The meat also should be clean and not contaminated with bacteria or other microorganism. Spices and seasoning must be properly measured according to approved weights according to the recipe. They not only have direct impact on food habits and expectations of sausage consumers but also must complement each other as an identity to the product (Savic, 1985; Marchello & Robinson, 2004).

The second key in sausage production is the chopping and grinding process. The quality and consistency of products can vary tremendously with the level of chopping. Grinding improves the uniformity of product by distributing the ingredients and making the particles similar in size (Savic, 1985; Marchello & Robinson, 2004).

According to Essien (2003), a low meat temperature and proper texture are ensured by adding ice to the formulation and creating the desired length of chopping time

The third step is the stuffing process. The raw sausage emulsion is encased either in artificial (cellulose or collagen) or in natural casings derived from slaughtered animals. Casings should be thoroughly selected in relation to the size and type. The filling must be firmly packed into a stuffing machine to ensure air pocket is eliminated. Sometimes air is removed beforehand in a vacuum mixer. The meat is extruded by the stuffing machine into the casing and tied immediately based on a fixed length (Henrickson, 1978; Savic, 1985).

The fourth step is the thermal processing process. Various cooking methods are available to cook the sausage. This includes the use of oven cooking, steaming, smoking, drying or a combination of all. Sausage is smoked and heated in order to pasteurize and extend its shelf life. Smoke imparts flavour-sustaining properties to sausages. The higher the level of smoke density, the more pronounced the flavour, even after weeks of storage. Smoking and heating fixes the colour and causes protein to move to the surface of the sausage and hold its shape when the casing is removed (Savic, 1985; Marchello & Robinson, 2004).

The final step is the packaging process. Cellulose casings often used in frankfurter sausage, are removed after cooking and showering with cold water. The sausage may be placed in specially designed packages and cartons in order to offer an acceptable visual and structural presentation of the product to consumers (Essien, 2003).

2.4.2 Current trend in sausage production

There has been a sudden shift in consumer eating habits in recent years as consumers are becoming more health-conscious; i.e. foods with lower levels of fat, less of sodium chloride, cholesterol, and caloric content. Nevertheless, the consumption of meat product is increasing rapidly and converged that the demand for meat exponentially skyrockets every year. In Malaysia, frankfurters are common sausage type products found in the market. However, eating processed meat products like sausages/frankfurters continually will affect health status and at the same time will increase risk of cancer (Chan et al., 2011).

Health conscious individuals modify their food habits by reducing the amount of fat content. According to Yang et al. (2007) and Baer & Dilger (2014), fat is one of the most variable raw materials in sausage products which makes up a large percentage of sausage composition. Fat is essential in the processing, textural, and sensory characteristics of sausage products as it strongly influence the binding properties, rheological and structural attributes of meats, especially in finely ground meat products. Thus, direct replacement of fat with non-meat proteins is used as an alternative approach towards fat reduction due to the excellent functional and nutritional properties that it imparts. Examples of non-meat protein used to attempt the detrimental effects of reducing the fat level in sausages are corn germ flours, fiber, soy protein, water and carbohydrates (Tokusoglu & Unal, 2003; Yang et al., 2007; Choi et al., 2011). Table 2.2 shows literature on the trend of healthier sausage products.

