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Production and Formulation of a New Low- fat Fermented Functional Camel Meat Sausage by *Lactobacillus casei, L. paracasei and L. rhamnsus*

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سپاس یزدان پاک را که مرا مشمول الطاف خویش نمود که با طی مراحل تحصیل به اخذ درجه دکتری نائل شوم به شکرانه این نعمت بزرگ الهی که با امکانات این مرز و بوم فراهم و نزد اینجانب به امانت گذاشته شده است، در پیشگاه ملت ایران به کتاب آسمانی خود، قرآن کریم، سوگند یاد می کنم که:

- ✓ در سراسر زندگی حرفه ای، به نحو احسن در راه اعتلای کشور ایران و جامعه بشری قدم برداشته و در این راه از هیچ کوششی دریغ نکنم.

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- ✓ همواره علم و دانش خود را به روز نگاه داشته و در ادای وظایف و تعهدات حرفه ای در حد توان سعی و تلاش خود را به کارگیرم.

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Dedication

*This Research Paper is lovingly dedicated first to (**Muhamad** and **al Muhammad**) especially (**Fatimah Al-Zahra** and **Um-Al-Banen**). Then dedicated this study to my respective mother, father, my sisters and my brothers, especially brother Wesam. My dear mother in a world of uncertainty, nothing means more than knowing your love only grows stronger. You're always on my mind, each and every day; even though you are ... I always miss you so much, **MOM.***

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Faleeha Hasan Hussein

ABSTRACT

This research study was conducted with the aim of development new formulation contain less fat fermented sausages was the reduction of fat content and the simultaneous addition of non-lipid fat replacers in order to minimize the potential health risks associated with the consumption of high –fat foods. Probiotic fermented sausages are safe and healthy meat products. Semi-dry fermented sausages were manufactured from beef and camel meat in four types: without adding starter culture (control); inoculated with *Lactobacillus casei*, *L. paracasei* and *L. rhamnsus*. All treatments were analyzed for the physico-chemical characteristics (Protein, Moisture, Fat, Ash, Lactic acid value and pH), microbiological features (Total Aerobic Count, Total Molds and Yeasts and Lactic Acid Bacteria Count) and sensory evaluation (color, flavor, texture and overall acceptability) were analyzed after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. Microbial analysis demonstrated the predominance of lactic acid bacteria in semi-dry fermented sausage during the cold storage which reached (7.95, 8.28 and 9.24) log CFU g^{-1} in samples of beef meat and (7.92,8.07 and 8.90) log CFU g^{-1} in samples of camel meat inoculated with *Lactobacillus casei*, *L. paracasei* and *L. rhamnsus* respectively at 4°C for 45 days. Chemical analysis of semi-dry fermented sausage showed a significant difference ($p \leq 0.05$) in moisture content which decrease in all samples during the period of cold storage at 4°C. However, all other parameters such as protein, fat and ash increased. The dropped in pH value in all samples because of producing lactic acid during the fermentation by lactic acid bacteria. Physicochemical, microbial and sensory characteristics of fermented sausage inoculated with *Lactobacillus paracasei* and *L. rhamnsus* are found to be better than other ones. Also, we could preserve the product at 4°C for 45 days. The sensory evaluation was appeared superiority in the semi-dry fermented sausage that had *Lactobacillus casei* and *L. paracasei* and *L. rhamnsus* compared with control.

Key words: Lactic acid bacteria; Production semi-dry fermented sausage; Quality characteristics.

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List of Abbreviations

AOAC	Association of Official Analytical Chemistry
ATP	Adenosine Tri Phosphate
a_w	Water Activity
BA	Biogenic Amines
BC	Beef inculated with *Lactobacillus casei*
BNS	Beef Non Starter culture
BPC	Beef inculated with *Lactobacillus paracasei*
BPEL	BioProcess Engineering Laboratory
CAC	Camel inoculated with *L. casei*
CAPC	Camel inoculated with *L. paracasei*
CANS	Camel Non Strater
CAR	Camel inoculated with *Lactobacillus rhamnsus*
CFU	Colony forming unit
CNC	coagulase negative cocci
DSM	Deutsche Sammlung von Mikroorganismen/ German collection of microorganisms
FAO	Food and Agriculture Organization of the United Nations
FSIS	Food Safety and Inspection Service
GI	Gastro-Intestinal
GRAS	Generally Recognized As Safe
LAB	Lactic acid bacteria
M& Y	Molds and Yeasts Count
MP	moisture-to-protein ratio
MRS	De Man Rogosa and Sharpe
NSLAB	Non-Starter Lactic Acid Bacteria
Ppm	Parts per million
R-Beef	Beef inoculated with *L. rhamnsus*
R-Camel	Camel inoculated with *L. rhamnsus*
RH	Relative Humidity
SD	Standard deviation
TAC	Total Aerobic Count
USDA	United States Department of Agriculture
WHO	World Health Organization
YGC	Yeast extracts Glucose Chloramphenicol

Chapter 1

Introduction and Objectives

1.1. Introduction

Meat and meat products are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients. Meat provides high quality protein, consisting of all essential amino acids, minerals and vitamins (Jalarama Reddy *et al.*, 2013). However, the high saturated fat content of such products results in a restriction of consumption for those who are prone to cardiovascular diseases and /or suffer from over weight (Furlán *et al.*, 2014). Yet, fat is an important constituent of human nutrition and contribute to the flavor, tenderness, juiciness, appearance, texture and shelf life of meat products. Thus, the challenge for meat industry is to develop low-fat meat products without compromising sensory and texture characteristics (Furlán *et al.*, 2014). The content of proteins in muscle tissue of different species of animals ranges from 15% to 20%. The content of amino acids in meat proteins is well balanced i.e. they content all amino the human organism (essential amino acids). Solely connective tissue proteins have low biological value since they contain little tryptophan and cysteine (non-essential amino acids). Meat processing, also known as further processing of meat, is the manufacture of meat products from muscle meat, animal fat and certain non-meat ingredients. Meat products may be canned meat products, sausages, patties, etc. with thousands of product types (Asmare, 2012). Meat and meat products are essential components in the diets of human beings; their consumption is affected by various factors. The most important ones are product characteristics (sensory and nutritional properties, safety, price, convenience, etc.) and consumer and environment-related characteristics (psychological, health, family or educational aspects, general economic

situation, climate, legislation, etc.) (Barat *et al.*, 2015). Camel meat is also relatively high in polyunsaturated Fatty acid (PUFA[1]) in comparison to other red meat (Gheisari and Ranjbar, 2013), which contributes to its health- promoting benefits. Consumption of camel meat can therefore lead to a reduction in total Fat and cholesterol intake and an increase in PUFA as compared with other conventional meat sources. Moreover, camel meat is also used for medicinal purposes in diseases such as hyperacidity, hypertension, pneumonia, and respiratory disease; it is also known as an aphrodisiac (Maqsood *et al.*, 2015). Such a diet can be expected to reduce cardiovascular diseases and improve health (Maqsood *et al.*, 2015). Thus, camel as a meat source seems to present a viable alternative to other red meats. Camel meat is known to be more beneficial for health because the meat contains lower fat and cholesterol levels than other red meats (Gheisari and Ranjbar, 2013). Camel meat is the least studied meat and is mistakenly believed to be of lower nutritive value and quality than other types of red meat (Abdelhadi *et al.*, 2013). Comparative technical information shows that the fat content of camel meat is considerably less than beef (Sahraoui *et al.*, 2017), low in cholesterol, vitamin E and high in protein and water holding capacity (Soltanizadeh *et al.*, 2010). Camel meat is similar in taste and texture to beef (Barat *et al.*, 2015). Microbial contamination can lower the quality of fresh minced camel meat; shorten its shelf life and result in economic loss and probably health hazards. Low temperature storage is one of the primary preservation methods to maintain meat freshness, because the rates of microbiological, chemical and biochemical changes are reduced at decreased temperatures (Maqsood *et al.*, 2015).

[1] Poly Unsaturated Fatty Acid

The demand for meat products with lower fat contents or healthier fatty acid compositions has increased in recent years due to new guidelines recommending reduced saturated fat intake and consumers' desire to lose weight. Several alternative strategies have been used in the manufacture of these products, such as substitution of saturated fat with vegetable oils and the use of fat replacers, the substitution of animal fat with vegetable oils has been suggested to improve the fatty acid profile and to decrease the cholesterol levels of meat products. Several vegetable oils have already been used as fat substitutes, such as olive, flaxseed, corn, soybean, and canola oil .However, the simple replacement of animal fat with vegetable oil does not alter the lipid content or caloric value of the products. Thus, reducing the amount of added oil, combined with the use of non-lipid fat substitutes, could be an option in the production of dry-fermented sausages with low fat content and acceptable Physicochemical and sensory characteristics (Menegas *et al*., 2013).

1.1.1. Fermentation

Fermentation is one of the oldest food preservation methods. Fermentation was a significant method to extend storage life. Worldwide, the main fermented foods are dairy products, meats, fish and vegetables (Lu, 2010). Application of fermentation has been known from ancient time in many cultures, the biological driver being food preservation to prevent starvation when fresh food was limited. Moist foods will not remain edible for long, owing to microbial colonisation. Moreover, deteriorated foods can be poisonous for humans. Fermentation is a low cost food preservation technique that can apply to a wide range of moist foods, including meat. Fermented foods still play an important role in the human diet, because fermented foods are shelf stable, have low energy consumption, are easily

digested, have unique sensory properties and nutritional benefits. Actually, the market demand for fermented foods has increased recently, as consumers regard fermented foods as natural and beneficial for health (Lu, 2010). Fermented meat products are mainly fermented meat sausages, using cultures of LAB[1] either endogenous to the food or added. In general, fermented sausages are made from comminuted meat, salt, glucose and LAB. With the growth of LAB, the generation of lactic acid rapidly reduces the pH to between 4.0 and 4.5. This pH, and presumably the concomitant effect of lactate as undissociated lactic acid and bacteriocins, inhibits the growth of other microbial species, but not the LAB (Lin, 2017). The main fermentative microbiota is lactic acid bacteria (LAB) including *Lactobacillus*, *Pediococcus*, *Enterococcus*, *Leuconostoc*, *Lactococcus* and *Weissella* (Reis *et al*., 2012). Most of the meat products produced today is based on traditional practices. These products are attractive to consumers because they offer a wide variety of colors, flavors, and textures. There are many factors affecting the sensory characteristics of meat products such as the meats used as raw materials (genetic type, feed. age. sex, and rearing system), microorganisms selected as microbial starters for the fermentation and type of processing technologies (cooking, drying, ripening, smoking, etc.) (Ahmad and Amer, 2013). In fermented meat products, like dry fermented sausages, starter proteases play an important role on proteolysis. Proteolysis contributes to the consistency of the product by the degradation of the myofibrillar structure and to its taste through the accumulation of small peptides and free amino acids. These amino acids directly contribute to flavor or indirectly as precursors of flavor compounds through amino acid degradation reactions (Ahmad and Amer, 2013). The extent of proteolysis is variable and mainly depends on the raw materials and

[1] Lactic Acid Bacteria

processing conditions and, in the case of fermented meats, the type of starters added (Ahmad and Amer, 2013). There are many specific flavors due to the high number of available aromatic plants such as pepper, paprika, mustard, nutmeg, cloves, oregano, rosemary, thyme, garlic, onion, and so on. These compounds have a high impact on the aroma of fermented products (Ahmad and Amer, 2013).

1.1.2. Fermented sausages

Quality of traditionally fermented sausage is influenced by many factors such as selection of raw material, metabolic activity of epiphytic flora and physico-chemical properties developed during ripening, smoking and drying of meat. Lactic acid bacteria are essential for dry sausage production. Their ability to lower the pH of the mixture by producing acid from sugars leads to the development of the desirable organoleptic properties prevents the growth of pathogens and ensures the stability and safety of the final product (Borovic *et al.*, 2015). Even if their initial levels varied their final levels were close to the one of industrial products manufactured with starter cultures**.** In traditionally fermented European sausages, facultative homofermentative lactobacilli constitute the predominant flora through ripening (Borovic *et al.*, 2015). One of the most important fundamentals for the development of civilization was food preservation and storage. The transformation of raw materials to more-or-less stable foods by drying and fermentation was well known in many ancient cultures and used for different foods such as meat. Fermented sausage is one of the oldest known forms of processed meat products and is very popular in many areas around the world. Sausages were invented as a means of making the most of leftovers of meat and entrails. One of the most common fermented sausages is salami. Dry fermented sausages can

be defined as meat products that are manufactured by selecting, chopping, and mincing lean and fat, with or without offal, adding condiments, spices, additives and starter culture (optional). The ingredients are stuffed into casings, ripened, cured, and in some cases smoked (Leroy *et al.*, 2006). Fermented sausages are industrially or artisanally produced meat products preferred by many consumers for their aroma and taste. Their manufacturing technology is more or less similar all over Europe. The product is then fermented and ripened under controlled conditions for a period which may last from ten days up to a few months during this period various physicochemical, microbial and organoleptic changes occur and the product gradually develops characteristic color, flavor, taste and texture, is stabilized and finally it is ready to be placed in the market. Various types of fermented sausages are manufactured using different spice mixtures, starter cultures and types of raw materials and meat (Papavergou, 2011). The conditions during fermentation, ripening and storage of fermented sausages (acidic pH, high availability of free amino acids due to high protein content of raw materials and extended proteolysis, occurrence of high populations of fermenting or contaminating microbial groups with amino acid decarboxylating activity, long ripening times etc.) may favor bacterial amino acid decarboxylation and accumulation of biogenic amines (BA[1]) in the final product (Toldrá and Reig , 2007).

1.1.3. Lactic acid bacteria

Lactobacilli are food-grade organisms witnessing a long history of safe use being widely consumed with fermented foods and beverages Lactobacilli display a naturally wide range of antibiotic resistance and in most cases it is not of the transmissible type. Lactobacilli possess inhibitory

[1] Biogenic Amines

properties against enteropathogens thanks to production of several antimicrobial compounds (Caggia *et al.*, 2015). Lactobacilli are normal residents of the human gastrointestinal (GI[1]) tract. Many species belonging to the genera *Lactobacillus* with health beneficial properties have been introduced as probiotics. Lactobacilli are rod-shaped, Gram-positive lactic acid bacteria that are classified as "generally recognized as safe" (GRAS[2]) (Lin, 2017). Lactic acid bacteria (LAB) play an important role in sausage fermentation because they induce flavor and texture changes and have a preservative effect, resulting in an increased shelf life of the product. The fermentation of carbohydrates inherent in sausage starting material leads to the production of lactic acid, which decreases the pH in the fermented sausages. The low external pH disturbs the homeostasis of the pathogenic and spoilage bacteria and restricts their growth. Microbial interference by LAB also can occur due to the production of bacteriocins (Liu *et al.*, 2010). Lactic acid bacteria are an order of bacteria exhibiting an enormous capacity to degrade different carbohydrates and produce lactic acid as their main end product (Lin, 2017). They have been historically used in food fermentation and preservation as acidification inhibits the growth of spoilage agents. (Lin, 2017). Common lactic acid bacteria in fermented meat sausages: their role and diversity LAB include a diverse group of Gram-positive non-spore forming cocci, coccobacilli or rods, with common morphological, metabolic and physiological characteristics (Reis *et al.*, 2012). They are facultative anaerobic with variable oxygen tolerance in different species. LAB growth depends on the presence of fermentable carbohydrates. They are classified as homofermentative or heterofermentative based on end products of glucose metabolism. While

[1] Gastro-Intestinal
[2] Generally Recognized As Safe

homofermentative LAB convert glucose mainly to lactic acid using the glycolysis (Embden–Meyerhof–Parnas or Embden–Meyerhof) pathway, the heterofermentative LAB use the phosphoketolase (6-phosphogluconate) pathway and convert glucose to lactic acid, carbon dioxide and ethanol or acetic acid. LAB is a diverse group of organisms with diverse metabolic capacity. This diversity makes them easily adaptable to a wide range of conditions, allowing them to thrive in acid foods' fermentations (Reis *et al.*, 2012). LAB not only produce organic acids (principally lactic acid) but also antimicrobial substances known as bacteriocins e.g. nisin. These substances are cationic amphipathic peptides that able to damage cell membranes of susceptible microorganisms, resulting in cell leakage and metabolic inhibition (Lu, 2010). Some species of *Lactobacillus*, including *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus johnsonii*, *Lactobacillus reuteri*, and *Lactobacillus rhamnosus*, have been used as probiotics (Zhang *et al.*, 2016). Increased attention has been paid to the probiotic abilities of *Lactobacillus* (Zhang *et al.*, 2016). The bacteria which play a significant role and commonly found in fermented sausages are lactic acid bacteria (Ahmad and Amer, 2013). These microorganisms are used as starter cultures, promoting meat fermentation (Ahmad and Amer, 2013). Lactic acid bacteria improve safety and stability of the product, enhance colour stability, prevent rancidity and release various aromatic substances (Ahmad and Amer, 2013). Spontaneously fermented European sausages are mostly obtained with the use of homofermentative *Lactobacillus sakei* and/or *Lactobacillus curvatus* strains (Trząskowska, *et al.*, 2014). Also, a number of successful attempts were made at adding e.g. *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus rhamnosus* and other strains to processed meat products (Trząskowska, *et al.*, 2014). The species

Lactobacillus casei, *Lactobacillus paracasei*, and *Lactobacillus rhamnosus* are phylogenetically and phenotypically closely related (Reale *et al.*, 2015). These bacteria are able to colonize a variety of fermented food products, representing the dominant component of non-starter lactic acid bacteria (NSLAB[1]) in long ripened cheeses and traditional fermented milk products (Reale *et al.*, 2015). Numerous bacterial strains belonging to the species *L. casei*, *L. paracasei* and *L. rhamnosus* are used as starter cultures in milk fermentation, as adjunct cultures for intensification and for acceleration of flavour development in dairy products and for their probiotic characteristics (Reale *et al.*, 2015). Moreover, the probiotic properties have been proven for strains belonging to this species (Reale *et al.*, 2015). LAB constitute a group that has been greatly associated with fresh meat and cooked meat products but represents a controversial cohort of microbial species that either contribute to generation of offensive metabolites and the subsequent organoleptic downgrading of meat (Pothakos *et al.*, 2015) or serve as bioprotective agents with strains of certain species demonstrating reduced spoilage capacities and inhibitory activity against spoiling microbiota (Pothakos *et al.*, 2015). Thus LAB is optimal candidates to develop regardless the packaging system, while their occurrence is dependent on the competitive microbes and the influence of the storage conditions upon the latter (Pothakos *et al.*, 2015). LAB are widely found in nature, adapted to different carbohydrate rich environmental niches, as well as to terrestrial and marine animals. In food products, LAB are found in dairy products (yogurt and cheese), in fermented vegetables (olives, sauerkraut), in meat and fermented meats products and in sourdough bread (Fraqueza, 2015). LAB plays a recognized role in fermented foods preservation and safety, thus promoting final products microbial stability. The preservation ability

[1] Non-Starter Lactic Acid Bacteria

of LAB is based on competition for nutrients and the production of antimicrobial active metabolites such as organic acids (mainly lactic acid and acetic acid), hydrogen peroxide and peptidic compounds (bacteriocins) (Fraqueza, 2015).

1.1.4. Probiotic

The products fermented by probiotics such as lactic acid bacteria (LAB) are consumed far and wide. The probiotic potential of LAB has been proved in various fermented foods (Xiong *et al.*, 2014). Probiotic strains should also have desirable antibiotic resistance and sensitivity patterns, antagonistic toward potentially pathogenic microorganisms and have metabolic activities beneficial to the well-being of the host (Verdenelli *et al.*, 2009). Characteristics ascribed to a probiotic strain are, in general, strain specific and individual strains have to be tested for each property. The most studied probiotics are the lactic acid bacteria, Particularly *Lactobacillus* and *Bifidobacterium*. Most of *Lactobacillus* species are normal and non-pathogenic inhabitants in human and animal intestine and their presence is important for the maintenance of the intestinal microbial ecosystem (Verdenelli *et al.*, 2009). Probiotics targeted for human consumption are usually isolated from humans or animals because strains from these origins can better adapt to the conditions encountered in the human/animal GI tract, which enables more successful gut colonization (Argyri *et al.*, 2013). However, certain food-associated *Lactobacillus* strains have probiotic characteristics even though they do not belong to the gut microbiota (Solieri *et al.*, 2014). According to the FAO[1]/WHO[1] (2002), a bacterial strain should fulfill a number of

[1]Food and Agriculture Organization of the United Nations

requirements to be considered probiotic; these requirements must be verified by in vitro and in vivo tests. In vitro tests are useful for the selection of strains that have greater probiotic potential; these tests increase knowledge regarding the strain as well as the mechanisms underlying the beneficial effects (Vijayakumar *et al.*, 2015). *Lactobacillus* spp. belongs to the group of lactic acid bacteria (LAB) and has a long history of use in the production of dairy products due to their ability to convert lactose into lactic acid (Casarotti *et al.*, 2017). In addition to their use as technological agents in the food industry, some *Lactobacillus* species can confer health benefits to the host when they are administered adequately as probiotics. Probiotics are currently defined live microorganisms that, when administered in adequate amounts, confer health benefit on the host" (Hill *et al.*, 2014) and can adjust the host body's microecological balance, improve intestinal function, and stimulate digestion and immune function. *Lactobacillus* was the earliest discovered probiotic of the three types of probiotics, which also include *Bifidobacterium* and Gram-positive cocci (Zhang *et al.*, 2016). Numerous studies have proposed the use of probiotics to improve gut health in the treatment of inflammatory bowel diseases and in the prevention of antibiotic-induced diarrhoea (Silva *et al.*, 2017). Although probiotics have been extensively studied and commercialized, and are the subject of national and international regulations, there is no agreement concerning the amount of probiotic bacteria necessary to produce their beneficial effects. Generally, probiotic food products must contain 10^6 CFU/mL[2] or CFU/g[3] (Casarotti *et al.*, 2017). Nevertheless, some authors state that beneficial effects can be achieved even when bacteria lose their viability (Casarotti *et al.*, 2017). Although a large

[1] World Health Organization
[2] Colony Forming Unit/ml
[3] Colony Forming Unit/gram

number of probiotic strains are available for commercial use worldwide, the isolation and characterization of new strains from different species is desirable; thus, many studies in this field have been published in recent years (Casarotti *et al*., 2017).

"Functional foods" refers to foods with health-promoting ingredients enriched beyond the normal traditional nutrients. These foods contain a reasonable quantity of some bioactive components, such as probiotic, prebiotic, and symbiotic components (AdebayoTayo and Akpeji, 2016). Probiotics are introduced as microorganisms which show the beneficial effects on the host health when consumed in sufficient amounts. Among probiotics, LAB group, through secreting the bioactive compounds, can be utilized as preservative agents. Therefore, the majority of probiotics include LAB. The LAB is a functional heterogeneous bacterial group which is linked to traditional dairy and food fermented products (Haghshenas *et al*., 2016). Probiotics exert their benefits through four different mechanisms of action: (a) interference with pathogenic bacteria by competing with nutrients and adhesion sites, (b) improvement of the barrier function of the epithelial lining, (c) immunomodulation, and (d) influence on other organs of the body through the immune system. (Hill *et al*., 2014). A starter culture: Is an inoculum of beneficial microorganisms added to food products such as meat, milk, and vegetables to improve food quality and safety. Starter cultures provide rapid lactic acid development from fermentation of sugars added to the sausage (Visessanguan *et aI*., 2004a) resulting in a decreased pH that retards the growth of most spoilage microorganisms and enhances flavor and texture of the final product (Meng and Schaffner, 1997). There is a linear relationship between numbers of lactic acid bacteria and pH. Acidity has been reported to contribute to the

decline of undesirable microbes in dry fermented sausages. This is due to the homeostatic disturbance of pathogenic and spoilage organisms resulting from the low pH of their external environment (Leistner, 2000). The use of starter culture therefore, helps to stabilize the fermentation by controlling the microflora of the food **(**Font de Valdez *et aI.*, 1990). Another advantage of using starter cultures is that fermentation and ripening process are more easily controlled when culture characteristics and starting concentrations are known Common fermentation cultures include species of *Lactobacillus, Pediococcus,* and *Staphylococcus.* Not all lactic bacteria are capable of producing bacteriocins, so adding a mixture of starter cultures could draw on the beneficial effects of each. Mixed starter cultures allow manufacturers to exploit both biological (bacteriocin) and environmental properties (PH and water activity) of fermentation cultures in controlling pathogens.

1.1.5. Sausages

Sausages and sausage products have evolved into a wide variety of flavors, texture, and shapes resulting from variation in ingredients and manufacturing processes. Sausage is known to be the oldest and most enduring form of processed meat and is widely consumed throughout the world (Asmare and Admassu, 2013). Many of the sausage products available in the marketplace are simply cured, comminuted products, meaning they contain curing salts and undergo many of the same processing steps as do fermented sausages, but they are not fermented (such as; frankfurters, bologna, and breakfast sausages). In contrast, there are fermented meat products such as: moist sausages (Lebanon bologna, mortodella, etc.), semi-dry sausages (summer sausages, Thuringer cervelat, etc.), and dry sausages (pepperoni, salami, chorizo, etc.). The origin of

fermented meats goes far back in Ancient Romans and Greeks and in fact, the origin of words of sausage and salami were from Latin expressions *salsicia* and *salumen*; respectively (Toldra, 2002). Sausages are a type of food consisting of ground meat, animal fat, salt, spices, fillers, extenders and binders that are usually packed in polymeric casing. Sausage-making is a very old food preservation technique and is a logical outcome of efficient butchery. Sausage-makers put to use meat and animal parts that are edible and nutritious, but not particularly appealing, and that allow the preservation of meat that cannot be consumed immediately (Fox, 1987). As meat is highly perishable, its storage and marketing requires considerable energy input in the form of chilled or frozen storage. Development of shelf-stable ready-to-eat products would not only save energy, but would also be a valuable contribution to the growth of the meat industry (Ahmad and Srivastava, 2007). Modern sausage production is independent of environmental conditions. Temperature, humidity, moisture, and acidity are continuously and carefully controlled. Microorganisms with specific desired properties can be grown separately and added to sausages. Recipes and ingredients for particular types of sausages can be obtained from the literature and there are limits to amounts of additives regarded as acceptable. To combat meat-borne pathogens and still preserve the desired sensory properties of the product, meat processors have adopted "hurdle technology" that combines different preservation methods to inhibit spoilage and pathogenic microorganisms without compromising the safety, nutritional, sensory, and shelf life of meat products.

In recent years, increased concerns about the potential health risks associated with the consumption of high fat foods has led the food industry to develop new formulations or modify traditional food products to contain

less fat (Furlán *et al*., 2014). Health organizations all over the world have promoted the choice of a diet low in saturated fat and cholesterol and moderated in total fat, as a means of preventing cardiovascular disease which constitutes one of the main causes of mortality in the world. One of the strategies for the development of low-fat fermented sausages was the reduction of fat content and the simultaneous addition of non-lipid fat replacers to minimize texture defects (Muguerza *et al*., 2004).

1.2. The Objectives of This Study

1.2.1. Main Objective

The main objective of this study was the production and formulation of a new-low fat functional fermented sausage from (beef and camel) meat with probiotics, use three genes of probiotics (*Lactobacillus casei, L. paracasei and L. rhamnosus*).

1.2.2. Specific Objectives

The specific objectives were to:

1-Study composition of physico-chemical of semi-dry fermented sausages which inoculated with (*Lactobacillus casei, paracasei and rhamnosus*) compared to control, during 45 days at 4°C cold storage.

2- Analyze microbiological population of semi-dry fermented sausages which inoculated with (*Lactobacillus casei, paracasei and rhamnosus*) compared to control, during 45 days at 4°C cold storage.

3- Evaluate sensory characteristics of semi-dry fermented sausages which inoculated with (*Lactobacillus casei, paracasei and rhamnosus*) compared to control, during45 days at 4°C cold storage.

4- Select the best sample of semi-dry fermented sausages.

Chapter 2

Literature review

2.1. Fermented of Meat

In contrast to the lactic acid fermentation that occurs in milk, the meat fermentation has been, until recently, considerably less well studied and understood. In fact, the use of pure, defined starter cultures in the fermented meats industry is a relatively recent development (begun only in the 1950s and '60s). Before the use of meat starter cultures, the most common way to start the fermentation practice was, as noted above, backslopping. Backslopping works for several reasons. First, backslopping ordinarily selects for those bacteria that are well suited for growth in the sausage environment. Strains that are slow to scavenge for carbohydrates, are inhibited by fermentation acids, or are sensitive to salt or nitrites are not maintained. Instead, they are displaced by more competitive bacteria that have particular metabolic and physiological advantages in that environment. Thus, even a prolific acid-forming strain does have much of a chance if it is not also tolerant of salt and nitrite and able to grow in a low oxygen environment. Second, the bacterial population that becomes established during repeated transfers and fermentations is heterogenous in nature, consisting of multiple species and strains (Hutkins, 2006). If, for example, one such strain were to suddenly die or otherwise be lost due to the presence of bacteriophages or an inhibitory agent, then the remaining strains, acting as back-up, would still be able to complete the fermentation. Finally, backslopping is effective due simply to the size of the inoculum, which is usually around 5%, but which can be as high as 20% of the total mass. Such a large inoculum provides reasonable assurance that the desired organisms will overwhelm the background flora and that undesirable interlopers will have little chance of competing. Despite backslopping's long history and wide use, there are several drawbacks associated with it.

Such products often have inconsistent quality and fermentations can be unreliable and difficult to control. In large production facilities in Europe, and especially in the United States, short and consistent fermentation times, standardized production schedules, and consistent product quality have become essential (Hutkins, 2006). A manufacturer producing a single, small batch of product can tolerate delays, and may even come to appreciate differences in product quality. However, inconsistent product quality and production delays due to sluggish fermentations are unacceptable for large manufacturers who have substantial employee payrolls and tight production schedules. Above all, the entire backslopping process can be considered microbiologically risky, since any deviation from the norm (i.e., slow or delayed fermentation) may permit growth of *Staphylococcus aureus*, *Listeria monocytogenes*, *C. botulinum*, or other pathogens of public health significance. Although obvious, the following point cannot be over-emphasized: sausage is made from raw meat that may well contain pathogenic organisms. If a cooking step is not included, fermentation represents the primary means of preservation and the main barrier against pathogens. Since the actual fermentation can take a long time (from twelve to thirty-six hours and in some cases, even longer), a slow or failed fermentation may not be discovered right away, permitting growth of pathogenic bacteria. If the bacteria are able to reach high levels, even subsequent acid production (or even a heating step) may not be sufficient to inactivate these pathogens. It is important to mention, as noted above, that it is entirely possible to produce fermented sausages without any type of culture at all (Hutkins, 2006). This practice is not uncommon, and is still practiced in many parts of Europe. One can simply prepare the sausage mixture and wait for the natural lactic flora to take over. This method is successful because the formulation and fermentation conditions

(i.e., salt, nitrate, low temperature and an anaerobic environment) provide sufficient selection of desirable lactic acid bacteria. However, for obvious food safety reasons, none of the large, modern operations in the United States rely on natural fermentations. Finally, there is one other way to produce sausages that have a tangy or acid-like flavor without a starter culture and without relying on the indigenous microflora. The meat mixture can be directly acidified using a foodgrade acidulant (e.g., glucono-δ-lactone), which results in a product with sensory properties that mimic (somewhat) those of fermented sausage (Hutkins, 2006). In ancient Rome, leftover fresh meat was blended with salt then extruded into skin casing and hung inside a room to dry. People only knew that these dry sausages had a special flavour but did not understand the fermentation role of lactic acid bacteria. Around the same time, a fermented dry sausage named Lap Cheong was developed in China (Leistner, 1986). None of these styles required cooling to minimise microbial colonisation, and this was presumably the reason for their widespread adoption, particularly as meat animals could not survive extreme winters without huge energy and resources supply. Fermentation captured and preserved the food value of an animal. In earlier times, the fermentation relied on the naturally occurring microflora on meat. The natural fermentation of sausage required experienced masters to control the fermentation quality. In modern practice, starter cultures with a known bacterial profile are applied (Zeuthen, 2008) to guarantee fermentation. Starter cultures reduce the fermentation time, product variance and cost of fermented products. In addition, starter cultures improve the safety of fermented products, owing to their rapid dominance in the fermenting sausage (Lu, 2010). Fermented meat in South East Asia (called sour meat) seems to be growing in popularity as consumers search for more varieties in their food choices.

Guidelines proposed in the U.S. (American Meat Institute, 1982; Hui, *et al.*, 2004) for making fermented dry or semi-dry sausages include a definition of dry sausage as chopped or ground meat products that due to bacterial action, reaches a pH of 5.3 or less. The drying removes 20 to 50% of the moisture resulting in a moisture-to-protein ratio (MP[1]) of no greater than 2.3 to 1.0. In Germany, cured meat can be safely stored at 10°C. According to USDA[2]/FSIS[3], dry salami must have a MP ratio of 1.9 to 1, pepperoni- 1.6 to 1, and jerky 0.75 to 1, to be labeled as such. Semi-dry sausages are similar except that they have a 15 to 20% loss of moisture during processing. Semi-dry sausages also have a softer texture and a different flavour profile than dry sausages. Because of the higher moisture content, semi-dry sausages are more susceptible to spoilage and are usually fermented to a lower pH to produce a very tangy flavour (Asmare, 2012). Some sausages are heavily smoked and some have a high sugar level giving them a sweet flavour. Semi-dry products are generally sold after fermentation (pH of 5.3 or less). These are heated, and do not go through a drying process (a_w) is usually 0.86 or higher. They are also usually smoked during the fermentation cycle and have a maximum pH of 5.3 in less than 12 hours. If the semi-dry sausage has a pH of 5.0 or less and a moisture protein ratio of 3.1 to 1 or less, it is considered to be shelf stable (USDA/FSIS Food Labeling Policy Manual) but most semi-dry products require refrigeration (2°C; 37ºF). In Europe, fermented meat with a pH of 5.2 and a water activity (a_w) of 0.95 or less is considered shelf stable. To decrease the pH (below 5.0) with limited drying, the U.S semi-dry products are often fermented rapidly (12 hours or less) at a relatively high temperature (32-46ºC; 90-115ºF). In Europe, fermentation is slower (24

[1] moisture-to-protein ratio
[2] United States Department of Agriculture
[3] Food Safety and Inspection Service

hours or more) at a lower temperature and results in a higher pH. These differences in speed of fermentation and final pH result in products having different flavour. The primary objectives of fermentation include extending shelf life of material, enhancing flavor, and inhibiting spoilage and pathogenic microorganisms. During fermentation, microorganisms convert carbohydrates and other sources of carbon in food material to ethanol and carbon dioxide or organic acids. Metabolic by-products from the microorganisms involved in fermentation preserve the food, enhance flavor and increase consumer acceptability (Rahman, 2007). Preservation methods such as the use of irradiation, blanching, and heating are also used in the food industry to control pathogens and spoilage microorganisms. Food fermentation may be preferred over some of the above methods, although some processors consider irradiation and heating methods as more effective in controlling pathogens than fermentation. One of the reasons why fermentation is preferred is the additional flavors that fermentation products impart, its role in developing a more nutritive and digestible product, and its role in enhancing food preservation, as the low pH inhibits the growth of microbial pathogens (Norma and Hotchkiss, 1998). Fermentation encourages the growth of beneficial microbes and their metabolites in foods. Fermentative bacteria, especially lactic acid bacteria, contribute to the texture and flavor of the fermented food product. Fermentation is an inexpensive method of food preservation and does not require sophisticated facilities. It can therefore be practiced anywhere in the world by people with little or no training. Fermented foods may be classified by their physical, chemical or biological properties. Steinkraus (2002) classified fermented foods by types of organisms used in the process, the biochemistry of the food, and the state of the food (e.g. liquid or solid). Campbell-Platt (1987) had earlier expanded the classification to

include cereal products, beverages, dairy foods, legumes, fruits and vegetables, fish, and meat products. Based on classification, processors identify the strains of lactic cultures that produce the results in different fermented foods (Table 2-1).

Table 2-1 Some common fermented foods

Food	Microorganism responsible
Dairy products	
Yogurt	Lactic acid bacteria
Cheese	Lactic acid bacteria
Butter milk	Lactic acid bacteria
Fermented vegetables	
Pickle cucumber	Lactic acid bacteria
Sauerkraut	Lactic acid bacteria
Pickled olives	Lactic acid bacteria/ (yeasts)
Fermented animal products	
Fermented sausage	Lactic acid bacteria
Fermented fish	Lactic acid bacteria
Bakery products	
Bread	Yeasts
Sourdough bread	Lactic acid bacteria+ yeasts
Sauces	
Vinegar	Yeasts + acetic acid bacteria
Soy sauce	Moulds + yeasts + lactic acid bacteria

Source: (Guizani and Mothershaw, 2007)

2.1.1. Fermented sausages

Fermented sausages were prepared from comminuted mixture of meat; fat salt spices and sugar using bacterial culture there allowed to undergo fermentation under strict conditions of temperature and humidity (Ahmad and Amer, 2013). Fermented sausages made from other animal meats are rare, but can be found in the Middle East. As a religious prohibition edict, pork is not allowed for consumption by Muslims, thus other animal meats are used for sausage making, such as sheepmeat (Lu, 2010). The characteristic flavour of fermented sausage is due to LAB fermenting the

carbohydrate source to lactic acids and other organic acids that are together responsible for the typical sour flavour. Furthermore, after the pH is lowered by the growth of LAB, the tertiary and, secondary structure of meat proteins is adversely affected, commonly known as denaturation. Some proteins are hydrolysed to generate peptides and free amino acids (Nishimura *et al.*, 1988). In addition, fat oxidation progresses slowly throughout fermentation, and the addition of salt, sugar, and spices contribute to flavour. Thus, these combinations define the flavour of fermented sausages (Lu, 2010). Processing of fermented sausages includes meat and fat selection, grinding, mixing, stuffing, fermentation, drying and/or smoking and packaging for retail distribution. Bones and intermuscular fatty tissue must be removed from the meat, and connective tissue and membranes trimmed off. Meat and fat are chilled or frozen and comminuted to the desired particle size. A grinder machine or a bowl-cutter is used. The grinder machine consists of a rotating set of knives and a chopping plate that confer the desired meat and fat particle size, whereas the cutter consists of a rotating set of knives in a rotating bowl. Grinding is usually carried out under vacuum to avoid drying and changes in lean and fat color, that are request to be red and white, respectively (Vignola *et al.*, 2010).

Once meat and fat have been ground, they are mixed with additives: curing salts (NaCl, nitrates and nitrites), ascorbic acid, colorants, sugars (lactose, dextrose), spices, aromatic herbs, and starter cultures: lactic acid bacteria (LAB) and/ or coagulase negative cocci (CNC[1]). The process can be carried out in the bowl cutter during the chopping or in a vacuum mixer machine after the meat and fat have been ground in the grinder machine,

[1] coagulase negative cocci

depending on the product. Meat fermentation is a low-energy, biological acidulation, preservation method, which results in unique and distinctive meat properties, such as flavor and palatability, color, microbiological safety, tenderness, and a host of other desirable attributes of fermented sausages. Changes from raw meat to a fermented product are caused by "cultured" or "wild" microorganisms, which lower the pH. Because this is a biological system and it is influenced by many environmental pressures that need to be controlled to produce a consistent product. Some of these factors include a fresh, low-contaminated, consistent raw material; a consistent inoculum; strict sanitation; control of time, temperature and humidity during production; smoke; and appropriate additives (Toldra and Reig, 2007). Lactic acid originates from the natural conversion of glycogen reserves in the carcass tissues and from the added sugar during product fermentation. A desirable fermentation product is the outcome of acidulation caused by lactic acid production and lowering the water activity (a_w[1]) caused by the addition of salt (curing) and drying. Both natural and controlled fermentations involve lactic-acid bacteria (LAB) (Ockerman and Basu, 2007). Another technologically important bacterial group in sausages fermentation is coagulase negative cocci (CNC). Actually, most starter cultures, today, consist of LAB and/ or CNC, selected for their metabolic activity. The reduction of pH and the lowering of water activity are both microbial hurdles that produce a safe product (Ockerman and Basu, 2007). After fermentation sausages are air dried or smoked depending on the sausage type. Air drying is generally used for Spanish, French, Italian and Greek fermented sausages. The drying for these products is highly dependent on temperature, air velocity, and caliber of sausages, which are responsible of the duration of the process. Today, a wide variety of

[1] Water Activity

fermented sausages are produced; the variations depend on raw materials, microbial populations, and processing conditions.

2.1.2. Principles of fermented sausage manufacture

There are actually only a few general steps involved in fermented sausage manufacture. First, the ingredients are selected, weighed, mixed, and stuffed into casings. Second, the stuffed sausages are held under conditions necessary to promote the fermentation. Third, the sausage is subjected to one or more post fermentation steps whose purpose is to affect flavor, texture, and preservation properties. These latter steps can range in duration from as little as one week in the case of moist or semi-dry sausages to more than two months for very dry, strongly flavored sausages such as Italian salamis. Table 2-2 shows the desirable properties of meat starter cultures.

Table 2-2 Desirable properties of meat starter cultures

Bacteria	Fungi
Non-pathogenic	Non-pathogenic
No toxins produced	No toxins produced
Grows well in meat	Competitive at the surface
Stable	
Produces good flavor	Proteolytic and lipolytic
Nitrate/nitrite resistant	Moldy aroma
Salt-tolerant	
Bioprotective	
Easy to identify	

Source: (Hammes and Knauf, 1994)

2.1.3. History of sausage making

Sausage processing evolved as an economic means of food preservation that also converts pieces of meat into more palatable products. According to the Oxford encyclopedia of Foods and Drinks (2004), the name sausage came from the Latin word salsus, which means salted. This is because sausage processing began as a method of preserving meat with salt. Records however show that Babylonians and the people of China consumed sausage as early as 1500 BC (Pederson, 1971). Meat trimmings were ground, salted, stuffed into casings, and dried to develop the desired flavor. The evolution of cured sausage began by adding either sodium Chloride or saltpeter (potassium nitrate), and by the late 1800s scientific study showed that saltpeter was beneficial in sausage preservation. A variety of unique sausage fermentation techniques emerged in other parts of the world. For example nham, a Thai fermented sausage is fermented in leaves instead of casings. The common element for all fermentation techniques is the unique role microorganisms play in the Processing of meat products. Intensive research into the ecological and biochemical properties of these microbes contributed to the cultivation and use of lactic bacteria in sausages. Researchers and processors in the United States embraced the use of lactic bacteria in sausage as early as 1930s and this later gained popularity through the work of Jensen and Paddock in 1940.

Meat products have been among the fastest growing components of the global agriculture and the food industry (Tallard, 2006). Sausage is known to be the oldest and most enduring form of processed meat. The origin of fermented meats goes far back in Ancient Romans and Greeks manufactured fermented sausages, and in fact, the origin of words like sausage and salami may proceed from the Latin expressions *salsicia* and

salumen, respectively (Toldra, 2002). The production and consumption of fermented meats expanded throughout Europe in the middle ages and were adapted to climatic conditions (e.g., smoked in northern Europe and dried in Mediterranean countries). Today, a wide variety of fermented sausages are produced; the variations depend on raw materials, microbial populations, and processing conditions (Table 2-3).

Table 2-3 Examples of fermented meats with different dryness degrees

Product	Type	Examples	Weight loss (%)	Drying/ Ripening
Un dry fermented sausages	Spreadable	German teewurst, Frische mettwurst	<10	No drying
Semidry fermented sausage	Sliceable	Summer sausage, Lebanon bologna	<20	Short
Dry fermented sausage	Sliceable	Hungarian and Italian salami, pepperoni, French saucisson	>30	Long

Source: (Farnworth, 2008)

2.1.4. Manufacture of traditional and modern methods of sausage

Traditionally, sausage manufacture was confined to homes and small commercial settings. The ground meat was processed with indigenous herbs and spices. Fermentation of meat depended on adventitious bacteria present in the butcher's premises. This means that the quality of sausage produced at the time relied on the type and amount of beneficial microorganism present at the time of processing. Each manufacturer followed family recipes in preparing sausages to their own taste **(**American Meat Institute Foundation, 1960). Therefore, flavor was determined by each manufacturer's location and family recipes. Modern sausage production is independent of environmental conditions. Temperature, humidity, moisture, and acidity are continuously and carefully controlled.

Microorganisms with specific desired properties can be grown separately and added to sausages. Recipes and ingredients for particular types of sausages can be obtained from the literature and there are limits to amounts of additives regarded as acceptable.

2.1.5. Meat starter cultures

Once microbiologists began to study and identify the microorganisms present in fermented sausages in the 1940s, it became clear that lactic acid bacteria were the primary organisms responsible for the fermentation. This conclusion was based on the fact that the predominant organisms isolated from naturally fermented sausages were species of *Lactobacillus*. When the isolates were propagated and re-inoculated into fresh meat, a well fermented sausage could be produced with all the expected characteristics. Patents, based on using these bacteria as meat starter cultures, were assigned in Europe and the United States. However, application of this technology was initially unsuccessful (Hutkins, 2006). This was because the *Lactobacillus* strains that had been identified and used successfully in trial situations were difficult to mass produce in a form convenient for sausage manufacturers. For any organism to function well as a starter culture, it must not only satisfy the performance criteria, but it must also be present in high numbers and be viable at the time of use. In the case of the *Lactobacillus* cultures, cell viability following lyophilization (or freeze drying, the main form of starter culture preservation) was poor, leading to slow and unacceptable fermentation rates (Hutkins, 2006). Demand for a culture that could be used for fast, consistent, large-scale production of fermented sausage eventually led to the discovery of other lactic acid bacteria that not only had the relevant performance characteristics, but also the durability required for commercial applications. In addition,

improvements in starter culture technology and the development of frozen concentrated cultures enhanced culture viability such that *Lactobacillus* starter cultures are also now available. Initially, the *Lactobacillus* strains that were isolated from fermented sausage and used as starter cultures. Although the different *Lactobacillus* strains used as starter cultures perform the same basic role—to ferment sugars and produce organic acid—they vary with respect to several important physiological and biochemical properties (Hutkins, 2006). These differences influence how they are used as starter cultures. First, different species have different temperature optima and different thermal tolerances. For example, *Lactobacillus sake* and *L. curvatus* are considered psychrotrophic, meaning they are capable of growth, albeit slowly, at temperatures as low as 4°C.Thus, they are suitable for fermentations conducted at cool ambient temperatures. The ability of some strains to produce peroxides also is a serious problem. Depending on the starter culture strain and the level of oxygen in the environment, hydrogen peroxide can be formed directly in the fermenting sausage. Hydrogen peroxide can react with heme proteins in the muscle tissue to form undesirable, green pigments. In addition, hydrogen peroxide and peroxide radicals promote lipid oxidation, a serious flavor defect (Hutkins, 2006).