Table 2.2 Trend of healthier sausage products

Types of sausage	Objectives of the study	Reference
Low-fat cooked sausage	Evaluation and comparison of the compositional, nutritional and sensory properties of low-fat sausages produced with different meats (beef and chicken) and other ingredients (sunflower oil and tomato juice).	Yilmaz et al., (2002)
Low fat frankfurter	Evaluation on the effect of using a mixture of locust bean/xanthan gum and replacing pork back fat with olive oil on the stability and quality characteristics of reduced and low-fat frankfurters.	Luruena-Martınez, et al., (2004)
Spanish dry-fermented sausages (salchicho'n)	Investigation on the effect of the addition of orange fiber on the fermentation and dry-curing phases of a Spanish dry-fermented sausage in relation to stability (physico-chemical and microbiological profiles).	Fernández-López, et al., (2008)
Italian salami	Investigation on the levels of NaCl, Na, and other minerals of Italian salami from the market, and to explore a possible technological intervention aimed to improve their mineral composition in terms of reduced Na content.	Zanardi et al., (2010)
Reduced-fat emulsion sausages	Evaluation on the replacing animal fat with water and brown rice fiber by studying proximate composition, pH, colour, cooking yield, emulsion stability, textural profile, viscosity, and sensory characteristics of reduced-fat emulsion sausage.	Choi et al., (2011)
Italian type chicken sausage	Evaluation on the antimicrobial and antioxidant effects of lysozyme extracted from egg white as a replacer of nitrite.	Herath et al., (2015)
Fermented dry sausage	Development of no nitrite added fermented dry sausages, using extract from the overground part (stems, leaves and flowers) of *Kitaibelia vitifolia* as a functional ingredient.	Kurćubić et al., (2014)
Pork frankfurter	Determination on the effect of rice bran and walnut extract on textural and rheological properties of cooked meat emulsions made with pork backfat, canola oil or canola-olive oil blends.	Álvarez et al., (2012)

According to World Health Organization (WHO) recommendations, the dietary sodium chloride intake should not be more than 5g/day/person. Therefore, a technological intervention based on sodium chloride partial replacement by other chloride salts was used especially in dry fermented sausage, as a strategy to decrease the sodium content of cured meat products (Zanardi et al., 2010). Sodium reduction requires partially substituting the sodium chloride by other compounds into meat derivatives that have similar effects on sensory, technological and microbiological properties. However, the extent to which sodium levels can be reduced is limited, depending on the type of product (Wirth, 1991). Some compounds have been used for this purpose other than sodium, such as potassium and magnesium salts. Although total substitution of sodium chloride does not seem possible because of sensory reasons, a combination of sodium, potassium and magnesium salts may produce satisfactory results (Jiménez-Colmenero et al., 2001).

On the other hand, dietary fibers are added in sausage production not only for their desirable nutritional properties but also for functional and technological properties (Fernández-Ginés et al., 2003). Based on Malaysian Recommended Nutrient Intake (NCCFNM, 2005), the dietary fiber (DF) intake should be around 20-30g DF/ day. Nevertheless, the findings by Ng et al. (2010) reported that the amount is high and difficult to achieve by most Malaysians. The finding also showed low mean DF intake ranging from 10.7 g to 16.1g/dy among Malaysian adults.

Sodium or potassium nitrite is widely used as a curing agent in sausages and other cured meat products. Nitrite hampers spoilage and delays the development of oxidative rancidity, develops the characteristic flavour of cured meats by reacting with myoglobin, and stabilizes the red colour (Norman & James, 2004). Nitrite, however, can react with secondary or tertiary amines in meat to form carcinogenic, teratogenic and mutagenic N-nitroso compounds (NNC) (Kurćubić et al., 2014; Herath et al., 2015). According to Sebranek & Bacus (2007) and Yilmaz & Zorba (2010), researches on nitrites reduction were successfully applied. Schrader (2010) reported that there are uncured no-nitrate meat products available in the marketplace with a higher level of safety than the traditional products.

2.4.3 Application of vegetables/ fruits as ingredient in sausage products

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, flavours, antibacterial and antioxidants substances (Balasundram et al., 2005). 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. According to Yogesh et al. (2012), 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.

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 colour, odour, flavour and a reduced shelf life (Coronado et al., 2002). However, the use of synthetic antioxidants is associated with toxicity and adverse effects related to human health (Isaza et al., 2011;

Lorenzo et al., 2013). 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. Table 2.3 reports the current research on vegetables/ fruits used as ingredients in sausage production.