2.1.6. Lactic acid bacteria used in the fermentation of meat

Lactic acid bacteria ferment carbohydrates into lactic acid. This efficient carbohydrate fermentation coupled to substrate level phosphorylation is essential feature of lactic acid bacteria (LAB) metabolism. Generally, LAB produce lactic acid as their main fermentation end-product via glycolysis (the Embden-Meyerhof pathway) or the 6-phosphogluconate/phosphoketolase pathway and they are named

homo- or heterofermentative LAB, respectively (Kandler, 1983, Axelsson, 1998). LAB is generally catalase negative but also catalase positive strains exist (Engesser and Hammes, 1994). They prefer nutritionally rich environments and are natural inhabitants of the mammalian gastrointestinal tract. Several food products are manufactured using *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *streptococcus*, or *Pediococcus* strains (Daly and Davis, 1998) and therefore actually by their metabolic products (Table 2-4).

Table 2-4 Commercial significance of metabolic products LAB

Metabolite	Beneficial	Deleterious
Lactic acid	Preservation, sensory improvement	Acidification
Acetic acid	Aroma	Off-taste
Diacetyl	Aroma (dairy products)	Off-taste
CO_2	Preservation, flavour enhancement	Gas production(blowing)
H2O2	Preservation	Discolouration
Biogenic amine	--------	Health(food intoxications)
slime	Stabilization(e.g. yoghurt)	sensory
Methane thio, H2S	Aroma	Off-taste and odour
Bacteriocins	Preservation	Health? inhibition of beneficial LAB
Wide-spectrum	Inhibition of pathogens and spoilage	Health? Resistance of intestinal
antimicrobials	microorganisms	microorganisms

Source: (Holzapfel *et al.*, 1995)

2.2. Probiotic

Probiotic foods are a group of health-promoting, so-called functional foods, with large commercial interest and growing market shares (De Vuyst *et al.*, 2008). In general, their health benefits are based on the presence of selected viable strains of lactic acid bacteria (LAB), that, when taken up in adequate amounts, confer a health benefit on the host (De Vuyst *et al.*,

2008). They are administered mostly through the consumption of fermented milks or yoghurts (De Vuyst *et al.*, 2008). In addition to their common use in the dairy industry, probiotic LAB strains may be used in other food products, including fermented meats (De Vuyst *et al.*, 2008). Although the concept is not new, only few manufacturers consider the use of fermented sausages as carriers for probiotic LAB (De Vuyst *et al.*, 2008).

2.3. Ingredients of fermented sausages

It is entirely possible to manufacture a fermented sausage with just a handful of ingredients. In fact, only five ingredients are essential: meat, sugar, salt, culture, and a curing agent. Fermented meats can indeed be made without adding a culture, but most large-scale manufacturers would not dream of making product without a culture. Likewise, fermented sausages also can be manufactured in the absence of the curing agent. However, these agents, either in the form of nitrite or nitrate, perform such important microbiological and organoleptic functions that they are nearly universally used. That being said, there is a small (but growing) market among organic foods proponents for reduced or even nitrite-free meat products. Even if the organoleptic properties provided by nitrite could be provided by other agents (a big if), removing nitrite from cured meat products would expose these products to a potentially serious food safety threat. Still, provided that other barriers are in place, especially low pH and low temperature, theoretically safe products can be produced (Hutkins, 2006).

2.3.1. Meat selection and grinding

Of the ingredients listed above, the main ingredient is obviously the meat, which contributes not only the protein and the bulk of the product matrix, but also the fat, which provides much of the flavor. The fat-containing cuts usually are chopped or ground separately from the leaner portions to impart a desired appearance and flavor. The grind also affects texture and accordingly determines the type of product. For example, some sausages (e.g., Plockworst) have large visible fat particles, whereas others (e.g., cervelat) are ground to fineness such that the fat particles are so small as to be indistinguishable from the sausage matrix. The fat and lean portions may even be derived from different animals. Beef fat contains more unsaturated lipids than pork fat, and is more susceptible to oxidation reactions that may result in undesirable rancid flavors. Obviously, there is a trend to use less expensive cuts, but high quality meats are often still used (Hutkins, 2006). A basic requirement for producing uniform processed meat products is proper selection and preparation of meat ingredients. Animal tissues vary widely in moisture, protein, and fat content, in pigmentation, and in the ability to bind water and fat. Thus, the processor must know the properties and composition of the various available meat tissues in order to arrive at the correct meat formulation. Lean meat 65-75% is commonly added in the mix of most industrial sausages but, this ratio is variable (Asmare, 2012).

Using the meat as a food dates back to the beginning of human history. With increasing prosperity increased diversity in human tempers. That's why food manufacturers such as meat products for the presence in household consumption basket to produce meat products and various dimensions of their greed's. Over the time, the researchers came to the

conclusion that processing of meat into meat products improves the product's shelf-life and microbiological safety (Ghajarbeygi *et al*., 2016**)**. In the study of Yaghoubifar *et al*. (2009) mentioned per capita consumption of sausages for every Iranian citizen is about 4 kg. The nutritional value of meat products is mainly due to the energy supplied by these products, and also to their high biological value proteins, vitamins and minerals (Muguerza *et al*., 2004). In addition, meats are most important in supplying iron and other vital substance for human consumption (Rogov *et al*., 1989). Camel meat is also relatively high in polyunsaturated fatty acids (PUFA) in comparison with beef **(**Dawood and Alkanhal, 1995).This is an important factor in reducing the risk of cardiovascular disease, which is related to saturated fat consumption (Giese, 1992). Comparative technical information shows that the fat content of camel meat is considerably less than beef, low in cholesterol and high in protein. Camel meat is similar in taste and texture to beef (Williams, 2002). Meat is one of the best source of proteins, vitamins and minerals which are essential nutrients required for proper growth and maintenance. Meat has been defined as the flesh of animals which are suitable as food (Forrest *et al*., 2001). This includes all processed or manufactured products which might be prepared from these tissues i.e. meat may be fresh, cured, dried or otherwise processed. Meat is one of the highly perishable foods because of its high nutritional contents, enzymatic action and the presence of microorganisms (bacteria, yeasts moulds) which may result in oxidative rancidity, discoloration, mouldiness, off flavour, sliminess etc. The major source of these deteriorative changes being microorganisms, this renders the meat unacceptable and unfit for human consumption (Forrest *et al*., 2001). Methods of preserving meat include the use of high temperature e.g. canning, low temperature e.g. chilling, freezing and pasteurization, drying e.g. hot air drying, wind and

sun drying, smoking, use of radiation and the use of chemical preservatives. It has been reported that drying reduces the moisture content to a level that prevents the growth of microorganisms especially fungi and bacteria (K'Opondo, 2011). All handling and storage methods are therefore primarily concerned with minimising microbial contamination and retarding microbial growth and activity. The amino acid content of meat includes Isoleucine, Leucine, Lysine, Methionine, Cystine, Phenylalanine, Valine, Threonine**,** Tryptophan, Arginine, Histidine, and Alanine among others which make it better than other foods as source of protein (Lawrie, 1985). Meat being highly nutritious and having high moisture content with nearly neutral pH serves as a good culture medium for most microorganisms and as such, it is classified among perishable foods whose contamination with spoilage organisms is almost unavoidable (Ikeme, 1990). This makes meat preservation more difficult than other kinds of food. Dried meat is commonly prepared in the North from where it is transported to other parts of the country for consumption. According to (Fakolade and Omojola, 2008) there was an increase in microbial load of organisms on dried meat samples after six months of storage due to moisture absorption from the environment**.** Meat from healthy animals is used in fermented sausage production. Animal species, anatomical region of the animal, composition, and microbiological quality have a great influence on the functional characteristics of meat. Bones should be removed, connective tissue membranes trimmed off, and soft intermuscular fatty tissue detached. Meat and fat are chilled (grinding technology) or frozen (bowl chopper technology) and comminuted to the desired particle size. The cutter (a rapid rotating set of knives in a slowly rotating bowl) has become the established means of chopping (Asmare, 2012).

2.3.2. Sugars

The next essential ingredient is the sugar or carbohydrate. Although glucose, in the form of the polymer glycogen, is initially present in muscle tissue of slaughtered animals, glycogen stores are quickly depleted during the postmortem period. Thus, fresh meat contains little fermentable sugar and addition of sugar is necessary. Sugars are commonly added to fermented sausages, among which the most often used are dextrose, glucose, sucrose, and lactose, as well as corn syrup and different starches (Rust, 2007). Glucose is most common in the United States, and is added to about 1% to 2% of the total batter weight (Hutkins, 2006). The main role of sugars in fermented meat products is to act as substrates for LAB to produce lactic acid and influencing the rate of pH decline. The quantity (dextrose a minimum of 0.75% is often recommended), of sugar influences the rate and extent of acidulation, and also contributes favorably to flavor, texture, and product yield properties (Asmare, 2012**)**. Since the amount of acid produced by the lactic culture is directly related to the amount of available glucose, the sugar concentration in the batter can be adjusted, in general, to give a particular final pH. Also, higher sugar levels promote faster fermentations, which are preferred in the United States. In contrast, many European fermented sausage manufacturers prefer less tanginess and more diverse flavor development. Achieving these characteristics require slower fermentation rates, thus less rapidly fermentable sugar is added (as little as 0.1% to 0.2%) (Hutkins, 2006).

2.3.3. Other Ingredients

Adding ingredients to sausages, like any form of food processing, is meant to increase consumer acceptability of the product. Adding different ingredients produces a variety of sausage types. These ingredients not only

improve the sensory properties of sausage but also serve as preservatives and to help ensure the stability of the product. However, adding one ingredient does not necessarily improve the sensory and safety attributes of sausages. A combination of ingredients like curing salt, seasonings, and starter cultures have proved to be beneficial and acceptable for producing safe and stable sausages (Dogbatsey, 2011) . Some of the key ingredients and their roles are summarized in the Table 2-5 (Dogbatsey, 2011).

Table 2-5 Key non-meat ingredients and their uses

Non Meat Ingredients	Uses	Examples
Salt	Preservation	Sodium Chloride
	Flavoring	Potassium Chloride
Spices	Antioxidant	Ginger
	Flavoring	Cloves
	Antimicrobial Agent	Black Pepper
Curing Agents	Improve Color	Sodium nitrite
	Retards rancidity	Sodium nitrate
Starter Cultures	Fermentation	*Lactobacillus plantarum*
	Antimicrobial Agent	*Pediococcus acidilactici*
		Staphylococcus carnosus

2.3.3.1. Salt

Salt is an essential ingredient in all types of sausage products (fermented or not). Salt, added in concentrations of 2.4% to 3%, performs several critical functions. First, it is responsible for extracting and solubilizing the muscle proteins, which are ordinarily in an insoluble form. Once extracted and solubilized, the proteins form a “sticky” film around the meat particles, creating an emulsion-type structure. Second, salt is very important for flavor and texture purposes of the end product, and is often used as a carrier for curing and flavoring agents (Gerhard, 2006; Hutkins, 2006). Finally, salt is the primary means, at least initially, for controlling the microflora. Salt will also increase the bind (myosin extraction which forms a gel between meat particles and meat and fat particles). Smith and

Palumbo (1973) investigated the effect of different concentrations of sodium chloride on the survival of spoilage bacteria in Lebanon bologna. They found that higher concentrations of sodium chloride inhibited the growth of spoilage organisms in the absence of starter cultures. This is because salt lowers water activity, making water unavailable to the microorganisms for growth. Plasmolysis also occurs, as water flows out of the cell membrane when the osmotic potential is disrupted.

2.3.3.2. Nitrite

Sodium nitrite is a vital ingredient for meat curing, but nitrates can sometimes be used as a replacement. For safety and health reasons, nitrite use in dry sausage is controlled at 156 Ppm[1] as the allowable limit. Nitrites were therefore encouraged as a replacement for nitrates even though some meat processors still use nitrates. Sodium nitrite or nitrate is added to meat products for two reasons. First, the bright color of cured meat product is retained through a series of reactions. Thus, nitrite is converted to nitric oxide, which then combines with myoglobin, a pigmented protein in meat responsible for natural meat color (Gotterup *et aI.*, 2007). Once combined, the nitric oxide myoglobin produces the bright red color of the cured meat. Second, sodium nitrite or nitrate is added to meat due its antimicrobial effects on pathogens such as the ability of nitrite to inhibit formation and growth of *Clostridium botulinum* spores (Dykhuizen *et aI.*, 1996). Christiansen *et aI.* (1974) reported an inverse relationship between the amount of nitrite and *Clostridium* cell counts. These authors noted the reduction was dependent on both temperature and salt concentration. Unfortunately, undesirable compounds are produced by humans who consume meats containing nitrates and nitrites. These compounds, called

[1] Parts per million

nitrosamines, may induce a disorder known as methemoglobinemia, a condition where blood oxygen is not transported efficiently because hemoglobin is converted to methemoglobin (Hord *et aI.*, 2009). Cassens (1995) also linked consumption of nitrite and nitrate intake from food to cancer in young children. Several countries therefore continue to monitor and control nitrate and nitrite levels in foods. The American Cancer Society concluded that nitrite levels in American foods do not cause significant cancer among Americans (American Cancer Society Dietary Guidelines Advisory Committee, 1996). After an appropriate heat treatment, other processes needed for human food consumption is prepared. With the advancement of science and the importance of increasing the shelf life of food, the researchers had to develop chemical preservatives to increase shelf life of food. Among these, nitrites are preservatives which were initially due to the favorable effects on the color and flavor of meat products had been used; but later it became more important effect of *clostridium*. The indiscriminate use of chemical sustaining food producers has led to regulatory agencies to determine the permissible acceptance limit for chemical substances used in their food. Nitrite is a compound that exists in living systems and also, it is one of the most active intermediate species in the nitrogen cycle, where suffers a surprising metamorphosis from a vilified substance that generates carcinogenic Nnitrosamines with amines and amides present in the stomach, to a life-saving drug that liberates a protective agent (nitric oxide or NO) during the hypoxic events (Bryan, 2006). As a high reactive compound, nitrite can function as an oxidizing, a reducing or a nitrosylating agent, and can be converted to a variety of

related compounds in meat, including nitrous acid, nitric oxide and nitrate. The World Health Organization has reported that the fatal dose of nitrite ingestion is between 8.7 μM[1] and 28.3 μM (Zhang *et al.*, 2013). In the same time, the Legislation of the European Union (Ghajarbeygi *et al.*, 2016) suggests a maximum supplement which is allowed in the first stage of the food processing, and it imposes a maximum residue of 50 mg/kg nitrites and 250 mg/kg nitrates for those meat products (expressed as NaNO2), which has not been treated thermally. Nitrites and nitrosamines effects on human health due to the formation of nitrites used in meat processing about one-fifth less than two decades ago (Ghajarbeygi *et al.*, 2016). The results of the study of Ghajarbeygi *et al.* (2016) showed that a standard for the production of meat products cannot be fully complies in Iran (Kamkar *et al.*, 2005). Also, in the study of Babaei *et al.* (2011) the average nitrate in sausage was 81.14 and 115.1 mg/kg, respectively, which have said in this case the acceptance limit is nitrite 60 ppm (Ghajarbeygi *et al.*, 2016). Therefore, it should be a careful monitoring and continuous, integral part of maintaining health and microbial quality of meat products (Ghajarbeygi *et al.*, 2016). But given the above people still have an interest in consumption of meat products which can be the source of this interest is the increase in population and new conditions of urban societies that physical access to fresh food such as fruit and vegetables lowered and people are forced for economic prosperity for their families, most of the day to spend outside the home and at work; So, to have the fast food **(**Ghajarbeygi et *al.*, 2016**)**.

1 Micrometer

2.3.3.3. Ascorbic acid

Sodium ascorbate and erythorbate are added as cure accelerators which speed up the formation of cured color and also act as antioxidants (Feiner, 2006).

2.3.3.4. Spices

Different spices and herbs have for centuries been added to foods in different concentrations to achieve characteristic flavors. In meat processing, spices are used alone or in combination with other ingredients like salts and sugars to give sausages and other meat products their desired flavor, pungency and color. Garlic, mace, rosemary, ground pepper, paprika, and ginger are spices that are most used in sausage manufacture (Verluyten *et aI.*, 2004). Other spices provide additional benefits when added to sausages. Chipault *et aI.* (1952) was the first to report the ability of rosemary and sage to exhibit profound antioxidant properties in meat. AI-Jay *et aI.* (1987) further evaluated the antioxidant properties of ten spices that are mostly used in dry sausage fermentation. They reported that clove, allspice, and black pepper exhibited antioxidant activity. This property of clove had earlier been attributed to the presence of eugenol (Kramer, 1985). According to Gray and Killinger (1966) the use of natural and artificial additives in foods can be used to minimize or inhibit the growth of food-borne microbes. The inhibitory capability of some spices has been attributed to their essential oils such as phenolic amide present in black pepper, tocopherols from oregano, diarylheptanoid found in ginger and eugenol in clove (Kim *et al.*, 1995). In a related study, Blank *et al.* (1987) discovered that ground clove eugenol has the tendency to decrease the rate and extent of *Bacillus subtilis* spore germination. Nutmeg, bay, and mace extracts added at levels of 125 ppm were found to inhibit *Clostridium*

botulinum toxin production in turkey frankfurters, whereas ground cinnamon, clove, mustard, garlic, and onion added at 0.5% had inhibitory effects on *Listeria monocytogenes* (Hall and Maurer, 1986). In addition to the sensory qualities spices impart to processed meat, they also serve as antioxidants and antimicrobial agents in meat products. Spices are used for flavor; act as effective antioxidants, to stimulate LAB activity by supplying manganese (Mn), and to inhibit undesirable organisms (Srinivassane, 2011). Table 2-6 shows the common spices used in sausage manufacture, their sources, and examples of resulting products.

Table 2-6 Common spices used in sausages, areas they are commonly found, and some products they are used to produce

Spice	Common Location Grown	Use
Garlic	India, U.S.A, Italy, Mexico	Frankfurters, Pork Sausages
Black Pepper	Singapore, Thailand	Polish and Smoke Sausages
Clove	Brazil, Sri Lanka, Tanzania	Bologna, Liver Sausage
Paprika	Hungary, Ethiopia, Spain	Polish Sausage, Bologna

Source: (Dogbatsey, 2011)

2.3.3.5. Starter culture

Most commercial cultures for sausage are supplied in either a frozen or lyophilized form. Frozen cultures, which are more common in the United States, are supplied as thick slurries in peel-back or flip-top cans ranging in size from 20 ml to 250 ml. Cell densities typically range from 10^8 to 10^9 cells per ml. A typical 70ml can is sufficient for about 150 kg of sausage batter. These cultures are shipped frozen under dry ice and users are instructed to store the cans at - 40°C or below. The cans should be thawed in cold water prior to use. Proper handling of frozen cultures is absolutely necessary to maintain culture viability and to ensure that culture performance (i.e., rapid fermentation) is not impaired. Lyophilized cultures, which are less commonly used, have the advantage of not requiring low-

temperature storage. They are stable at refrigeration or even ambient temperatures. As free-flowing powders, they are easily measured and distributed into the batter. These cultures, which can contain up to 10^{10} cells per gram, are usually more expensive, however, than frozen cultures (Hutkins, 2006). Beneficial microorganisms are vital in the manufacture of fermented sausages. They often present naturally in meat, but can also be added in the form of starter cultures. Meat fermentation using starter cultures ensures the presence of sufficient cell numbers to guarantee consistent and controlled fermentation. Adding these microbes also inhibits the growth of spoilage and pathogenic microorganisms by reducing pH, producing organic acids, and producing inhibitory bacteriocins. Other reasons why food processors use starter cultures are their ability to reduce variations between batches resulting in consistent product as well as Minimize processing time (Dogbatsey, 2011). The term starter culture was then adopted and used for bacterial inoculation because these cells are used to initiate the fermentation process (Cogan, 1995). Such microorganisms must be tolerant to nitrite and salts while exhibiting growth at optimal temperatures. They must be safe for consumption, and must not produce off odors. Some of the genera of starter cultures include *Lactobacillus, Lactococcus,* and *Pediococcus. Pediococcus* and *Lactobacillus* are used in the meat fermentation industries where the aim is to produce fast-ripening and low acid sausages (Dogbatsey, 2011). In general bacterial starter cultures have a variety of functions including:

1-Boosting acidity (decreasing pH)

2- Intensify the curing colour (acid environment catalyses curing reaction)

3- Counteract rancidity of fats (due to enzymatic impacts)

4- Development of flavor and taste

5- Texture improvement of ripened products (by supporting formation of protein gel in sausage mixes) (Asmare, 2012).

2.3.3.6. Ice water

Water is added to sausages like frankfurters to activate or solubilize muscle protein which is critical for meat emulsion stability. Water is also required to disperse salt, nitrite and other ingredients. Ice is commonly used to counteract the heating effects of the high cutting and shearing forces generated by the knives on the bowl cutter and to keep the temperature of the sausage mass down. Thus, ice aids in obtaining a homogeneous emulsion by activating the maximum amount of protein (Feiner, 2006). The amount of water to be added is limited to the extent that amount of added water plus fat content in frankfurters cannot exceed 40% (CFIA, 2003). Ice water is used as a carrier for the curing agents, and improves the extraction of meat protein and the hydration of meat-extender proteins. The amount of added ice, the roles of water in biological systems are numerous and the mechanisms of action and interaction of this molecule with organic molecules are not totally elucidated. Two fundamental functions can be distinguished (Gervais *et al*., 1996):

1-A solvent function on the level of the organism as well as of the cell provides nutrients and scavenges wastes, or metabolites, under the dissolved form.

2-A structural function which is implicated in the stability and the function of the biological structures organized at the molecular and cellular levels.

At the molecular level, the role of water is in the stabilization of the structure of the biopolymers; such as proteins, nucleotides and carbohydrates. At cellular level, the role of water molecules is in the

stabilization of the lamellar structure of the plasmic membranes and thus in the preservation of membrane permeability has been shown. In the intracellular medium, the molecules of water linked with other molecules, such as polyols, sugars or enzymes, contribute to the maintenance of the cellular volume, especially when the cell is placed in a hypertonic medium and particularly during desiccation or freezing conditions (Asmare, 2012).

2.3.4. Casings

Casings: are special cylindrical containers used to protect sausages and various meat products, In addition, casings also protect products during storage to give them shape and hold them together during further processing steps such as fermentation, smoking, drying, boiling, frying or roasting. Casings can be of natural origin or artificial. Natural casings are obtained from animal intestines derived from slaughtering. Manufactured artificial casings are made of cellulose, collagen or synthetic materials (Asmare, 2012).

2.3.4.1. Natural casings

These are derived almost from gastro-intestinal tracts of cattle, sheep, and goat. They are somewhat irregular, difficult to use with high speed stuffing equipment. They are very permeable to moisture and smoke; they shrink and thereby remain in close contact with the surface of a sausage as it loses moisture. Natural casings are highly contaminated with bacteria and are often preserved for later use by packing in salt or saturated salt brine (FAO, 1991).

2.3.4.2. Artificial casings

Artificial casings solve many of the problems associated with natural casings. Examples include collagen, cellulose and plastic casings.

Collagen casings are traditionally made from collagen extracted from the corium layer of bovine hides before being decalcified and ground. Acid is used to swell the collagen and make the dough easily extruded. The collagen dough contains functional additives such as cross linkers, plasticizers and cellulose fibers in order to have consistent extrusion (Nakyinsige *et al.*, 2012). Collagen casings can be edible or non-edible, depending on the type of sausage. For example, edible collagen casings are widely used for fresh and cooked sausages, while non-edible collagen casings, which are basically used for larger-diameter sausages, are removed prior to consumption (Feiner, 2006). The growth in artificial casings has increased steadily over the last 6 years due to improvements in productivity, food safety and cost, compared to natural casings, which are becoming more expensive due to the decline in the sheep population (Viscofan Annual Report, 2012). Collagen sausage casings are the most successful edible film commercially used in the meat industry. Collagen casings are made from natural collagen extracted from the corium layer of bovine hides with several processing steps. Collagen casings were developed as an alternative to natural casings because of numerous advantages in mechanical and physical properties compared to natural casings. However, collagen casings lack the gas barrier and antimicrobial properties that would enhance the quality and safety of sausages (Krkic *et al.*, 2012).

Cellulose casing are an artificial casing made from solubilized cotton or wood pulp. It is very uniform, strong, and not quite as susceptible to bacteria as other types of casings. Sausages produced by this method are easier to pack than those in variable animal casings due to their uniform size and weight. This is advantageous in both retail and professional markets (Asmare, 2012). Furthermore, they have good permeability to

moisture and smoke in wet environment (Romans *et al*., 2001). Cellulose casings must be removed from the finished product after cooking because they are nonedible (Nakyinsige *et al*., 2012).

Plastic casings have variety of layers ranging from one to five layers. The production of plastic casings requires sophisticated processing technology, especially for multiple layers of plastic casings. Plastic casings are good barriers against oxygen and moisture. They are easy to handle, easy to peel and have consistent diameters. However, plastic casings shrink during the cooling process and subsequently produce a wrinkled appearance that might limit consumer acceptance. The material used for monolayer plastic casings is polyamide and for multiple layers are polyamide and polyethylene (Feiner, 2006).

2.4. Manufacture of fermented sausage

For the manufacture of typical dry fermented sausages, lean meat (60-70%) and fatty tissue (30-40%) are comminuted, mixed with about 2.4-3% salt, curing agents, some sugar, spices and, in many cases, starter cultures. The mix is placed with inclusion of as little oxygen as possible, into vapour-permeable casings and subjected to a fermentation and drying process. Control of temperature and relative humidity is essential for the production of dry sausages. Ripening chambers with precisely adjustable temperature and humidity are expensive, particularly if they are equipped with smoke generators and smoke combustion devices. This is the main reason why today only a small proportion of fermented dry sausages are manufactured on an artisanal scale (Lucke, 1994).

2.5. Mixing and stuffing

Once lean meat and fat have been comminuted, curing salts, additives, other ingredients (sugars, spices/aromatic herbs), and starter cultures (LAB) are added to the meat batter and thoroughly mixed in the bowl chopper. Mixing should be sufficient to uniformly distribute ingredients; over mixing must be avoided. After the batter has been sufficiently mixed, it is moved to a stuffer, a device that pumps the mix into casings. The casings are essentially long tubes that give the product its characteristic shape. The diameter of the casings can vary from less than 1.5 cm to more than 9 cm. As the tubes are filled, they are tied off or cut to give desired section lengths, again depending on the product being made. Lengths can vary from 5cm to 100 cm. Shape and diameter size are important, not only because they are specific for a given product, but more importantly, because they influence the rate of drying, cooking, smoking, and ultimately the flavor and texture of the finished product (Asmare, 2012).

2.6. Sausage fermentation

Meat as a substrate for microorganisms. Lean meat contains considerable amounts of peptides and amino acids but only small amounts of glucose and glucose-6-phosphate. The content of these fermentable sugars, as well as the content of lactic acid and the pH, depend on the glycogen content of the muscle at slaughter and my vary considerably. As a rule, meat with a pH above 5.9 contains too little lactate and sugar for a safe fermentation; it binds water tightly and provides better conditions for growth of acid-labile bacteria (Michet, 2015). The solid-substrate fermentation inherent to meat means bacteria grow in micro-colonies, and therefore starter cultures need to out-compete the indigenous microflora of the raw material without the use of a kill-step (cooking), which would

change the appearance of the meat. The comminuted meat quickly becomes anaerobic in the interior, resulting in obligate aerobe growth to the surface (Michet, 2015).

Once the sausage batter is packed into casings, the material is moved into specially designed ripening chambers where the fermentation occurs. These facilities often referred to as the green room or smoke house or simply the house have controls for maintaining temperature, humidity, and air movement. Moreover, control systems in modern facilities are fully programmable so conditions can be ramped or adjusted depending on how the fermentation is proceeding or on the particular specifications of the manufacturers. Thermocouples and pH probes inserted directly into product samples can feed the appropriate information into a computer to provide constant monitoring, record-keeping, and feed-back control. Thus, the entire fermentation can proceed in the absence of a full-time operator (Hutkins, 2006). Fermentation parameters vary depending on the culture and the desired product qualities. In general, lower incubation temperatures require longer fermentation times. For example, at the low temperature range of 21°C to 24°C (70°F to 75°F), which is very near ambient, fermentation can take as long as two to three days. At 29°C to 32°C (85°F to 90°F), twelve to sixteen hours of fermentation will be required. In the United States, where faster overall production times are preferred, the incubation temperature can be as high as 37°C to 40°C (98°F to 102°F) for as little as twelve to eighteen hours. Since individual culture strains may have different temperature optima and tolerances, selection of cultures that perform under the selected conditions is critical (Hutkins, 2006). It is also important, for some applications that the fermentation rate not proceeds too fast. This is particularly relevant when flavor- or color-producing

organisms (e.g., *Micrococcus*) are used, because they may be inhibited by fast lactic acid producers. However, if the fermentation is too slow, there may be sufficient opportunity for pathogens or spoilage organisms to grow. Ultimately, the pH at the end of fermentation should be less than 5.1, which, by itself, will not make the product shelf-stable, but which does provide a reasonable protective barrier against most foodborne pathogens. In addition, fermented sausages at pH 5.0 to 5.1 will have only a slight tart flavor that may actually be indistinguishable from non-fermented products (Hutkins, 2006). Alternatively, depending on the culture, the incubation conditions, and the amount of substrate (i.e., fermentable sugar) provided, much higher acidities can be achieved. Typically, summer sausage has a pH around 4.9 but can be as low as 4.7.When the pH reaches 4.8 or less, a definite "tangy" flavor becomes apparent. Fermentation rooms are also equipped with air movement devices and humidistats to maintain the relative humidity (RH) at desired levels. Since the RH[1] in the atmosphere surrounding a food directly influences the water activity of that food (where $a_w \times 100 = RH$), as RH is decreased, the more moisture is lost to the atmosphere and the lower will be a_w in the sausage. Typically, the RH should be about 10% less than $a_w \times 100$ of the finished product (Hutkins, 2006).

Stuffed sausages are placed in fermentation chambers under controlled temperature and specified period, depending on the sausage type to be produced. The aim of the fermentation process is to transform the highly perishable substrate meat into a shelf stable and safe product ensuring an optimum nutritive value and sensory quality (Farnworth, 2008). Meat fermentation is a low-energy, biological acidulation, preservation method, which results in unique and distinctive meat properties, such as flavor and

[1] Relative Humidity

palatability, color, microbiological safety, tenderness, and a host of other desirable attributes of this specialized meat item. Changes from raw meat to a fermented product are caused by "cultured" or "wild" microorganisms, which lower the pH. Because this is a biological system and it is influenced by many environmental pressures that need to be controlled to produce a consistent product. Some of these factors include a fresh, low-contaminated, consistent raw material; a consistent inoculum; strict sanitation; control of time, temperature and humidity during production; smoke; and appropriate additives (Toldra and Reig, 2007). A desirable fermentation product is the outcome of acidulation caused by lactic acid production and lowering the water activity (a_w) caused by the addition of salt (curing) and drying. Both natural and controlled fermentations involve lactic-acid bacteria.

The fermentation step in the sausage production includes the period in the sausage process where pH decreases from approximately 5.7 to its lowest value, which could vary from 5.5 (hard salamis) to 4.6 (or even 4.2 in high-temperature fermented sausages) depending on the sausage type. Fermentation parameters vary depending on the culture and the desired product qualities. The fermentation lasts from less than 12 hours to several days depending on the sausage style. Sausages fermented at high temperatures (37°C or higher) reach quickly the lowest pH value **(**Varnam and Sutherland, 1995) but, temperatures of around 24°C result in a pH of 4.6 to 5.0, and lower temperatures usually in a higher pH at a slower speed. The amount of added sugar is the parameter of major influence on the lowest attainable pH but not on the speed (Puolanne, 1977).

2.7. Drying

It is a key operation especially for dry fermented sausage production. And the aim is to lose the desired amount of moisture in the shortest possible time without the occurrence of case hardening. The process of drying, and therefore the reduction in a_w, has a significant impact on taste, flavor, texture and color of the product. It is hard to define the point at which fermentation ends and the process of drying starts because a loss in weight of the product occurs right from the beginning of the fermentation process. Generally, the drying phase starts once microbiological stability is obtained. Good air movement is necessary to rapidly remove water vapor and any condensate that collects at the surface. The rate of drying is also critical. If the RH is too low and the temperature too high, drying will initially be rapid. However, at these high drying rates, the surface will become dehydrated and form a hard, water-impermeable skin. This phenomenon, called case hardening, results in slower drying and poor product quality, since water molecules are unable to diffuse through the hardened surface and are trapped within the sausage interior (Asmare, 2012).

2.8. Processing effects during dry fermented sausage production

Fermented sausages comprise chopped or ground meat that is mixed with other nonmeat ingredients such as curing salts, spices, cereals and flavoring components, and allowed to undergo a lactic fermentation in the course of a drying process. Production of fermented sausage involves salting, drying, and lactic acid fermentation and the products are generally eaten without cooking. Based on these relatively simple processing steps and widespread artisanal production, numerous fermented meats have been developed, in many ways (Table 2-7). Processing of fermented sausage

involves a wide range of physical and chemical treatment methods, normally combining a variety of methods (Pacific, 2007).

Table 2-7 Processing steps and application principles of fermented meat

Processing	Stage Processing Principle(s)
Comminution	Grinding of meat and fat and mixing with additives to form a batter for stuffing
Fermentation	Growth and development of microbial flora pH drop and acid gelation of meat proteins
Ripening and drying	Ripening for enzyme action and development of sensory quality Drying
Smoking	Imparting specific flavor and color Preservative effect

Adopted from (Asmare, 2012)

2.9. Physical changes

The acidulation produced during the fermentation stage induces protein coagulation and thus some water release. The acidulation also reduces the solubility of sarcoplasmic and myofibrillar proteins, and the sausage begins to develop consistency. The drying process is a delicate operation that must achieve equilibrium between two different mass transfer processes: diffusion and evaporation (Baldini *et al.*, 2000). Water inside the sausage must diffuse to the outer surface and then evaporate to the environment. The two rates must be in equilibrium because a very fast reduction in the relative humidity of the chamber would cause an excessive evaporation of the sausage surface that would reduce the water content on the outer parts of the sausage and cause hardening. This is typical of sausages with a large diameter because of the slow water diffusion rate. The cross section of these sausages shows a darker, dry, hard outer ring. On the other hand, when the water diffusion rate is much higher than the

evaporation rate, water accumulates on the surface of the sausage and causes wrinkled casings. This situation may happen in short-diameter sausages being ripened in a chamber with high relative humidity.

2.10. Chemical changes

There are different enzymes, from both muscle and microbial origin, involved in reactions related to color, texture, and flavor generation (Toldra and Reig, 2007).

2.10.1. Color

The color of the sausage depends on its moisture and fat content as well as its content of hemo protein, particularly myoglobin. Color is also influenced by pH drop rate and the ultimate pH, and may be also affected by the presence of spices like red pepper. An excess of acid generation by lactobacilli may also affect color. The characteristic color is due to the action of nitrite with myoglobin. Nitrite is reduced to nitric oxide, favored by the presence of ascorbate. Myoglobin and nitric oxide may then interact to form nitric oxide myoglobin, which gives the sausage its characteristic cured, pinky-red color (Scott and Hui, 2004).

2.10.2. Taste

The main nonvolatile compounds contributing to the taste of fermented meats. Sour taste, mainly resulting from lactic acid generation through microbial glycolysis, is the most relevant taste in fermented meats. Sourness is also correlated with other microbial metabolites such as acetic acid. Ammonia may be generated through the activity of deaminase and deamidase, usually present in yeasts and molds, reducing the intensity of the acid taste. Salty taste is usually perceived as a direct taste from salt

addition. ATP[1] derived compounds such as inosine monophosphate and guanosine monophosphate exert some taste enhancement, while hypoxanthine contributes to bitterness. Other taste contributors are those compounds resulting from protein hydrolysis (Asmare, 2012).

2.10.3. Texture

The development of the consistency of fermented meats is initiated with the addition of salt and pH reduction. The water-binding ability of myofibrillar proteins decreases as the pH level approaches their isoelectric point, and water is released. The solubility of myofibrillar proteins also decreases, with a trend towards aggregation and coagulation of the proteins, forming a gel. The consistency of this gel increases with water loss during drying. So there is continuous development of textural characteristics like firmness, hardness, and cohesiveness of meat particles during drying (Toldra, 2002).

2.10.4. Flavor

Little or no flavor is usually detected before meat fermentation, although a large number of flavor precursors are present. As fermentation and further ripening/drying progress, the combined action of endogenous muscle enzymes and microbial activity produces a high number of nonvolatile and volatile compounds with sensory impact. The accumulation of these compounds is increased and sensory perception enhanced as long as the process continues. Some compounds with sensory impact may be produced through further chemical reactions and the addition of spices also makes an intense contribution to specific flavors (Scott and Hui, 2004).

[1] Adenosine Tri Phosphate

2.10.5. Aroma

The origin of the aroma mainly depends on the ingredients and processing conditions. Different pathways are responsible for the formation of volatile compounds with aroma impact. Proteolysis creates many small peptides and free amino acids. Microorganisms can convert the amino acids leucine, isoleucine, valine, phenylalanine, and methionine to important sensory compounds with low threshold values (Asmare, 2012).

Chapter 3

Materials and methods

3.1. Raw material collection and samples preparation

3.1.1. Chemicals

Soy oil, sodium chloride, sodium nitrite, red pepper powder, sugar powder, black pepper powder, poly phosphate, garlic, special spices, ascorbic acid, starch and flour were obtained from Kadur Factory **(**Table 3-1). De Man Rogosa and Sharpe (MRS[1]) broth (Merck, Darmstadt, Germany), Nutrient agar media (Merck, Darmstadt, Germany**),** (YGC[2]) Yeast extract glucose chloramphenicol agar media (Merck, Darmstadt, Germany), (MRS) agar media (de Man, Rogosa and Sharp agar) (Oxoid LTD., Basing–Stoke, Hampshire, England) and pepton water were obtained from BioProcess Engineering Laboratory, Hexane, sulific acid, catalase, boric acid, phenolphthalein and NaOH were provided by chemistry Laboratory, Department of Food Science and Engineering, University of Tehran, Iran.

Table 3-1 Raw material and common ingredients used in semi-dry fermented sausages, formulation

Ingredients	Quantity (g)
meat	100
soy oil	15
sodium chloride	2.29
sodium nitrite	0.03
sugar powder	0.37
garlic	2
poly phosphate	0.86
red pepper powder	0.26
black pepper powder	0.17
ascorbic acid	0.1
starch	2.6
flour	8.6
special spice	0.54

[1] De Man Rogosa and Sharpe
[2] Yeast extract Glucose Chloramphenicol

3.1.2. Samples preparation

3.1.2.1. Meat purchasing

Fresh boneless (camel and beef) meat were obtained from local market in Karaj, Iran. The lean from these species were packed in separate plastic bags and transported to BioProcess Engineering Laboratory, Department of Food Science and Engineering, University of Tehran, Iran, under chill conditions. As soon as the meat arrived, it was held at refrigeration temperature until processed on that day.

3.1.2. 2. Starter culture preparation

Three bacterial strains: *Lactobacillus casei (*DSM 20011[1]), *L. paracasei (*DSM-20006[2]) and *L. rhamnosus* (LGGID-100271) were obtained from BioProcess Engineering Laboratory (BPEL[3]), University of Tehran, Iran. These bacteria were reactivated in de Man Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany), incubated at 37˚C for 24 hours. Under aseptic conditions, they were transferred to (MRS) agar by streaked and incubated at 37˚C for 48 hours. After purification and enumeration of these Lactic acid bacteria, isolated typical colonies were transferred from MRS agar to MRS broth overnight incubated at 37˚C for 24 hours following the same reactivated processing mentioned above, and the number of cell was approximately 1×10^{8} CFU.mL^{-1}(Figure 3-1). Each strain was centrifuged at 4025 ×g, 4°C for 10 min., and the obtained residual was washed two times with 0.85% saline solution water and used for inoculation separately in the product (Nanasombat and Wimuttigosol, 2012).

[1] Deutsche Sammlung von Mikroorganismen/ German collection of microorganisms
[2] Deutsche Sammlung von Mikroorganismen/ German collection of microorganisms
[3] BioProcess Engineering Laboratory

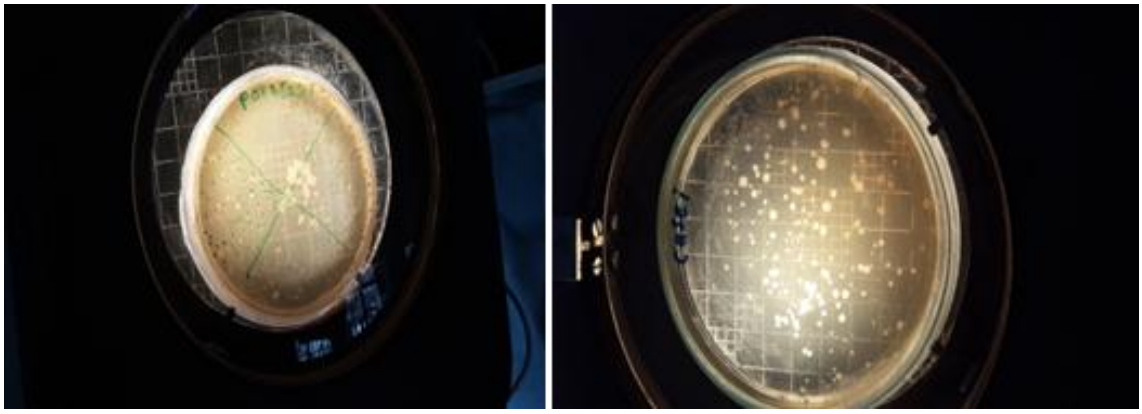

Figure 3-1 *L. paracasei* and *L.casei* after activation

a. Identification of the LAB isolates

The bacteria were identified by microscopic morphological checking tests. LAB isolates were Gram positive, catalase negative, rod shapes, non-spore former, non-motile. LAB Isolates which were used in this study were kept at 4°C in MRS broth until they were further tested. The purity of cultures was tested periodically and at the starting of each experiment by gram staining, and the strains belonging to the LAB group were identified (Ahmed and Elwy, 2015).

3.1.3. Sausage preparation

Four samples from each types of semi-dry fermented sausage were prepared as following: A control sample produced without added starter culture (BNS[1] and CANS[2]). Three other types of samples were produced with starter cultures containing one strain of these starter culture *Lactobacillus casei*, *L. paracasei* and *L. rhamnosus* respectively. Meat and other above-mentioned ingredients which were used in certain percentages

[1] Beef Non Starter
[2] Camel Non Strater

per Kg batter for production of semi-dry fermented sausages. The respective starter cultures were added to each sample as a 2 mL wet inoculums per Kg of batter. In control sample, 2 mL of sterile saline water were added per Kg of batter. A Naturin Cutter *(*Naturin, Germany) was used for preparation of batter; the cutter was sterilized before the preparation of meat mixture for each treatment. The spice mixture and other ingredients including starter culture were added and mixed with minced meat in a cutter for about 20 minute. The batter had been filled into artificial collagen casings of 20 mm diameter using a filling machine (Naturin, Germany) at 5˚C (Bozkurt and Bayram, 2006). Samples of produced sausages were fermented at 30°C for 24 hour, and then dried at two stages (at 60°C for 4 hour and at 75°C for 20 minute). These heating stages improve the quality (sensory evaluation) and inhibit bacterial development. They were, finally, stored in there refrigerator at 4°C as described by Ahmad *et al*. (2012). Sampling was performed by randomly choosing from each sausage group after 0, 10, 20, 30, 40 and 45 days in order to analyze physicochemical, microbiological and sensorial properties. The flow diagram of the process used in preparing the semi-dry sausages is as shown in Figure 3-2.

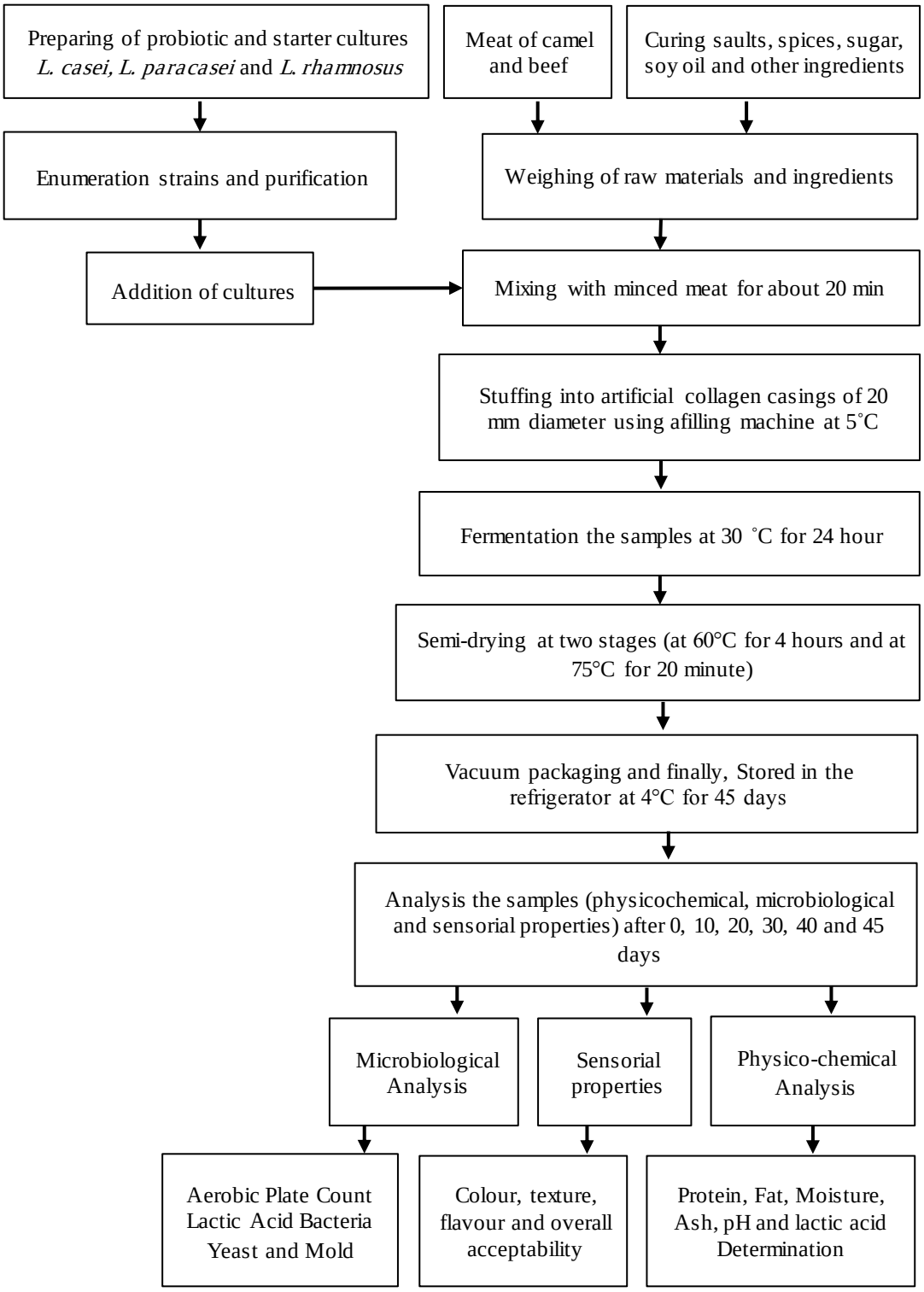

Figure 3-2 General flow diagram of processing of semi-dry fermented sausages

Figure 3-3 shows examples of semi-dry fermented sausages after formulation and before fermentation.