Table 2.3: Current research on vegetables/ fruits as ingredient in sausage products

Non-meat ingredients	Summary findings	Reference
Cherry extract	(i) Total phenols, reduce capacity and radical captive activity were significantly higher ($P<0.05$) in sausages with cherry extract.	Isaza et al., (2011)
Pumpkin fiber	(i) Chicken frankfurter with higher pumpkin fiber levels had lower lightness values ($P<0.05$), less cooking loss, lower emulsion stability, and lower colour scores ($P<0.05$).	Choi et al., (2012)
Avocado pulp and tomato paste	(i) Avocado pulp increases ($P<0.05$) the proportion of monounsaturated fatty acids in the pork frankfurter. (ii) Antioxidant activity increased ($P<0.05$) with incorporation of tomato paste, much higher than adding avocado pulp.	Valenzuela-Melendres et al., (2014)
Pleurotus sajor-caju (PSC) powder	(i) Frankfurter with 6% PSC had the lowest fat content (10.74%). (ii) Total dietary fiber of chicken frankfurter was increased in line with the levels of PSC powder (0.08 - 6.20%). (iii) The concentration of β-glucan increased in line with the levels of PSC powder (0.16%-1.43%).	Wan Rosli & Maihiza (2015)

Table 2.3: (Continued)

Carrot powder	(i) Incorporation of carrot powder did not significantly affect pH value of sausage samples. (ii) 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 & Ahmad (2015)
Pomegranate peels powder	(i) The addition of different concentrations of pomegranate peels powder caused high storage stability and reduced values of TBA and TVN of prepared beef sausage samples during refrigerated storage.	El-nashi et al., (2015)
Shiitake powder	(i) Shiitake incorporation did not cause colour and texture defects of frankfurters during storage. (ii) The TBARS values of frankfurters produced with shiitake were much lower than those of the control. (iii) The addition of shiitake retarded the growth of aerobic bacteria during storage. (iv) The shiitake incorporation significantly ($P<0.05$) improved the sensory; high in flavour, taste and acceptability scores.	Pil-Nam et al., (2015)

2.4.4 Functional and role of ingredients in sausage production

2.4.4(a) Sodium chloride

The principal function of sodium chloride or salt in the sausage formulation is to stabilize fat in emulsion products and determining the product texture that results from heat-set protein gelation (Sebranek, 2009). Sodium chloride is highly water soluble and forms sodium (Na^+) and chloride (Cl^-) ions in solutions. An ionic strength of 0.5 or more will cause muscle myofibrils to swell and disintegrate, depolymerize myosin filaments and solubilize the myofibrillar protein (Hamm, 1986). The presence of chloride ions in the protein structure also helps to increase water retention which has a significant impact on cooking yields, juiciness, tenderness, and mouthfeel of the product when consumed. On the other hand, sodium ion is responsible for the flavour that is derived from salt. It is not only playing a role in saltiness perception, but also increased intensity of other flavours that results in the presence of sodium (Ruusunen & Puolanne, 2005).

Salt concentration plays an important role in the control of microbial growth as it helps in lowering the a_w. The lower value of a_w would indicate the lower risk of growth of pathogenic microorganisms (Sebranek, 2009). Most of the commercial sausages in the market contain around 1.5-2.5% of salt (Hui et al., 2000). Based on WHO (2004), on a population basis, 5-6g/day of dietary salt is recommended for the general public due to established consumption of more than 6g of salt/day/person is associated with an increase in blood pressure. However, for genetically salt susceptible individuals and hypertensive persons, the range of salt intake in diets should be between 1-3g/day (Zanardi et al., 2010).

2.4.4(b) Binders, extenders and fillers

In the production of sausages, especially in lower or medium-grade sausages and loaves, ingredients commonly known as binders, extenders, and fillers are normally added. The type, amount, and quality of binders, extenders and fillers used in sausages vary depending on the origin (Savic, 1985).

Binders are proteinaceous agents enhancing water-binding properties and help to bind different materials in sausage products. Extenders are usually added in such an amount that they are able to increase the bulk or modify the quality of a sausage or loaf product. Meat extenders are primarily derived from plant proteins, usually from soybeans. According to Savic (1985) and Zayas (1997), various binders and extenders are added to sausage formulations for one or more of the following reasons rather than for their nutritional fortification: (i) to enhance fat and water binding properties, (ii) to improve the stability of the sausage emulsion, (iii) to improve gelling qualities of sausage, (iv) to reduce production costs, and (v) to improve sensory (flavour) and colour characteristics. The source of binders and extenders are usually originated from animal or plant source. Typical examples are gelatin, dry milk powder, sodium caseinate, soy protein isolate, vital wheat gluten, soy protein concentrate, dried or frozen whole egg or egg yolk, fresh egg white and blood plasma protein (Ranken et al., 1997; Zayas, 1997).