Figure 3-3 Examples of semi-dry fermented sausages after formulation

3.2. Methods of analysis

3.2.1. Microbiological analysis

The samples were analyzed based on the method described by American Public Health Association (APHA, 1992). After removing the casings from samples. 1 gram from each sample was taken and transferred under aseptic conditions to a glass tube containing 9 ml of sterile normal saline- peptone water (NaCl 0.85% and 0.1% peptone water). Serial decimal dilutions were prepared from (10^{-1} to 10^{-8}). Nutrient agar media (Merck, Darmstadt, Germany) were used to count Total Aerobic Counts (TAC[1]), Yeast extract glucose chloramphenicol agar media (YGC) (Merck,

[1] Total Aerobic Counts

Darmstadt, Germany) were used to moulds and yeasts counts [(M[1] &YC[2])] and (MRS) agar media (de Man, Rogosa and Sharp agar) (Oxoid ltd., Basing-stoke, Hampshire, England) were used for (LAB) with triplicates. A colony counter was used for the counting colonies grown in the incubated Petri-dishes after being incubated at 37˚C for 48 hours for TAC and LAB while [(M &YC)] were incubated at 25˚C for (3-5) days finally means and standard deviations of results were calculated after counting.

3.2.2. Physico- chemical analysis

3.2.2.1. Moisture content determination

The moisture content was determined by weighting 5 grams of samples in crucible known weight and drying in an oven (SW-90D, Sang Woo Scientific Co., Bucheon, Korea) at 105°C for 24 hours until reaching a constant weight, after that the samples were cooled in desiccators and their weighted in order to determine the moisture content, as mentioned in the method of AOAC (2006), which calculated according to the following equation:

$$Moisture\% = \frac{\text{sample weight - dried sample weight}}{\text{Fresh sample weight}} \times 100$$

3.2.2.2. Fat content determination

Fat content was measured by the Soxhlet method with a solvent extraction system (SOXTEC Avanti 2050 Auto System, Foss Tecator AB, Hoganas, Sweden) based on the method of AOAC (2006). 5 grams from

[1] Molds
[2] Yeasts Count

the samples were taken to soxhlet equipment. The samples were undergo to continuous extraction with a solvent hexane for 5 hours .The sample was then removed to the extractor and permissible to dry for 2 hours at 105˚c in a drying oven till no trace of hexane remained. The samples were then cooled and weighted for determined the fat percentage; the results were done as following:

$$Fat\% = \frac{\text{Fat weight}}{\text{Sample weight}} \times 100$$

3.2.2.3. Protein content determination

Total Protein content was determined according to Kjeldahl method with an automatic Kieldahl nitrogen analyzer (Kjeltec 2300 Analyzer Unit, Foss Analytical AB, Hoganas, Sweden) which is used to determine the amount of nitrogen (%) and to calculate the ratio of protein by multiplying the amount of nitrogen to the constant factor (6.25) as mentioned in the method of AOAC (2006). Selected randomly samples of semi dry fermented sausage and these samples were minced and weighted (15-20) mg[1] after removed the casing, then digested in Kjeldahl flask by adding10 gm[2] of catalysts (selenium) and 20 ml of concentration H_2SO4.The digestion was heated until a clear solution was obtained for about 3 hours. The digested samples were cooled and then100 ml of distilled water was added to each flask and shaken to avoid precipitation of sulfate in the solution. 50 ml[3] of boric acid containing methyl blue were put under condenser of each distilled unit. The digested and diluted solution was

[1] Abbreviation for milligram, a unit of measurement of mass in the metric system equal to a thousandth of a gram
[2] A gram is equal to the mass of one milliliter, one thousandth of a liter, of water at 4 degrees C
[3] a metric unit of volume equal to one thousandth of a liter.

transferred into the sample compartment of the distiller. The tubes were rinsed with two portions of about 5ml de-ionized water and the rinses were added into the solution. A 25ml of 40% sodium hydroxide solution was added into the compartment and washed with a small amount of water, stoppered and the steam switched on. About 100 ml solution of the sample was distilled, and then the receiver was lowered so that the tip of the condenser is above the surface of the distiller. The distillation was continued until a total volume of 150 ml is collected. The tip was rinsed with a few milliliter of water before the receiver was removed. Finally, the distilled liquid was then titrated opposite standardized (0.1) N sulphuric acid to a reddish color. The equation which used for calculation of Nitrogen was as follows:

$$Nitrogen\ content\ \% = \frac{(\mathrm{T} - \mathrm{B})\mathrm{xNX14.007} \times 100}{\text{Weight of sample (g)}} \times 1000$$

Where:

T= Volume in ml of standard sulfuric acid solution used in the titration for the test material.

B= Volume in ml of standard sulfuric acid solution used in the titration for the blank determination

N= Normality of standard sulfuric acid

14 = each ml is equivalent to 14 mg nitrogen.

1000= to convert from mg to g.

6.25= constant factor.

Protein content % = Nitrogen content % × 6.25

3.2.2.4. Ash determination

Prepared the samples as mention in the determination of protein (removed the casing and minced the semi-dry fermented sausage), then were weighted 5 grams of each sample and triplicates were put into dried crucible of known weight. The crucible was put inside a muffle furnace at 105˚C. The temperature was increased gradually till it reached (500-525) C˚ for 24 hour. Then the crucible was removed, cooled into desiccators and weighted. The ash percentage was calculated by the following the equation: As mentioned in the method of AOAC (2006).

$$Ash\% = \frac{\text{weight of crucible before ashing-weight of crucible after drying}}{\text{Sample weight}} \times 100$$

3.2.2.5. pH determination

The pH value of semi-dry fermented sausage was determined by weighted 10 g of each sample. Then the samples of semi-dry fermented sausage were homogenized in 90 ml of distilled water (Wang, 2000) and their pH value was measured by pH meter (Crison Instruments S.A., Alella, Spain) by submerging the electrode directly into the samples of semi-dry fermented sausage and the readings were recorded.

3.2.2.6. Water activity

Water activity (a_w) of samples was determined using a meter (model Cx-2, Deacagon Devices, Inc., Pullman, WA, Washington, USA) at 25°C (Bowser *et al.*, 2014). Weight (3-4) g of the samples of semi-dry fermented sausage and put into a sample cup. The result was read as soon as the

humidity and temperature values became stable. The mean value of three measurements was documented.

3.2.2.7. Lactic acid value

Lactic acid value was determined by filtration of the samples of semi-dry fermented sausage and then titrating with 0.1 N NaOH (1ml 0.1N NaOH = 0.0090 g lactic acid), contained phenolphthalein (0.1% in 95% ethanol w v^{-1}) as the indicator. NaOH was then added slowly to the sample until a pink colour appeared. Each ml of 0.1N NaOH is equivalent to 90.08mg of lactic acid as stated in AOAC (2000). The results were reported as percent lactic acid by using the formula.

$$\textit{Titratable acidity of lactic acid} = \frac{\textbf{ml NaOH} \times \textbf{N NaOH} \times \textbf{M.E} \times \textbf{100}}{\textbf{Volume of sample used}}$$

Ml NaOH = Volume of NaOH used

N NaOH = Normality of NaOH

M.E = Equivalenat factor = 90.08mg

3.2.3. Sensory analysis

Sensory evaluation (colour, texture, flavour and overall acceptability) was carried out for all samples of semi-dry fermented sausage inoculated and control using 10 trained panelists. According to Al-Ahmad (2014-2015), for each sample, a score sheet from 1-9; 1 represents very dislike while 9 is very like. Sensory evaluation was done again for the samples stored at 4°C after 0, 10, 20, 30, 40 and 45 days. After each the cold storage period (0, 10, 20, 30, 40 and 45) the samples of semi-dry fermented sausages were cut into slices of uniform size and coded the samples, which

were placed randomizly in white paper plate at room temperature to 10 panelists. These panelists were chosen from the stuff and students of department of food science and Engineering, university Tehran. The samples evaluated according to Sensory Evaluation Form (Table 3-2).

Table 3-2 Sensory Evaluation Form

3	Not acceptable	6	Acceptable some what	9	excellent
2	Not acceptable at all	5	Betwixt and between	8	Very acceptable
1	Dogmatic	4	Not acceptable little	7	Medium acceptance

Sensory evaluation

Sample. no	Color	Flavor	Texture	Overall Acceptability
1				
2				
3				
4				
5				
6				
7				
8				
9				

3.3. Study experimental design

The present study is in three parts:

1-The three starter cultures: *Lactobacillus* casei, *Lactobacillus paracasei and Lactobacillus rhamnosus* used in this research were provided by Department of Food Science and Engineering, University of Tehran, Iran. These starter cultures preparing and purification and then were added to batches of semi-dry sausages.

2- The manufacturing of semi-dry fermented sausages with two kind of meats (camel and beef) in four parts were prepared as following: One of these parts is a control sample produced without added starter culture, Three other samples were produced with starter cultures containing one

strain from each of starter culture *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* respectively).

3- Physicochemical properties, microbiological and sensory evaluation of reduced fat content semi-dry fermented functional (beef and camel) sausage producing by *Lactobacillus* casei, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* strains were studied after (0, 10, 20, 30, 40 and 45) days of cold storage at 4C˚.

The selection of potentially probiotic LAB *Lactobacillus* casei, *Lactobacillus paracasei* and *Lactobacillus rhamnosus* strains were carried out at the department of Food Science and Engineering. The semi-dry fermented sausages were manufactured at the Kadur factory (Karaj, Iran) and stored at the department of Food Science and Engineering for 45 days at temperature 4˚C. The samples were analysed microbiological at the microbiology laboratory, while the samples were analysed also physico-chemical and sensory at the chemistry Laboratory, department of Food Science and Engineering, University of Tehran, Iran. Bacterial strains and growth conditions were shown in (Table 3-3).

Table 3-3 Microbial group, media and incubation conditions for enumerating representative bacteria from semi-dry sausage

Microbial group	Medium	Incubation conditions
Lactic acid bacteria	MRS broth (PH Merck, Darmstadt, Germany)	at 37˚C for 24 hours
	MRS agar (OXOID LTD., BASING–STOKE, HAMPSHIRE, ENGLAND)	at 37˚C for 48 hours
Total aerobic count	Nutrient agar media (Merck, Darmstadt, Germany)	at 37˚C for 48 hours
Yeast and Mold	YGC) (Merck, Darmstadt, Germany)	at 25°C, for (3-5)

3.4. Statistical analysis

SPSS software (version 17.0) was used to determine the effect of refrigerated storage and the (LAB) on the quality characteristics of semi-dry fermented sausage. Each trial was repeated 3 times. The obtained data were analyzed by one-way (ANOVA[1]), and significant differences ($p \leq 0.05$) among the means were compared by using the Duncan test.

[1] Analysis of Variance

Chapter 4

Results and dissections

Production of semi- dry fermented sausages based on reduces fat content at fermentation time 24h. Subsequent changes in chemical, physico-chemical, and microbiological of sausage samples were observed, and the sensory analyses of final semi-dry fermented sausages were also analyzed. Comparisons were drawn in between control and samples inoculated with *Lactobacillus casei*, *L. paracasei* and *L. rhamnsus* for each type of meat (beef and camel). Sufficient enough to explain the variations of quality of (semi-dry fermented sausage) samples at various conditions are presented in the following varies Tables (4-3 to 4-25) and Figures (4-1to 4-9).

4.1. Chemical composition

4.1.1. Physico-chemical composition of raw materials

The Physico-chemical composition of beef and camel meat used as raw materials for production semi-dry fermented sausages were presented in (Table 4-1).

Table 4-1 Physico-chemical composition of camel and beef used in the semi-dry fermented sausages

Kind of meat	Moisture%	Protein%	Fat%	Ash%	pH	a_w
Beef	70.50	20.05	8.30	0.92	5.67	0.991
Camel	72.14	19.63	7.13	1.01	5. 73	0.983

The moisture content was higher in camel meat compared with beef (Table 4-1). The content of moisture of camel meat in preset study of value 72.14% was higher to that the value reported 71% by Kadim *et al.* (2006) but lower than the value (76.82 %) reported by Soltanizadeh *et al.* (2010) for semitendinosus muscle of camel. Babiker and Yousif (1990) found the moisture content of 75.81% for semitendinosus muscle of camel. The

importance of moisture in meats lies in its pronounced effects on the shelf life of meat, its processing potential and sensory characteristics. Higher moisture content of a meat may affect its juiciness and wetness, being an indication of its water-holding capacity. The protein content was lower in camel meat compared with beef in the current study (Table 4-1) and this was similar to the study of Soltanizadeh *et al*., (2010). The protein content was affected by species. Elgasim and Alkanhal (1992) reported that the camel has protein content slightly less than that of beef, lamb, and goat or chicken meats. The content protein of the value 19.63% for camel meat is lower than the value 21.4% reported by Kadim *et al*. (2006) and Soltanizadeh *et al*. (2010). This level of protein indicates that the camel meat is a source of high quality protein in harsh climate arid regions (Kadim *et al*., 2006). However, the value recorded for fat of camel meat in the present study lower than the content of fat of beef meat (Table 4-1). The fat content of camel meat (7.13%) was higher than The mean fat of 6.4% for camel's longissimus muscle recorder by Kadim *et al*. (2006) but Soltanizadeh *et al*. (2010) reported the mean of fat 0.72% for camel's semitendinosus muscle. This variability is a general trend for camel meat. Meats vary greatly in their fat content according to the animal species, age, diet and part of the carcass used (Valsta *et al*., 2005). The content of ash of camel meat slightly higher than the value of beef meat in the present study (Table 4-1). The content of ash of camel meat (1.01%) was similar to those of Kadim *et al*. (2006) but Soltanizadeh et *al*. (2010) recorded the mean of ash of 1.1% for camel meat. Kadim *et al*. (2006) confirmed that camel meat is healthy and nutritious as it contains low fat as well as being a good source of minerals. pH of the camel meat is higher than the beef meat but the water activity of beef meat is slightly higher than the camel meat (Table 4-1).

The physico-chemical composition of spices used in semi-dry fermented sausages was presented in (Table 4-2).

Table 4-2 Chemical composition of spice used in semi-dry fermented sausages

Kind of spice	Moisture%	a_w
Black pepper	8.08	0.375
Spice	6.63	0.416

The value of moisture content of black pepper is 8.08% in current study and this result agreement to those of Murthy and Bhattacharya (1998) who found the contents of moisture range between (8-32%, dry weight basis) of black pepper (*Piper nigrum L.*) seed but those of Shreelavaniya and Kamaraj (2017) found physical properties of black pepper in the moisture range of 81.3 - 8.65 % were determined on wet basis (w.b.).

Black pepper is one of the oldest and most widely used spices in the world. Black pepper has the characteristics of pepper aroma and flavors due to their chemical substances especially the volatile oil (Aziz *et al.*, 2012).

Water activity of black pepper 0.375 in present study was lower than the value 0.409 of black pepper recorded by Peter (2001). The terms moisture content and water content are often used interchangeably and represent a measure of the quantity of water in a product. Controlling water activity in spices maintains proper product structure, texture, stability, density, and rehydration properties. Knowledge of the water activity of spices as a function of moisture content and temperature is essential during processing, handling, packaging and storage to prevent the deleterious phenomena of caking, clumping, collapse and stickiness. Water activity is a critical control point for the safety and quality of spices. It is easily measured using highly accurate instrumentation and should be an integral

part of the quality control system for any spice manufacturer according to Decagon Devices Inc. (2010).

4.2. Physico-chemical analysis

4.2.1. Physico-chemical analysis of semi-dry fermented sausages based on beef meat

Table 4-3 shows the results of the physical and chemical analyses of semi-dry fermented beef sausage samples inoculated by *Lactobacillus casei* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 15.42% at the end of storage period. The moisture content in all samples significantly decreased (range 62.78–60.93%) during the cold storage period at 4°C. The trend in fat content; it was similar to protein content in a significantly ($p \leq 0.05$) increased in all samples during the cold storage at 4°C. Fat content ranged between 16.38 and 17.51% for semi-dry fermented sausage during the cold storage period at 4°C. Ash content was almost significantly ($P \leq 0.05$) increased in all samples during the cold storage period at 4°C. Ash content ranged between 2.29 and 2.54% during the cold storage period at 4°C.

Table 4-3 Physico-chemical analysis of semi-dry fermented beef meat sausages inoculated with *Lactobacillus casei* during the cold storage at 4˚C for 45 days

storage/day	Protein%	Moisture%	Fat%	Ash%	PH	Lactic acid%
0	13.24±0.01^{f}	62.78±0.06^{a}	16.38±0.01^{f}	2.29±0.01^{d}	5.45±0.01^{a}	0.478±0.001^{f}
10	13.78±0.02^{e}	62.16±0.04^{b}	16.51±0. 01^{e}	2.45±0.02^{c}	5.41±0. 01^{b}	0.546±0.001^{e}
20	14.50±0.02^{d}	61.92±0.24^{b}	16.86±0.02^{d}	2.46±0.02^{bc}	5.40±0.02^{bc}	0.579±0.001^{d}
30	14.61±0.01^{c}	61.86±0.09^{b}	17.00±0.09^{c}	2.48±0. 05^{bc}	5.38±0.02^{c}	0.637±0.001^{c}
40	15.24±0.01^{b}	61.33±0.50^{c}	17.38±0.01^{b}	2.50±0.03^{ab}	5.32±0. 01^{d}	0.671±0.002^{b}
45	15.42±0.05^{a}	60.93±0.41^{c}	17.51±0.01^{a}	2.54±0.04^{a}	5.18±0.02^{e}	0.759±0.002^{a}

***Values are means of three replicates ± standard deviation.**

****Means with different superscript letters in the same column represent significant differences (p≤0.05).**

The results of the physical and chemical analyses of semi-dry fermented beef sausage inoculated with *L. paracasei* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are presented in Table 4-4. The protein content was significantly (p≤0.05) increased in all samples until it reached 15.75% at the end of storage period, while moisture content decreased in all samples during the cold storage period at 4°C, (range 61.40–60.55%). The trend in fat content was similar to protein content which showed a significant (p≤0.05) increase in all samples during the cold storage at 4°C. Fat content was in the range of 16.40-17.85% for semi-dry fermented sausage during the cold storage period at 4°C. Ash content was almost significantly (p≤0.05) increased in all samples during the cold storage at 4°C. Ash content ranged between 2.48 and 2.63% during the cold storage period at 4°C (Table 4-4).

Table 4-4 Physico-chemical analysis of semi-dry fermented beef meat sausages inoculated with *Lactobacillus paracasei* during the cold storage at 4˚C for 45 days

storage/day	Protein%	Moisture%	Fat%	Ash%	PH	Lactic acid%
0	13.79±0.02^{f}	61.40±0.03^{a}	16.40±0.02^{f}	2.48±0.02^{e}	5.26±0.01^{a}	0.480±0.002^{f}
10	13.91±0.01^{e}	61.08±0.27^{b}	16.75±0.02^{e}	2.51±0.03^{de}	5.22±0.01^{a}	0.571±0.001^{e}
20	14.69±0.02^{d}	60.91±0.12^{b}	16.91±0.04^{d}	2.54±0.01^{cd}	5.15±0.02^{b}	0.588±0.001^{d}
30	14.92±0.01^{c}	60.78±0.20b^{c}	17.33±0.02^{c}	2.56±0.01^{bc}	5.02±0.08^{c}	0.659±0.001^{c}
40	15.70±0.02^{b}	60.75±0.20b^{c}	17.67±0.03^{b}	2.60±0.03^{ab}	4.98±0.02^{c}	0.728±0.005^{b}
45	15.75±0.01^{a}	60.55±0.08^{c}	17.85±0.02^{a}	2.63±0.03^{a}	4.82±0.01^{d}	0.869±0.002^{a}

*Values are means of three replicates ± standard deviation.

**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

The results of physical and chemical analyses of semidry fermented beef sausage samples produced without starter culture (control) after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are shown in Table 4-5. The storage period effected significantly ($p \leq 0.05$) on protein content in all samples until it reached 17.24% at the end of storage period, while no significant differences were observed in moisture content during the cold storage. The moisture content decrease in the samples was in range of 63.39-61.92% during the storage period at 4°C. There were significant differences ($p \leq 0.05$) in the fat content of the samples control during 45 days at 4°C. Fat content ranged between 16.37 and 17.35% for the controls during the cold storage period at 4°C. Ash content was in the almost increased in all samples during storage period at 4°C. Ash content range of 2.56-2.68% during the cold storage period (Table 4-5). The control sample had higher protein content compared to the samples inoculated with *L. casei and L. paracasei*.

Table 4-5 Physico-chemical analysis of semi-dry fermented beef meat sausages (control) during the cold storage at 4˚C for 45 days

storage/day	Protein%	Moisture%	Fat%	Ash%	PH	Lactic acid%
0	16.74±0.02^{f}	63.39±0.32^{a}	16.37±0.02^{e}	2.56±0.02^{b}	5.51±0.01^{a}	0.388±0.001^{f}
10	16.78±0.02^{e}	62.62±0.59^{b}	16.48±0.03^{d}	2.57±0.02^{b}	5.48±0.03^{ab}	0.510±0.001^{e}
20	16.85±0.02^{d}	62.48±0.09^{b}	16.54±0.12^{d}	2.58±0.005^{b}	5.44±0.04^{bc}	0.572±0.001^{d}
30	16.93±0.02^{c}	62.32±0.42^{b}	16.89±0.02^{c}	2.60±0.03^{b}	5.39±0.01^{cd}	0.631±0.001^{c}
40	17.00±0.02^{b}	62.05±0.07^{b}	17.03±0.04^{b}	2.65±0.05^{a}	5.35±0.02^{d}	0.645±0.001^{b}
45	17.24±0.02^{a}	61.92±0.05^{b}	17.35±0.03^{a}	2.68±0.02^{a}	5.24±0.04^{e}	0.718±0.002^{a}

*Values are means of three replicates ± standard deviation.

**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

Table 4-6 shows the results of the physical and chemical analyses of semi-dry fermented beef meat sausage samples inoculated by *Lactobacillus rhamnsus* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 15.85% at the end of storage period. The moisture content in all samples significantly ($p \leq 0.05$) decreased (range 60.90–60.06%) during the storage period. The trend in fat content; it was similar to protein content in a significantly ($p \leq 0.05$) increased in all samples during storage at 4°C. Fat content ranged between 16.48 and 17.87% for semi-dry fermented sausage during the cold storage period. Ash content was almost significantly ($P \leq 0.05$) increased in all samples during the storage period at 4°C. Ash content ranged between 2.49 and 2.66% during the refrigerated storage period (Table 4-6).

Table 4-6 Physico-chemical analysis of semi-dry fermented beef meat sausages inoculated with *Lactobacillus rhamnsus* during the cold storage at 4°C for 45 days

storage/day	protein%	moisture%	Fat%	Ash%	PH	Lactic acid%
0	14.15±0.02^{e}	60.90±0.05^{a}	16.48±0.03^{f}	2.49±0.01^{c}	4.47±0.02^{a}	0.712±0.002^{f}
10	14.45±0.02^{e}	60.81±0.01^{b}	16.83±0.02^{e}	2.55±0.03^{bc}	4.45±0.05^{b}	0.981±0.001^{e}
20	15.15±0.02^{d}	60.79±0.03^{e}	16.94±0.02^{d}	2.56±0.05^{ab}	4.44±0.02^{bc}	1.009±0.001^{d}
30	15.51±0.01^{c}	60.56±0.03^{c}	17.58±0.04^{c}	2.58±0.03^{a}	4.43±0.03^{bc}	1.142±0.002^{c}
40	15.75±0.03^{b}	60.42±0.04^{e}	17.83±0.01^{b}	2.64±0.01^{a}	4.41±0.02^{bc}	1.223±0.003^{b}
45	15.85±0.02^{a}	60.06±0.05^{f}	17.87±0.03^{a}	2.66±0.03^{a}	4.35±0.03^{c}	1.415±0.003^{a}

*Values are means of three replicates ± standard deviation.

**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

The results of the physical and chemical analyses of semi-dry fermented beef meat sausage samples produced without starter culture (control) after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are presented in Table 4-7**.** The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 15.91% at the end of storage period, while moisture content was significantly ($p \leq 0.05$) decreased in all samples during the storage period, (range 63.96–61.22%). The trend in fat content was similar to protein content which showed a significant ($p \leq 0.05$) increase in all samples during storage at 4°C. Fat content was in the range of 11.93-14.46% for semi-dry fermented sausage during the cold storage period. Ash content was almost significantly ($p \leq 0.05$) increased in all samples during storage at 4°C. Ash content ranged between 2.51 and 2.67% during cold storage period (Table 4-7).

Table 4-7 Physico-chemical analysis of semi-dry fermented beef meat sausages (control) during the cold storage at 4°C for 45 days

storage/day	protein%	moisture%	Fat%	Ash%	PH	Lactic acid%
0	14.27±0.02^{d}	63.96±0.03^{a}	11.93±0.03^{f}	2.51±0.04^{b}	4.54±0.02^{a}	0.498±0.001^{f}
10	15.23±0.03^{c}	63.40±0.02^{b}	13.23±0.03^{e}	2.58±0.05^{b}	4.52±0.02^{a}	0.846±0.002^{e}
20	15.75±0.03^{b}	63.07±0.02^{c}	13.35±0.05^{d}	2.60±0.02^{ab}	4.51±0.01^{a}	0.866±0.002^{d}
30	15.78±0.01^{b}	62.25±0.02^{d}	13.72±0.02^{c}	2.63±0.02^{ab}	4.48±0.01^{a}	1.019±0.002^{c}
40	15.87±0.02^{a}	61.86±0.02^{e}	13.87±0.01^{b}	2.66±0.02^{ab}	4.47±0.07^{ab}	1.047±0.002^{b}
45	15.91±0.03^{a}	61.22±0.02^{f}	14.46±0.03^{a}	2.67±0.017^{a}	4.42±0.02^{b}	1.093±0.003^{a}

*Values are means of three replicates ± standard deviation.

**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

4.2.1.1. pH and lactic acid values

pH and lactic acid values for the semi-dry fermented beef meat sausage inoculated with *L. casei* during storage at 4°C are shown in Table 4-3. pH value of all samples significantly ($p \leq 0.05$) decreased during the refrigerated storage (range 5.45-5.18) while lactic acid values significantly ($p \leq 0.05$) increased (range 0.478-0.59%) during the cold storage. Results of pH and lactic acid values for beef meat samples inoculated with *L. paracasei* during the storage period at 4°C are indicated in Table 4-4. pH value in all samples decreased significantly ($p \leq 0.05$) during the cold storage at 4°C (range 5.26-4.82) while lactic acid values significantly ($p \leq 0.05$) increased during this time (range 0.480-0.869%). Results of pH and lactic acid values for the control samples during the storage period at 4°C are shown in Table 4-5. pH values in all samples of semi-dry fermented beef meat sausage significantly ($p \leq 0.05$) decreased while lactic acid values significantly ($p \leq 0.05$) increased during cold storage at 4°C for 45 days.

The control samples of semi-dry fermented beef meat sausage had higher value (5.51-5.24) compared to the samples inoculated with *L. casei* and *L. paracasei*. This decrease in pH values was due to the production of lactic acid during fermentation by lactic acid bacteria. The increase in lactic acid values in all the samples is the result of dropping of pH values during storage at 4°C. The control samples had lower values of lactic acid (0.388-0.718%) compared to those inoculated with *L. casei* and *L. paracasei* during the cold storage period.

pH and lactic acid values for the semi-dry fermented beef meat sausage inoculated with *L. rhamnsus during* storage at 4°C are shown in Table 4-6. pH value of all samples significantly ($p \leq 0.05$) decreased during the cold storage at 4°C (range 4.47-4.35) while lactic acid values significantly ($p \leq 0.05$) increased (range 0.712-1.415%) during the cold storage. The control samples of semi-dry fermented beef meat sausage had higher value (4.54-4.42) compared to the samples inoculated with *L. rhamnsus* (Table 4-7). This decrease in pH values was due to the production of lactic acid during fermentation by lactic acid bacteria. The increase in lactic acid values in all the samples is the result of dropping of pH values during storage at 4°C. The control samples had lower values of lactic acid (0.498-1.093%) **(**Table 4-7**)** compared to those inoculated with *L. rhamnsus* during the cold storage period.

4.2.2. Physico-chemical analysis of semi-dry fermented sausages based on camel meat

Tables 4-8, 4-9 and 4-10 showed the results of the physical and chemical analyses of semi-dry fermented camel meat sausages inoculated with *Lactobacillus casei*, *Lactobacillus paracasei* and control but Tables 4-11 and 4-12 showed the results of the physical and chemical analyses of

semi-dry fermented camel meat sausages inoculated with *Lactobacillus rhamnsus* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. Table 4-8 explains the results of the physical and chemical analyses of semi-dry fermented camel meat sausage samples inoculated by *Lactobacillus casei* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 12.82% at the end of storage period. The moisture content in all samples decreased (range 63.80–62.26%) during the cold storage period at 4°C. The trend in fat content; it was similar to protein content in a significantly ($p \leq 0.05$) increased in all samples during the cold storage at 4°C. Fat content ranged between 15.40 and 16.39% for semi-dry fermented sausage during the cold storage period at 4°C. Ash content was almost significantly ($P \leq 0.05$) increased in all samples during the storage period at 4°C. Ash content ranged between 2.44 and 2.64% during the cold storage period at 4°C.

Table 4-8 Physico-chemical analysis of semi-dry fermented sausages inoculated with *Lactobacillus casei* during the cold storage at 4°C for 45 days

storage/day	protein%	moisture%	Fat%	Ash%	PH	Lactic acid %
0	10.88±0.03^{e}	63.8±0.03^{a}	15.40±0.03^{f}	2.44±0.01^{e}	5.48±0.01^{a}	0.248±0.001^{f}
10	12.33±0.03^{a}	63.08±0.05^{b}	15.57±0.01^{e}	2.49±0.01^{d}	5.42±0.01^{b}	0.540±0.003^{e}
20	12.39±0.01^{c}	63.04±0.21^{b}	15.93±0.03^{d}	2.53±0.02^{c}	5.41±0.04^{b}	0.570±0.003^{d}
30	12.68±0.01^{b}	62.89±0.16^{b}	16.04±0.04^{c}	2.57±0.005^{b}	5.40±0.0 3^{c}	0.590±0.001^{c}
40	12.78±0.02^{a}	62.65±0.17^{c}	16.18±0.01^{b}	2.6±0.02^{b}	5.36±0.01^{c}	0.670±0.001^{b}
45	12.82±0.02^{a}	62.26±0.02^{d}	16.39±0.02^{a}	2.64±0.03^{a}	5.21±0.015^{d}	0.702±0.001^{a}

*Values are means of three replicates ± standard deviation.

**Means with different superscript letters in the same column represent significant differences ($p \leq 0.05$).

The results of the physical and chemical analyses of semi-dry fermented camel meat sausage inoculated with *L. paracasei* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are presented in Table 4-9. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 14.58% at the end of cold storage period at 4°C, while moisture content decreased in all samples during the storage period, (range 62.63–61.68%). The trend in fat content was similar to protein content which showed a significant ($p \leq 0.05$) increase in all samples during storage at 4°C. Fat content was in the range of 16.35-17.13% for semi-dry fermented sausage during the cold storage period at 4°C. Ash content was almost significantly ($p \leq 0.05$) increased in all samples during storage at 4°C. Ash content ranged between 2.53 and 2.66% during cold storage period at 4°C (Table 4-9).

Table 4-9 Physico-chemical analysis of semi-dry fermented camel meat sausages inoculated with *Lactobacillus paracasei* during the cold storage at 4°C for 45 days

storage/day	protein%	moisture%	Fat%	Ash%	PH	Lactic acid%
0	13.75±0.01^{e}	62.63±0.04^{a}	16.35±0.01^{f}	2.53±0.03^{d}	5.33±0.01^{a}	0.302±0.001^{f}
10	13.80±0.01^{d}	62.57±0.09^{a}	16.46±0.01^{e}	2.54±0.04^{d}	5.23±0.01^{b}	0.568±0.001^{e}
20	14.07±0.05^{c}	62.29±0.05^{b}	16.50±0.03^{d}	2.56±0.04^{bd}	5.21±0.01^{c}	0.581±0.003^{d}
30	14.15±0.02^{b}	62.14±0.05^{c}	16.70±0.02^{c}	2.57±0.01^{bd}	5.09±0.01^{d}	0.643±0.001^{c}
40	14.56±0.02^{a}	61.73±0.05^{d}	16.75±0.02^{b}	2.61±0.02^{b}	5.07±0.01^{d}	0.679±0.002^{b}
45	14.58±0.01^{a}	61.68±0.08^{d}	17.13±0.01^{a}	2.66±0.01^{a}	4.91±0.02^{c}	0.784±0.001^{a}

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

The results of physical and chemical analyses of semidry fermented camel meat sausage samples produced without starter culture (control) after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are shown in Table 4-10. The storage period effected significantly ($p \leq 0.05$) on protein content in all samples until it reached 15.90% at the end of cold storage period at 4°C, also significant differences ($p \leq 0.05$) were observed in moisture content during the cold storage. The moisture content of the samples was in range of 63.85-62.68% during the storage period at 4°C. There were significant differences ($p \leq 0.05$) in the fat content of samples during 45 days at 4°C. Fat content ranged between 15.05 and 16.37% for the controls during the cold storage period at 4°C. Ash content was in the almost increased in all samples during storage period at 4°C. Ash content range of 2.60-2.72% during the cold storage period at 4°C (Table 4-10).

Table 4-10 Physico-chemical analysis of semi-dry fermented camel meat sausages (control) during the cold storage at 4°C for 45 days

storage/day	protein%	moisture%	Fat%	Ash%	PH	Lactic acid%
0	15.16±0.02^{f}	63.85±0.02^{a}	15.05±0.1^{e}	2.60±0.03^{c}	5.56±0.01^{a}	0.207±0.002^{f}
10	15.26±0.01^{e}	63.78±0.03^{a}	15.43±0.03^{d}	2.63±0.02^{cd}	5.50±0.01^{b}	0.222±0.002^{e}
20	15.39±0.02^{d}	63.47±0.02^{b}	15.73±0.03^{c}	2.65±0.02^{bc}	5.48±0.02^{c}	0.540±0.002^{d}
30	15.53±0.02^{c}	63.00±0.1^{c}	15.97±0.03^{b}	2.68±0.01^{ab}	5.44±0.02^{d}	0.574±0.002^{c}
40	15.58±0.02^{b}	62.86±0.01^{d}	15.99±0.01^{b}	2.70±0.03^{a}	5.36±0.010^{e}	0.602±0.001^{b}
45	15.90±0.02^{a}	62.68±0.01^{e}	16.37±0.02^{a}	2.72±0.02a	5.34±0.01^{e}	0.624±0.001^{a}

*Values are means of three replications ± standard deviation (SD[1]).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

[1]Standard Deviation

Table 4-11 showed the results of the physical and chemical analyses of semi-dry camel meat fermented sausage inoculated with *Lactobacillus rhamnsus* after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C. The protein content increased significantly ($p \leq 0.05$) in all samples until it reached 14.93% for samples inoculated with *Lactobacillus rhamnsus* during the cold storage period at 4°C (Table 4-11). The moisture content of semi-dry fermented sausage was decreased significantly ($p \leq 0.05$) in all samples until it reached 61.04% for semi-dry fermented sausages inoculated with *L. rhamnsus* (Table 4-11**).** The trend in the fat content of samples was similar to protein content that increases significantly ($p \leq 0.05$) in all samples during the cold storage period at 4°C until it reached 17.17% for semi-dry fermented sausages inoculated with *L. rhamnsus* **(**Table 4-11). Ash content was almost increased significantly ($p \leq 0.05$) in all sample until it reached 2.68% for semi-dry fermented sausages inoculated with *L. rhamnsus* in the final of cold storage period at 4°C (Table 4-11).

Table 4-11 Physico-chemical analysis of semi-dry fermented camel meat sausages inoculated with *Lactobacillus rhamnsus* during the cold storage at 4°C for 45 days

storage/day	protein%	moisture%	Fat%	Ash%	PH	Lactic acid%
0	13.82±0.03^{f}	62.46±0.03^{a}	16.45±0.02^{f}	2.55±0.03^{d}	4.48±0.01^{a}	0.622±0.002^{f}
10	13.93±0.01^{e}	61.91±0.01^{b}	16.69±0.02^{e}	2.56±0.02^{cd}	4.46±0.02^{ab}	0.808±0.001^{e}
20	14.33±0.01^{d}	61.83±0.01^{c}	16.88±0.02^{d}	2.58±0.02^{bcd}	4.45±0.01^{abc}	0.931±0.001^{d}
30	14.41±0.02^{c}	61.66±0.01^{d}	16.95±0.01^{c}	2.60±0.03^{abc}	4.44±0.02^{bc}	1.079±00.2^{c}
40	14.66±0.02^{b}	61.47±0.03^{e}	17.07±0.01^{b}	2.66±0.01^{ab}	4.43±0.03^{bc}	1.099±0.001^{b}
45	14.93±0.02^{a}	61.04±0.02^{f}	17.17±0.02^{a}	2.68±0.02^{a}	4.41±0.01^{c}	1.112±0.002^{a}

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

The results of the physical and chemical analyses of semi-dry fermented camel meat sausage samples produced without starter culture (control) after 0, 10, 20, 30, 40 and 45 days of cold storage at 4°C are presented in Table 4-12. The protein content was significantly ($p \leq 0.05$) increased in all samples until it reached 15.58% at the end of cold storage period at 4°C, while moisture content decreased in all samples during the cold storage period at 4°C, (range 64.72–61.95%). The trend in fat content was similar to protein content which showed a significant ($p \leq 0.05$) increase in all samples during the cold storage at 4°C. Fat content was in the range of 10.42-12.92% for semi-dry fermented sausage during the cold storage period at 4°C. Ash content was almost significantly ($p \leq 0.05$) increased in all samples during storage at 4°C. Ash content ranged between 2.56 and 2.69% during the cold storage period at 4°C (Table 4-12).

Table 4-12 Physico-chemical analysis of semi-dry fermented camel meat sausages (control) during the cold storage at 4°C for 45 days

storage/day	protein%	Moisture%	Fat%	Ash%	PH	Lactic acid%
0	13.91±0.04^{f}	64.72±0. 21^{a}	10.42±0.02^{d}	2.56±0.02^{d}	4.71±0.02^{a}	0.497±0.002^{f}
10	14.28±0.02^{e}	64.66±0.91^{a}	11.55±0.02^{c}	2.60±0.01^{d}	4.67±0.03^{ab}	0.653±0.003^{e}
20	14.47±0.02^{d}	64.55±0.35^{a}	11.94±0.01^{b}	2.62±0.02^{cd}	4.64±0.03^{bc}	0.850±0.002^{d}
30	14.55±0.03^{c}	64.45±0.46^{a}	12.67±0.04^{a}	2.65±0.02^{bc}	4.62±0.02^{c}	0.967±0.003^{c}
40	15.26±0.03^{b}	64.41±0.48^{a}	12.77±0.02^{a}	2.68±0.04^{ab}	4.61±0.03^{c}	0.986±0.003^{b}
45	15.58±0.01^{a}	61.95±0.41^{b}	12.92±0.52^{a}	2.69±0.03^{a}	4.55±0.02^{d}	1.057±0.002^{a}

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

The increase in protein content of semi-dry fermented sausages can be due to the proteolytic activities of enzymes produced by microorganisms

during the fermentation and ripening, which can cause increase in the bioavailability of amino acids (Moretti *et al.*, 2004). This increase in protein content in time can be due to the reduction in moisture values. Our results coincide with those of Asmare and Admassu, who reported increase of protein content in all samples of dry fermented sausages (Asmare and Admassu, 2013); this can be attributed to the decrease of water content and high concentration of nutrients during processing. Decrease of moisture content led to an increase in protein, fat, and ash contents in all samples during the storage period at 4°C. Our findings are in agreement with those of Ahmad *et al.* (2012), who demonstrated the lowering down of moisture content of semi-dry fermented sausage. The control sample beef meat had lower fat content compared to the fermented sausages inoculated with *L. casei* and *L. paracasei*. Fat contributes to nutritional (source of essential fatty acids, lipo-soluble vitamins, and energy), organoleptic (flavor, texture, and mouth-feel), and technological properties (release of moisture) in meat products (Olivares *et al.*, 2010). Our results are in consistency with the findings of Asmare and Admassu (2013), who reported that fat content was also significantly ($p \leq 0.05$) increased in all dry-fermented sausages. The highest ash content was found in the control sample compared to others. This increase in ash content could be due to the use of salts (NaCl and NaNO2) in the sausage batters. Salt (NaCl) is one of the major ingredients in dry- fermented sausages: it plays an essential role in assuring the microbiological stability, and influences the final taste, color and texture; these values of ash increase in the end products due to drying process (Zanardi *et al.*, 2010). Our findings are almost in consistency with those of Hemat *et al.* (2010), who reported a significant ($P \leq 0.05$) increase in mineral components among the treatments.

4.2.2.1. pH and lactic acid values

pH and lactic acid values for the samples of semi-dry fermented camel meat sausages inoculated with *Lactobacillus casei, L. paracasei* and control during the cold storage period at 4°C are shown in Table 4-8, 4-9 and 4-10**.** The pH of all samples decreased significantly ($p \leq 0.05$) during the cold storage period at 4°C. Fewer declines in pH value in all samples of semi-dry fermented sausages inoculated with *L. paracasei* until it reached (4.91) at the end of cold storage period at 4°C (Table 4-9). The production of lactic acid during fermentation by lactic acid bacteria led to decreasing in pH values. The amount of lactic acid increased significantly ($p \leq 0.05$) in all samples of semi-dry fermented sausages during the cold storage period at 4°C. Control samples had a lower value of lactic acid 0.624% (Table 4-10) compared to those inoculated with *L. casei* and *L. paracasei* which had 0.702% and 0.784% respectively at the end of cold storage period at 4°C (Table 4-8 and 4-9**)**.

pH and lactic acid values for the semi-dry fermented camel meat sausage inoculated with *Lactobacillus rhamnsus* and control during the cold storage period at 4°C are shown in Table 4-11and 4-12. pH value of all samples significantly ($p \leq 0.05$) decreased during the cold storage period at 4°C (range 4.48-4.41) in the samples of semi-dry fermented camel meat sausage inoculated with *Lactobacillus rhamnsus*, while lactic acid values significantly ($p \leq 0.05$) increased (range 0.622-1.112%) during the cold storage period at 4°C. The control samples of semi-dry fermented camel meat sausage had higher value (4.71-4.55) during the cold storage period at 4°C compared to the samples inoculated with *L. rhamnsus* (Table 4-12). This decrease in pH values was due to the production of lactic acid during fermentation by lactic acid bacteria. The increase in lactic acid values in all

the samples is the result of dropping of pH values during storage at 4°C. The control samples had lower values of lactic acid (0.497-1.057%) (Table 4-12) compared to those inoculated with *L. rhamnsus* during the cold storage period at 4°C.

LAB utilize the carbohydrate portion of the meat as source of energy to produce acids and thus lower the pH, improving the texture of the products, providing stability against the proliferation of food spoilage and pathogen microorganisms and producing some aromatic compounds (Bacus, 1986) through production of lactic acid and a number of other antimicrobial and organoleptic compounds (e.g. acetic acid, ethanol, acetoin, carbon dioxide and pyruvic acid) (Albano *et al*., 2009). The results of this study are in agreement with report of Ahmad *et al*. (2012), who demonstrated that refrigerated storage significantly ($p \leq 0.05$) decreased the pH of semi-dry fermented sausage.

4.3. Microbiological analysis

4.3.1. Lactic acid bacteria count based on beef meat

Figure 4-1 shows the numbers of Lactic acid bacteria count (LAB) log CFU g-1 for beef semi-dry fermented sausage inoculated with *Lactobacillus casei*, *Lactobacillus paracasei* and control during the cold storage at 4˚C for 45 days**.** There was a significant increase ($p \leq 0.05$) in the numbers of LAB during the cold storage period at 4°C in all the samples of semi-dry fermented beef meat sausage such that they became predominate flora in the final products during the cold storage period at 4˚C (Figure 4-1). The control samples had lower number of LAB (range 6.47-6.81 log CFU g^{-1}) during the cold storage period at 4˚C comparing to the samples

inoculated with *L. casei* and *L. paracasei* (range 6.95 - 7.95 and 7.15-8.28 log CFU g^{-1}, respectively) (Figure 4-1).