As for fillers, they are carbohydrate products, which have the ability to adsorb extensive quantities of water presence in sausages. However, they are not good emulsifier. Common fillers used in sausage manufacture include cereal flours and starches derived from rice, corn, potato, rusk and others. Addition of flours in sausage formulation helps to form a firm and tight sausage structure. Potato flour for example, helps to bind moisture in cooked and emulsion-type sausages, but in fresh sausages, it causes a springy and resilient effect after drying. Corn flour on the other hand, contributes to good slicing characteristics. Savic (1985) also found that addition of pre-soaked rusk provides an even moisture distribution in the sausage, and yeastless bread may enhance both the texture and flavour of the sausage.

2.4.4(c) Cold water

Cold water is usually added into sausage formulation to improve the consistency of the mixture and to substitute for fat in low fat sausage products (Hui & Evranuz, 2012). In addition, it is also added during sausage making to help distribute non-meat ingredients and to increase the product yield (Hui et al., 2001). The amount of water added by the manufacturer varies depending on the amount of fat in sausage formulation. According to Hui & Evranuz (2012), the maximum fat content in cooked sausage is limited to 30%, and the amount of fat and water combined is limited to 40%.

2.4.4(d) Phosphate

Phosphates are widely used in meat industry to improve water binding, increase water holding capacity and to retard the development of oxidative rancidity in meat products. These include phosphate derivatives i.e sodium tripolyphosphate, tetrasodium pyrophosphate, sodium hexametaphosphate, sodium acid pyrophosphate, dissodium phosphate and others (Savic, 1985). Alkaline phosphate salts (sodium tripolyphosphate and tetrasodium pyrophosphate) elevate the pH of meat, thus improving its water-holding power.

Polyphosphates also act as buffers; they sequester cations and raise the ionic strength of the solution. Tetrasodium pyrophosphate interacts directly with actomyosin dissociating it into myosin and actin. Sodium tripolyphosphate has a similar effect, but is active after a short delay for enzymatic hydrolysis to tetrasodium pyrophosphate, while sodium hexametaphosphate does not interact at all. The actin and myosin, dissociated by tetrasodium pyrophosphate and sodium tripolyphosphate, are then solubilized by salt and thereby their water binding capacity is improved. The water is immobilized in the pores of formed gel structure during heat coagulation of highly solubilized protein (Savic, 1985; Molins, 1990). Although both sodium tripolyphosphate and tetrasodium pyrophosphate are superior to all other phosphates, sodium tripolyphosphates have a higher solubility and are less susceptible to form insoluble precipitates (Savic, 1985).

2.5 Quality characteristics of sausage

2.5.1 Texture profile analysis

Texture measurement in the form of texture profile analysis would help to give hardness, springiness, cohesiveness, adhesiveness and chewiness of a sample. Many processing factors affect the texture profile of a sausage such as types and amount of ingredients, additives, heat treatment, and equipment used (Yetim, 2000). According to Chambers & Bowers (1993), hardness is the most important attributes to the consumer, as it decides the commercial value of a sausage. In the ingredients point of view, myofibrillar protein (myosin and actin) plays an essential role in producing the desirable texture and good water holding capacity of sausages. It is affiliated to its ability to produce 3-dimensional gels upon heating and following cooling. The gelation of muscle protein involves partial denaturation and subsequent permanent aggregation of myosin heads during formation of disulfide bonds and helix-coil transitions of the tail part of the molecule, resulting in a three dimensional cross-linked network formation (Sarteshnizi et al., 2015). In addition, factors such as pH, salt concentration, and non-protein polymer ingredients are other factors that determine the formation of three-dimensional gels (Sun et al., 2011; Sun & Holley, 2011).

Apart from that, the inclusion of non-meat ingredients into the sausage formulations interacts with meat proteins also affects the thermal denaturation of meat proteins, which finally modify the physical quality of cooked meat products such as