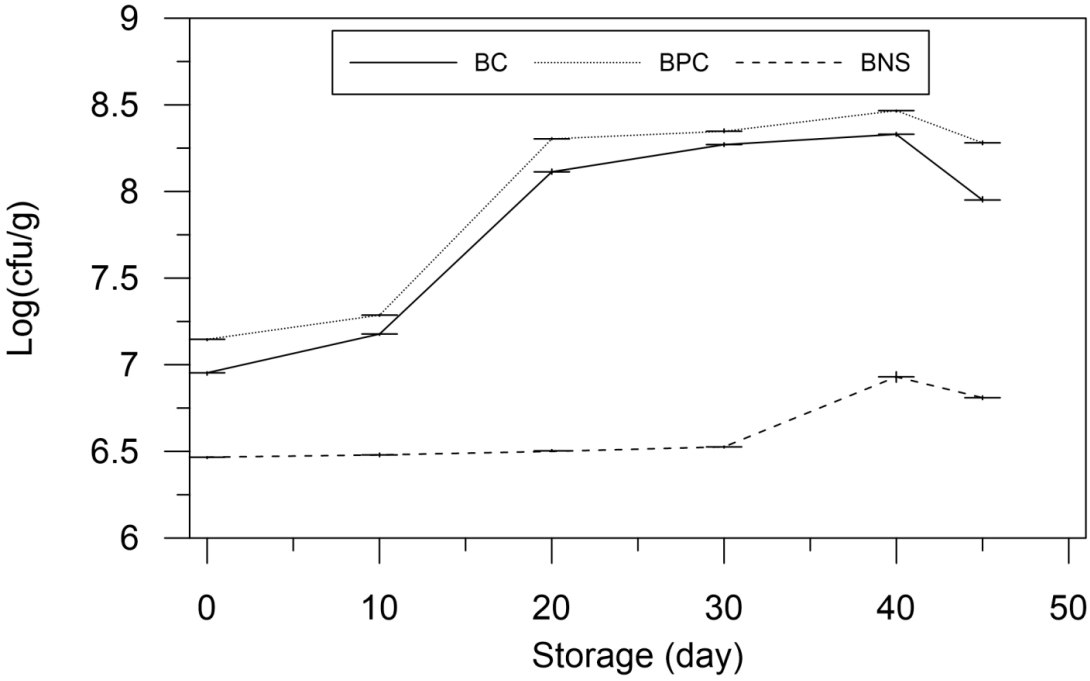

Figure 4-1 Microbial numbers of Lactic acid bacteria count (LAB) log CFU g-1 for semi-dry fermented beef meat sausage inoculated with *Lactobacillus casei* (BC), *Lactobacillus paracasei* (BPC) and Control (BNS) during the cold storage at 4˚C for 45 days

Table 4-13 shows the numbers of Lactic acid bacteria count log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus rhamnsus* and control during the cold storage at 4˚C for 45 days. There was a significantly increased ($p \leq 0.05$) in the numbers of LAB in all samples of semi-dry fermented beef meat sausage during the cold storage period at 4°C, such that they became predominate flora in the final products during the cold storage period at 4°C (Table 4-13). Control samples of semi-dry fermented beef meat sausage had lower numbers of LAB (range 7.67-8.01

log CFU g^{-1}) comparing to samples inoculated with *L. rhamnsus* (range 8.50 - 9.24 log CFU g^{-1}) during the cold storage period at 4°C (Table 4-13).

Table 4-13 Microbial numbers of Lactic acid bacteria count (LAB) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus rhamnsus* (BR) and control during the cold storage at 4˚C for 45 days

Storage/day	Control	BR
0	7.67±0.03^{e}	8.50±0.03^{e}
10	7.78±0.02^{d}	8.75±0.02^{d}
20	7.82±0.02^{d}	8.85±0.02^{c}
30	7.90±0.03^{c}	8.89±0.01^{c}
40	8.15±0.02^{a}	9.42±0.02^{a}
45	8.01±0.02^{b}	9.24±0.03^{b}

*Values are means of three replications ± standard deviation (SD)

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$)

4.3.2. Lactic acid bacteria count based on camel meat

Figure 4-2 shows the numbers of Lactic acid bacteria count (LAB) log CFU g^{-1} for semi-dry fermented camel meat sausage inoculated with *Lactobacillus casei*, *Lactobacillus paracasei* and control during the cold storage at 4˚C for 45 days. There was significantly increased ($p \leq 0.05$) in numbers of Lactic acid bacteria in all samples of semi-dry fermented camel meat sausage during the cold storage period at 4°C, and became the predominant flora at the end of the cold storage period at 4˚C. The control samples had lower numbers of LAB (range 6.38-6.67 log CFU g^{-1}) during the cold storage period at 4˚C comparing to the samples inoculated with *L. casei* and *L. paracasei* (range 6.85 -7.92 and 7.08-8.07 log CFU g^{-1}, respectively) at the end of the cold storage period at 4˚C due to the inoculation these samples with *L. casei and L. paracasei* (Figure 4-2).

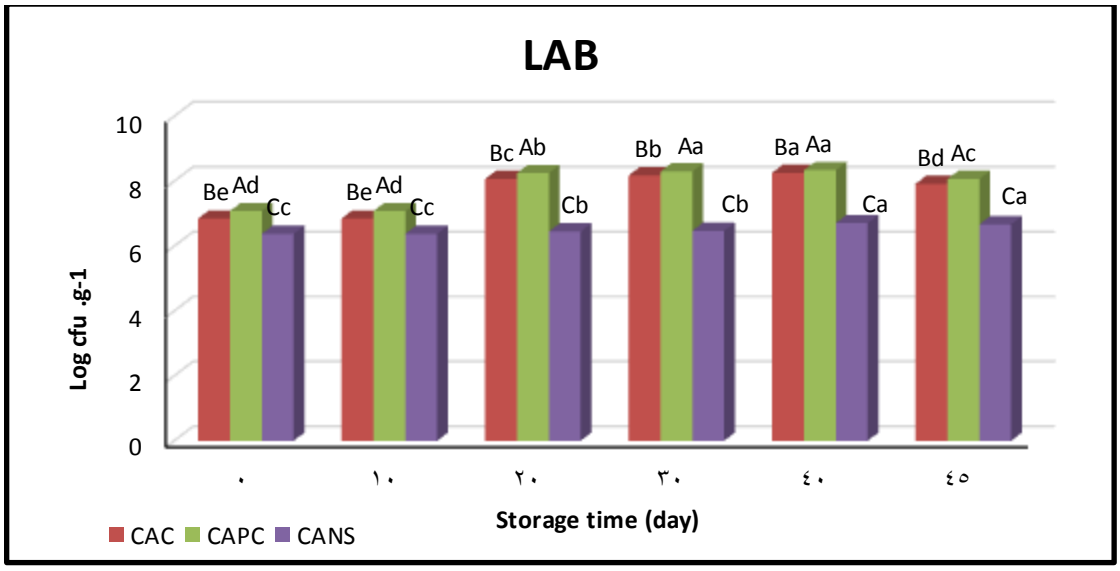

Figure 4-2 Microbial numbers of Lactic acid bacteria count (LAB) log CFU g^{-1} for semi-dry fermented camel meat sausage inoculated with *Lactobacillus casei* (CAC), *Lactobacillus paracasei* (CAPC) and Control (CANS) during the cold storage at 4˚C for 45 days

Table 4-14 shows the numbers of Lactic acid bacteria count (LAB) log CFU g^{-1} for semi-dry fermented camel meat sausage inoculated with *Lactobacillus rhamnsus* and control during the cold storage at 4˚C for 45 days. There was a significantly increased ($p \leq 0.05$) in the numbers of LAB in all the samples of fermented camel meat sausage during the cold storage period at 4°C, such that they became predominate flora at the end of the cold storage period at 4˚C (Table 4-14). The control samples had lower numbers of LAB (range 7.47-7.82 log CFU g^{-1}) comparing to the samples of semi-dry fermented camel meat sausage inoculated with *L. rhamnsus* (range 7.99-8.90) log CFU g^{-1} during the cold storage period at 4°C (Table 4-14).

Table 4-14 Microbial numbers of Lactic acid bacteria count (LAB) log CFU g^{-1} for semi-dry fermented camel meat sausage inoculated with *Lactobacillus rhamnsus* (CAR) and Control during the cold storage at 4˚C for 45 days

Storage/day	Control	CAR
0	7.47 ± 0.02^{e}	7.99 ± 0.01^{f}
10	7.50 ± 0.02^{e}	8.46 ± 0.02^{e}
20	7.63 ± 0.03^{d}	8.54 ± 0.02^{d}
30	7.71 ± 0.02^{c}	8.82 ± 0.02^{c}
40	7.94 ± 0.02^{a}	8.98 ± 0.01^{a}
45	7.82 ± 0.02^{b}	8.90 ± 0.03^{b}

*Values are means of three replications ± standard deviation (SD)

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$)

Lactic acid bacteria were able to resist the drying process and to maintain its growth during refrigerated storage stages. Increase in LAB numbers (which could be due to the environment of meat) is suitable for the growth of LAB and good adaptation of these bacteria with the fermentation conditions (Al-Ahmad *et al.*, 2014). Our results are in agreement with those of Ferreira *et al.* (2007) in fermented sausages, in which displayed a rapid increase in LAB count was noticed (Ferreira *et al.*, 2007). The number of LAB decreased at the end of refrigerated storage due to the exhaustion of sugar and the low temperature conditions (Fernández-López *et al.*, 2008), and also may be due to decrease in the moisture and increase in the acidity of semi-dry fermented sausage during the refrigerated storage (Al-Ahmad *et al.*, 2014).

4.3.3. Total aerobic count based on beef meat

Figure 4-3 shows the numbers of Total Aerobic Count (TAC) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus casei*, *Lactobacillus paracasei* and control during the cold storage at 4˚C for 45 days. Total aerobic count (TAC) of semi-dry fermented beef meat sausage significantly increased ($p \leq 0.05$) in all

samples during the cold storage period at 4°C (Figure 4-3). The control samples had lower numbers (range 5.91–6.60 log CFU g-1) compared to those samples inoculated with *L. casei* and *L. paracasei* (range 6.55-7.20 and 6.53-6.75 log CFU g-1, respectively) during the cold storage period at 4°C.

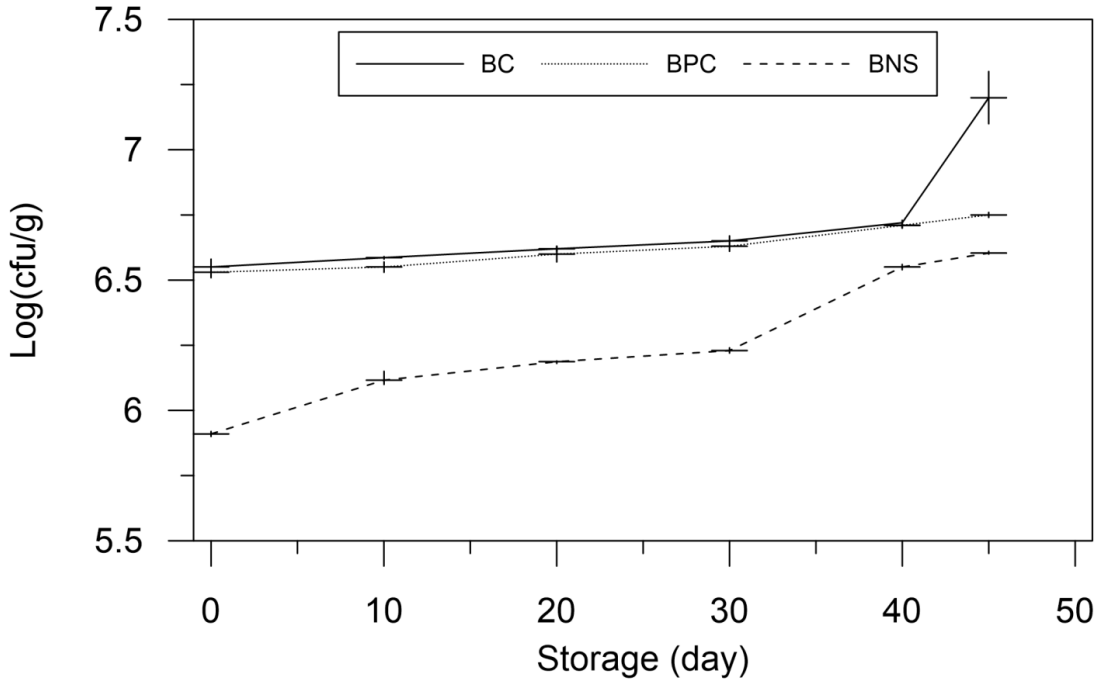

Figure 4-3 Microbial numbers of Total Aerobic Count (TAC) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus casei* (BC[1]), *Lactobacillus paracasei* (BPC[2]) and Control (BNS) during the cold storage at 4˚C for 45 days

Table 4-15 shows the numbers of Total Aerobic Count (TAC) log CFU g^{-1} for semi-dry beef meat fermented sausage inoculated with *Lactobacillus rhamnsus* and control during the cold storage at 4˚C for 45 days. The

[1] Beef inculated with *Lactobacillus casei*
[2] Beef inculated with *Lactobacillus paracasei*

control samples had lower numbers of TAC (range 6.20–6.65 log CFU g^{-1}) compared to those samples inoculated with *L. rhamnsus* (range 6.50-6.70 log CFU g^{-1}) during the cold storage period at 4˚C (Table 4-15).

Table 4-15 Microbial numbers of Total Aerobic count (TAC) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus rhamnsus* (BR) and Control during the cold storage at 4˚C for 45 days

Storage/day	Control	BR
0	6.20±0.02^{f}	6.50±0.03^{e}
10	6.40±0.03^{e}	6.52±0.02^{de}
20	6.45±0.02^{d}	6.55±0.02^{d}
30	6.50±0.01^{c}	6.60±0.03^{c}
40	6.60±0.02^{b}	6.65±0.02^{b}
45	6.65±0.03^{a}	6.70±0.03^{a}

*Values are means of three replications ± standard deviation (SD)

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$)

4.3.4. Total aerobic count based on camel meat

Figure 4-4 shows the growth of Total Aerobic Count (TAC) (log CFU. g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus casei* (CAC), *Lactobacillus paracasei* (CAPC) and control (CANS) during the cold storage at 4°C for 45 days. Total aerobic count of semi-dry fermented camel meat sausage were significantly increased ($p \leq 0.05$) in all samples during the cold storage period at 4˚C (Figure 4-4). Control sample had lower numbers (range 5.95-6.63) log CFU g^{-1} compared to those samples inoculated with *L. casei* and *L. Paracasei* which had numbers (range 6.60-7.23 and 6.57- 6.80 log CFU g^{-1} respectively) during the cold storage period at 4°C.

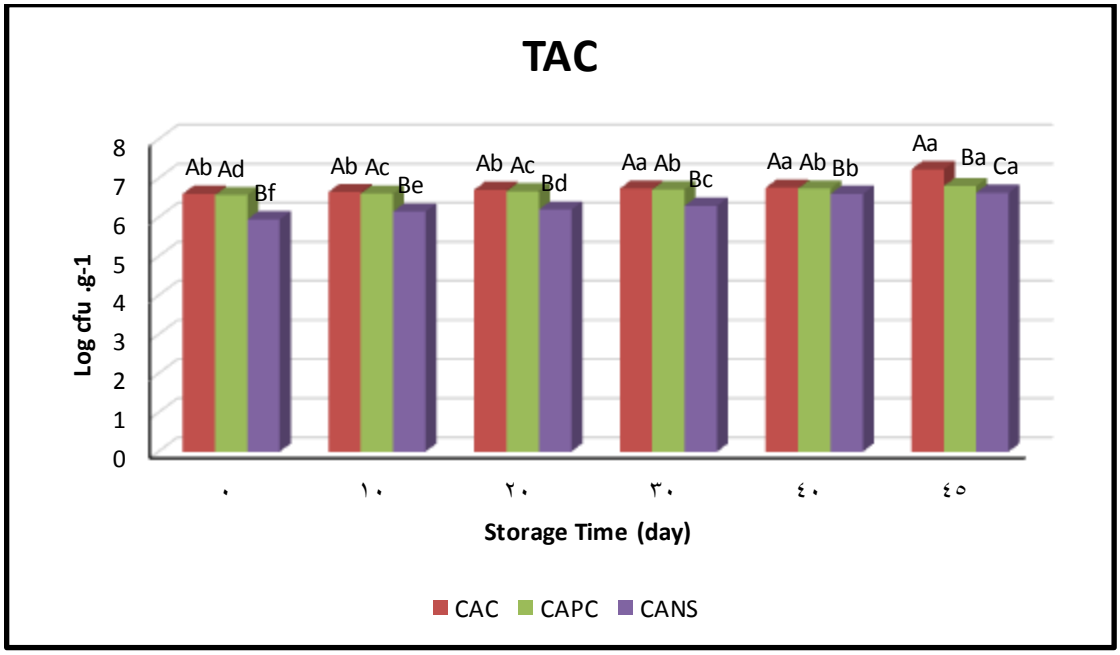

Figure 4-4 Growth of Total Aerobic Count (log CFU. g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus casei* (CAC), *Lactobacillus paracasei*) (CAPC) and control (CANS) during the cold storage at 4°C for 45 days

Table 4-16 shows the growth of Total Aerobic Count (TAC) (log CFU g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus rhamnsus* and control during the cold storage at 4°C for 45 days. Control samples had lower numbers of TAC (range 5.37-6.62) log CFU g^{-1} compared to those samples inoculated with *L. rhamnsus* which had numbers (range 6.50-6.75) log CFU g^{-1} during the cold storage period at 4°C (Table 4-16).

Table 4-16 Growth of Total Aerobic Count (log CFU g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus rhamnsus* (CAR[1]) and control during the cold storage at 4°C for 45 days

Storage/day	Control	CAR
0	5.37 ± 0.02^{d}	6.50 ± 0.03^{d}
10	6.42 ± 0.02^{c}	6.55 ± 0.02^{c}
20	6.47 ± 0.02^{b}	6.60 ± 0.04^{b}
30	6.58 ± 0.03^{a}	6.70 ± 0.02^{a}
40	6.60 ± 0.02^{a}	6.72 ± 0.02^{a}
45	6.62 ± 0.02^{a}	6.75 ± 0.03^{a}

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p\leq 0.05$).

Bacha *et al.* (2010) were observed similar loads of Total Aerobic Count (TAC) on other sausages. These increasing in (TAC) numbers can be due to the initial ingredients and the properties of used meat (Talon *et al.*, 2007). Microbial growth during storage is one of the main factors affecting the quality of meat products, leading to the spoilage and hence economic losses (Afshin *et al.*, 2011). Sausage may be contaminated after heat processing and during other processes such as slicing, packaging and peeling (Cygnarowicz-Provost *et al.*, 1994).

4.3.5. Molds and Yeasts count based on beef meat

Figure 4-5 shows the numbers of Molds and Yeasts count (M&Y) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus casei*, *Lactobacillus paracasei* and Control during the cold storage at 4˚C for 45 days. The numbers of molds and yeasts count were lower than LAB and TAC of semi-dry fermented beef meat sausages. The numbers of molds and yeasts count were significantly decreased ($p\leq0.05$) in all samples of semi-dry fermented beef meat sausages during the cold storage period at 4°C (Figure 4-5). The control samples had higher

[1] Camel inoculated with *Lactobacillus rhamnsus*

numbers of M&Y (range 4.17-3.22 log CFU g^{-1}) compared to the samples inoculated with *L. casei* and *L. paracasei* (range 4.14–2.26 and 4.15-2.50 log CFU g^{-1}, respectively) during the cold storage period at 4°C.

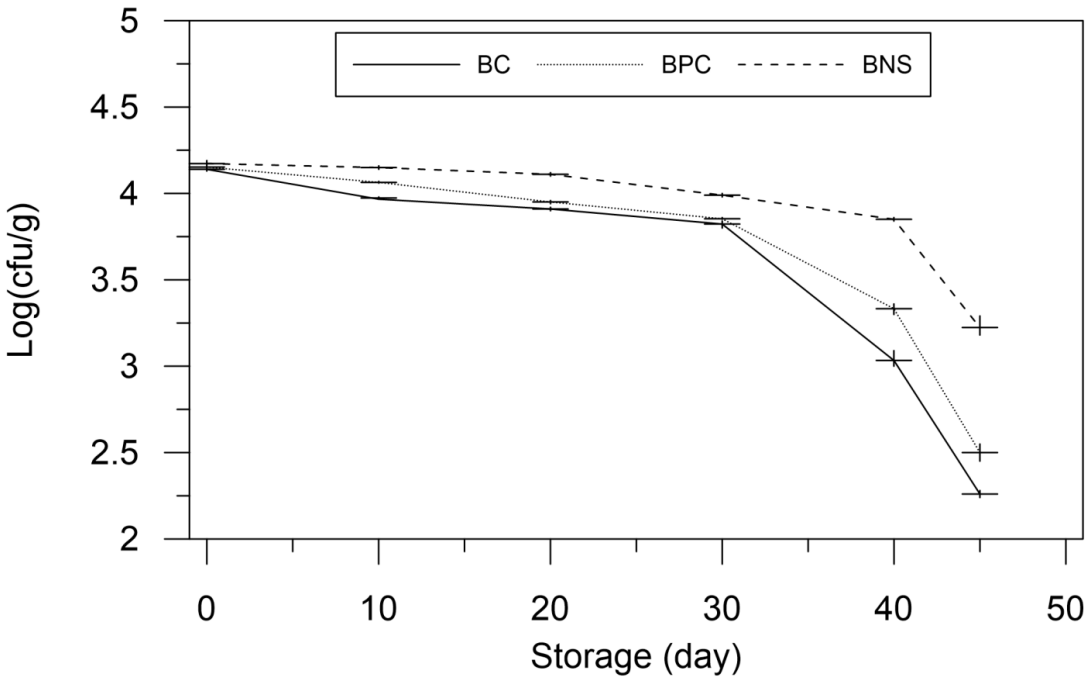

Figure 4-5 Microbial numbers of Molds and Yeast count (M&Y) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus casei* (BC), *Lactobacillus paracasei* (BPC) and Control (BNS) during the cold storage at 4˚C for 45 days

Table 4-17 shows the numbers of Molds and Yeast s count (M&Y) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus rhamnsus* (BR) and control during the cold storage at 4˚C for 45 days. The control samples of semi-dry fermented beef meat sausage had higher numbers of (M&Y) (range 4.74-3.32 log CFU g^{-1}) compared to the samples inoculated with *L.* rhamnsus (range 4.69–2.67 log CFU g^{-1}) during the cold storage period at 4˚C (Table 4-17).

Table 4-17 Microbial numbers of Molds and Yeasts count (M&Y) log CFU g^{-1} for semi-dry fermented beef meat sausage inoculated with *Lactobacillus rhamnsus* (BR) and control during the cold storage at 4˚C for 45 days

Storage/day	Control	BR
0	4.74±0.02[a]	4.69±0.03[a]
10	4.67±0.02[b]	4.47±0.02[b]
20	4.30±0.03[c]	4.00±0.03[c]
30	4.23±0.02[d]	3.95±0.02[d]
40	3.94±0.02[e]	3.85±0.02[e]
45	3.32±0.02[f]	2.67±0.02[f]

*Values are means of three replications ± standard deviation (SD)

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$)

4.3.6. Molds and Yeasts count based on camel meat

Figure 4-6 shows the numbers of Molds and Yeasts Count (log CFU g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus casei, Lactobacillus paracasei* and control during the cold storage at 4°C for 45 days. The numbers of molds and yeasts count were lower than LAB and TAC of semi-dry fermented camel meat sausages and were significantly decreased ($p \leq 0.05$) in all samples during the cold storage period at 4˚C (Figure 4-6). Control samples had higher numbers of molds and yeasts (range 4.16-3.17) log CFU g^{-1} compared to others samples inoculated with *Lactobacillus casei* and *Lactobacillus paracasei* which had numbers (ranged between 4.11-2.15) and (4.13-2.33) log CFU g^{-1} respectively during the cold storage period at 4˚C (Figure 4-6).

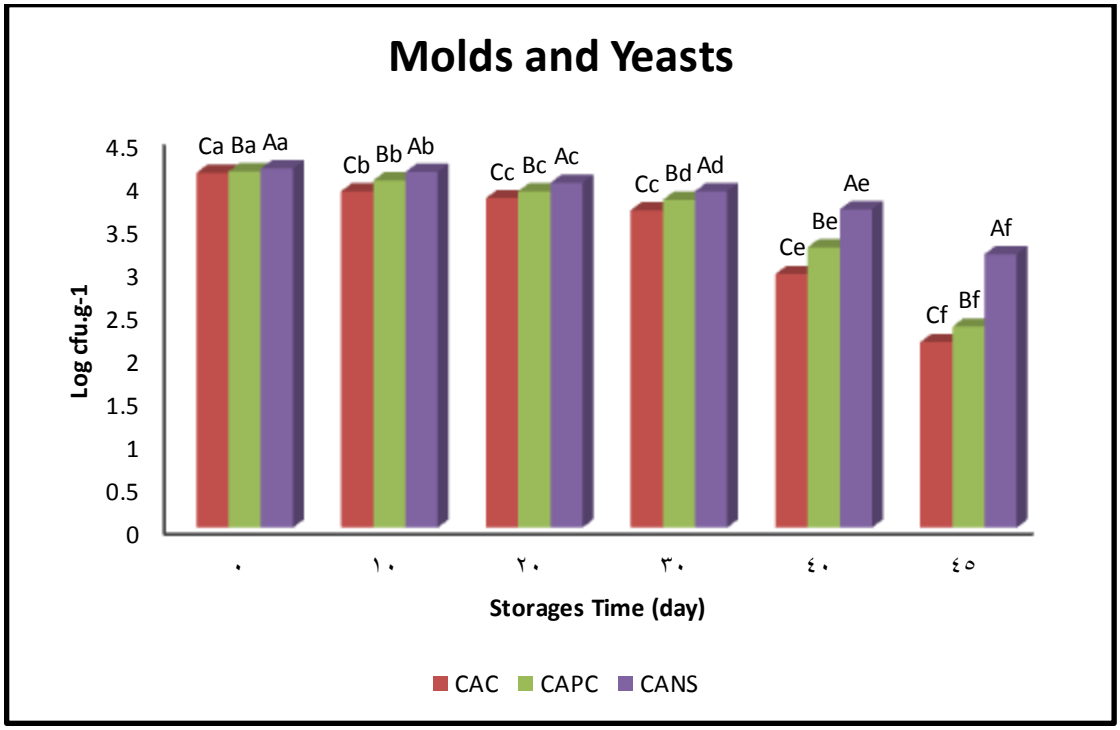

Figure 4-6 Molds and Yeasts Count (log CFU g^{-1}) for semi-dry fermented camel meat sausage inoculated with *Lactobacillus casei* (CAC[1]), *Lactobacillus paracasei* (CAPC[2]) and control (CANS) during the cold storage at 4°C for 45 days

Table 4-18 shows the numbers of Molds and Yeasts Count (log CFU g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus rhamnsus* and control during the cold storage at 4°C for 45 days. The control samples of molds and yeasts had higher numbers (range 4.42-3.30) log CFU g^{-1} compared to the samples inoculated with *L. rhamnsus* (range 4.22–2.40) log CFU g^{-1} during the cold storage period at 4°C (Table 4-18).

[1] Camel inoculated with *Lactobacillus casei*
[2] Camel inoculated with *Lactobacillus para casei*

Table 4-18 Molds and Yeasts Count (log CFU g^{-1}) for semi-dry fermented camel meat sausages inoculated with *Lactobacillus rhamnsus* (CAR) and control during the cold storage at 4°C for 45 days

Storage/day	Control	CAR
0	4.42±0.02[a]	4.22±0.02[a]
10	4.36±0.02[b]	4.10±0.03[b]
20	4.00±0.03[c]	3.97±0.02[c]
30	3.95±0.02[d]	3.90±0.02[d]
40	3.88±0.02[e]	[e] 3.80±0.02
45	3.30±0.02[f]	2.40±0.03[f]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

Our results are in agreement with report of Al-ahmad *et al*. (2014), who demonstrated reduced number of yeasts and molds in the starter cultures compared to the control in smoked fermented semi-dry sausages. This decline in the number of yeasts and molds in the samples inoculated with lactic acid bacteria compared with the control can be due to the competition among LAB, yeasts and molds (Al-Ahmad *et al*., 2014).

4.4. Sensory evaluation

The addition of starter culture improved the sensory properties of samples inoculated with starter culture compared to control. LAB resulted in- to increasing the lactic acid, and promoting the color, reducing the enzyme rancidity and improving the sensory properties of the final product (Al-Ahmad *et al*., 2014). There are many factors affecting the sensory characteristics of meat products such as the meats used as raw materials (genetic type, feed, age, sex, and rearing system), microorganisms selected as microbial starters for the fermentation and type of processing technologies (cooking, drying, ripening, smoking, etc.) (Ahmad and Amer, 2013). Sensorial analysis included the evaluation of color, flavor, texture and overall acceptability.

4.4.1. Sensory evaluation based on beef and camel meat

Tables 4-19, 4-20 and 4-21 show the sensory evaluation of semi-dry fermented beef meat sausage of samples inoculated with *L. casei, L. paracasei* and control, but Tables 4-22 and 4-23 show the sensory evaluation of semi-dry fermented beef meat sausage control and the samples inoculated with *L. rhamnsus* during the cold storage at 4°C for 45 days.

Figures 4-7, 4-8 and 4-9 show the sensory evaluation of semi-dry fermented camel meat sausage of samples inoculated with *L. casei, L. paracasei* and control, but Tables 4-24 and 4-25 show the sensory evaluation of semi-dry fermented camel meat sausage control and the samples inoculated with *L. rhamnsus* during the cold storage at 4°C for 45 days.

4.4.1.1. Color based on beef meat

Storage time had a significant effect ($p \leq 0.05$) on color scores in all the samples of semi-dry fermented beef meat sausage inoculated with *L. casei*. During the cold storage period at 4°C, the color scores significantly ($p \leq 0.05$) decreased (range 7.85- 6.75) (Table 4-19). As shown in Table 4-20, storage period had a significant effect ($p \leq 0.05$) on the color scores in the samples of semi-dry fermented sausage inoculated with *L. paracasei*, the color scores decreased (range 8.07-7.5). Storage period had a significant effect ($p \leq 0.05$) on the color scores in all samples of semi-dry fermented sausage (control) (Table 4-21). The control samples had lower color scores (range 7.75- 6.16) compared to those samples inoculated with *L. casei* and *L. paracasei* during the cold storage period at 4°C (Table 4-21).

The control samples of semi-dry fermented beef sausage had lower color scores (range 7.80- 6.70) (Table 4-22) compared to those inoculated with *L. rhamnsus* range (8.40-7.84) during the cold storage period at 4°C (Table 4-23).

Table 4-19 Sensory evaluation of semi-dry fermented beef meat sausages inoculated with *Lactobacillus casei* during the cold storage at 4°C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	7.85±0.01[a]	6.83±0.01[a]	7.82±0.02[a]	7.33±0.01[a]
10	7.76±0.01[b]	6.5±0.03[b]	7.8±0.04[a]	7.00±0.01[b]
20	7.5±0.03[c]	6.37±0.02[b]	7.5±0.02[b]	6.56±0.01[c]
30	7.4±0.02[d]	6.16±0.01[c]	6.6±0.03[c]	6.33±0.03[d]
40	7.00±0.1[e]	5.71±0.18[d]	6.46±0.01[d]	6.30±0.02[d]
45	6.75±0.02[f]	5.63±0.02[e]	6.13±0.02[e]	6.22±0.03[e]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

4.4.1.2. Color based on camel meat

Lactic acid bacteria result in increasing the lactic acid and promote the color of the product and reduced enzyme rancidity of fat and improving the sensory evaluation of final product (Heinz and Hautzinger, 2009). During the cold storage period at 4˚C, the score values of color significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented camel meat sausage. Control samples had lower score values of color (range 7.50-6.10) compared to those inoculated with *L. casei* and *L. paracasei* which obtained (range 7.53-6.13 and 7.77-6.66) respectively, during the cold storage period at 4˚C (Figure 4-7, 4-8 and 4-9).

Control samples of semi-dry fermented camel meat sausage had lower score values of color (range 7.50-6.10) (Table 4-24) compared to those samples inoculated with *L. rhamnsus* which obtained (range 8. 35-

7.55) during the cold storage period at 4˚C (Table 4-25). The decrease in color scores during storage may be due to the lipid oxidation and subsequent oxidized compounds reacting with amino acids during the non-enzymatic browning of the product (Ahmad and Amer, 2013). Our results are in agreement with the findings of Ahmad and Amer (2013), who reported that during the cold storage, scores of color significantly ($p \leq 0.05$) decreased in the semi-dry fermented sausages.

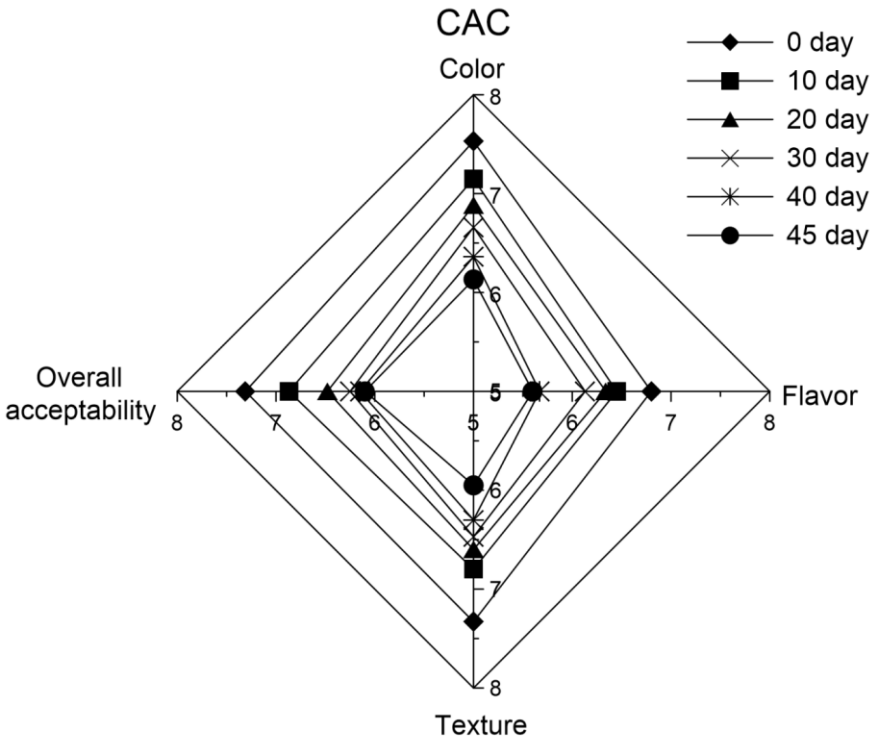

Figure 4-7 Sensory evaluation of semi-dry fermented camel meat sausages inoculated with *Lactobacillus casei* during the cold storage at 4°C for 45 days

4.4.1.3. Flavor based on beef meat

During the cold storage period at 4°C, the flavor scores significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented beef meat sausage inoculated with *L. casei* (range 6.83- 5.63) during the cold storage period at 4°C (Table 4-19). There were significant differences ($p \leq 0.05$) in flavor scores in all samples of semi-dry fermented beef meat sausage inoculated with *L. paracasei*, which had the highest flavor scores during the cold storage, as shown in Table 4-20. The flavor scores decreased in all samples (range 6.87-5.83) during the cold storage period at 4°C. There were significant differences ($p \leq 0.05$) in the flavor scores of semi-dry fermented beef meat sausage (control) and decrease in all samples during the cold storage (Table 4-21). The control sample had lower scores (range 6.66-5.60) compared to those inoculated with *L. casei* and *L. paracasei* during the cold storage period at 4°C.

Table 4-22 shows significant differences ($p \leq 0.05$) in the flavor scores of semi-dry fermented beef meat sausage (control) and decrease in all samples during the cold storage period at 4°C. The control sample had lower scores (range 6.80-5.60) compared to those inoculated with *L. rhamnsus* (range 8.00-7.40) during the cold storage period at 4°C (Table 4-23).

Table 4-20 Sensory evaluation of semi-dry fermented beef meat sausages inoculated with *Lactobacillus paracasei* during the cold storage at 4°C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	8.07±0.06[a]	6.87±0.02[a]	8±0.1[a]	8±0.01[a]
10	8±0.02[b]	6.53±0.04[b]	7.86±0.02[a]	7.8±0.03[b]
20	7.85±0.01[c]	6.4±0.04[c]	7.66±0.03b	7.16±0.01[c]
30	7.83±0.01[c]	6.18±0.01[d]	6.66±0.02[c]	7±0.01[d]
40	7.76±0.01[d]	6.13±0.01[e]	6.5±0.02[d]	6.5±0.03[e]
45	7.5±0.03[e]	5.83±0.02[f]	6.16±0.01[e]	6.44±0.04[e]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

4.4.1.4. Flavor based on camel meat

During the cold storage period at 4˚C, the score values of flavor significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented camel meat sausage. Control samples had lower score values of flavor (range 6.63-5.51) compared to those samples inoculated with *L. casei* and *L. paracasei* which scores (range 6.8- 5.60 and 6.85-5.80) respectively, during the cold storage period at 4˚C (Figure 4-7, 4-8 and 4-9).

Control samples of semi-dry fermented camel meat sausages had lower score values of flavor (range 6.70-5.50) **(**Table 4-24) compared to those samples of semi-dry fermented camel meat sausages inoculated with *L. rhamnsus* which scores range 7.95-7.10 during the cold storage period at 4˚C **(**Table 4-25).

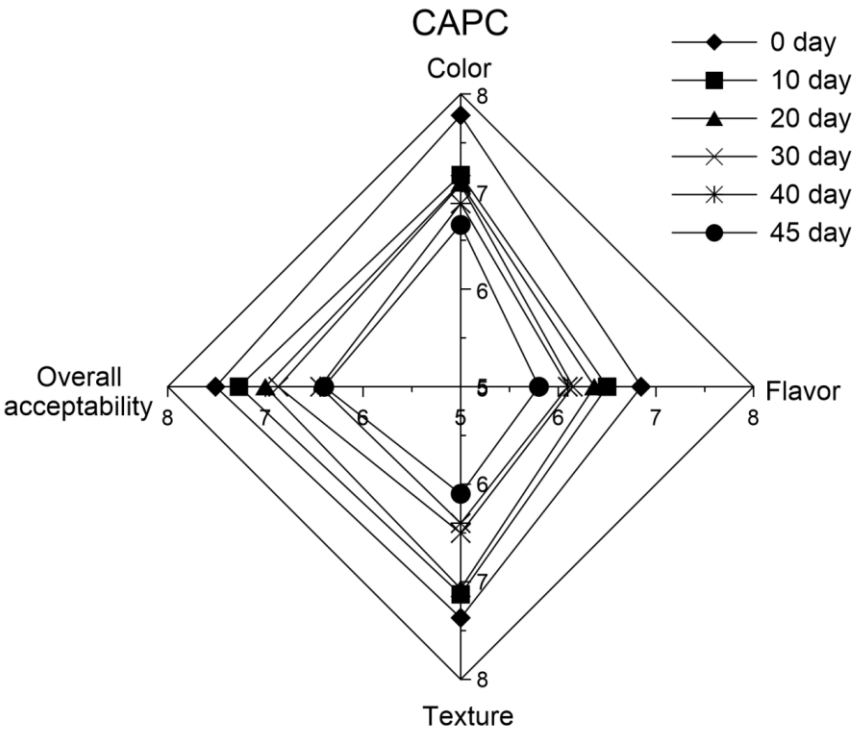

Figure 4-8 Sensory evaluation of semi-dry fermented camel meat sausages inoculated with *Lactobacillus paracasei* during cold storage at 4°C for 45 days

The characteristic flavor of fermented sausages mainly originates from the breakdown of carbohydrates, lipids, and proteins through the action of microbial and endogenous meat enzymes **(**Ahmad and Amer, 2013). The development of flavor is also influenced by several variables such as product formulation (especially spices), processing conditions, and starter culture (Ahmad and Amer, 2013). Our results are corresponding with those obtained by Ahmad and Amer (2013), who indicated a decrease of flavor for semi-dry sausage during the refrigerated storage.

4.4.1.5. Texture based on beef meat

Texture is a predominant element of the quality and acceptability of foods. It is perceived from the sensory impressions of the physical properties of a material, its nature, composition and behavior on

deformation received from the senses of touch, sight and hearing (Ahmad and Amer, 2013). As shown in Table 4-19, texture scores of semi-dry fermented beef meat sausage. There were significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented sausage inoculated with *L. casei* (range 7.82-6.13) during the cold storage period at 4°C, also there were significant differences ($p \leq 0.05$) in the texture scores of semi-dry fermented beef meat sausage inoculated with *L. Paracasei* and texture scores decreased in all samples, (range 8.0-6.16) during the cold storage period at 4°C (Table 4-20). During the cold storage period at 4°C, the scores of texture decreased significantly ($p \leq 0.05$) in all samples of semi-dry fermented beef meat sausage (control) (Table 4-21). The control sample had lower score of texture (range 7.50-6.00) compared to those samples inoculated with *L. casei* and *L. paracasei* during the cold storage period at 4°C (Table 4-21).

During the cold storage period at 4°C, the scores of texture significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented beef meat sausage (control) (Table 4-22). The control samples had lower score of texture (range 7.80-6.10) compared to those samples inoculated with *L. rhamnsus* which had (8.13-7.53) during the cold storage period at 4°C (Table 4-23).

Table 4-21 Sensory evaluation of semi-dry fermented beef meat sausages control during the cold storage at 4°C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	7.75±0.01[a]	6.66±0.01[a]	7.5±0.03[a]	7.25±0.02[a]
10	7.4±0.03[b]	6.3±0.03[b]	6.85±0.02[b]	6.75±0.01[b]
20	7.00±0.09[c]	5.83±0.01c	6.66±0.01[c]	6.50±0.04[c]
30	6.83±0.01[d]	5.66±0.01[d]	6.5±0.03[d]	6.30±0.03[d]
40	6.38±0.03[e]	5.63±0.03[d]	6.34±0.02[e]	6.07±0.12[e]
45	6.16±0.02[f]	5.6±0.02[e]	6.00±0.1[f]	5.83±0.02[f]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

4.4.1.6. Texture based on camel meat

Texture is a powerful element of the quality and acceptability of foods. It is perceived from sensory impressions of the physical properties of material, its nature, composition and behavior on deformation received from senses of touch, sight and hearing (Ahmad and Amer, 2013). During the cold storage period at 4°C, the scores of texture significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented camel meat sausage (Figure 4-7, 4-8 and 4-9). Control sample had lower scores of texture (range 7.16-5.67) compared to those samples inoculated with *L. casei* and *L. paracasei* which had scores of texture (range 7.33-5.95 and 7.37-6.10) respectively during the cold storage period at 4°C.

Control samples of semi-dry fermented camel meat sausage had lower scores of texture (range 7.30-5.90) (Table 4-24) compared to those samples inoculated with *L. rhamnsus* which had scores of texture (range 8.10-7.45) during the cold storage period at 4°C **(**Table 4-25). The significant decrease in texture during storage may be due to changes in the disulfide bonds and contents of amino acid (Ahmad and Amer, 2013). Increasing levels of fat constantly improved the scores of texture (as obtained by Ahmad and Amer (2013) during the refrigerated storage (2°C) indicated that the scores of the

texture of semi-dry fermented sausages incorporated with 20% and 25% fat significantly ($p \leq 0.05$) decreased.

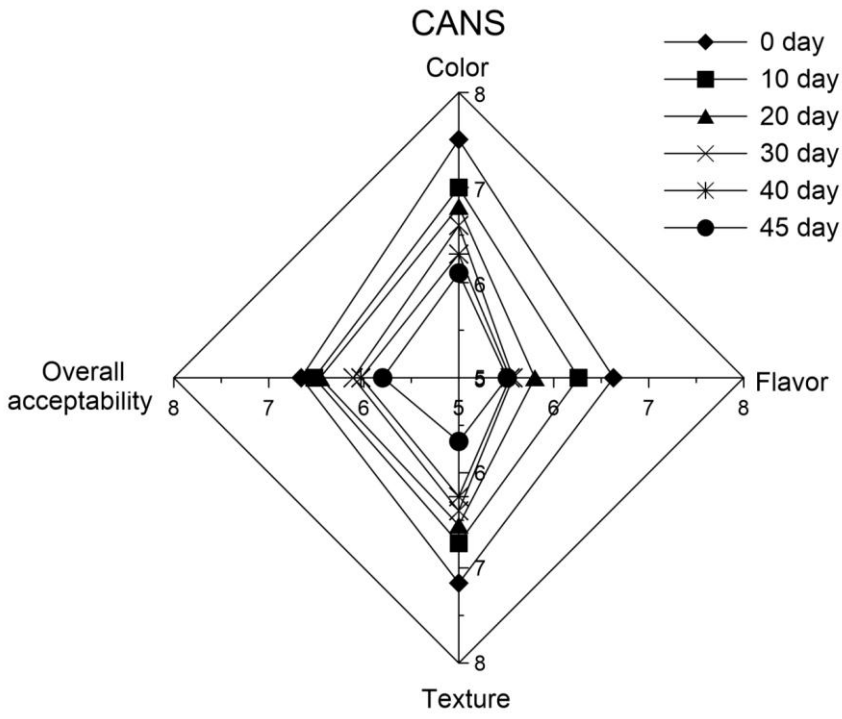

Figure 4-9 Sensory evaluation of semi-dry fermented camel meat sausages control (CANS) during the cold storage at 4°C for 45 days

4.4.1.7. Overall acceptability based on beef meat

According to Table 4-19, overall acceptability scores significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented beef meat sausage inoculated with *L. casei* and ranged between 7.33-6.22 during the cold storage period at 4°C. The samples of semi-dry fermented beef meat sausage inoculated with *L. paracasei* had the highest overall acceptability scores during the storage period at 4°C. Overall acceptability scores significantly decreased ($p \leq 0.05$) in all samples of semi-dry beef meat

fermented sausage inoculated with *L. paracasei* (range 8.0-6.44) during the cold storage period at 4°C (Table 4-20). The control samples of semi-dry fermented beef meat sausage had lower overall acceptability scores (range 7.25-5.83) compared to those samples inoculated with *L. casei* and *L. paracasei* during the storage period at 4°C (Table 4-21).

The control samples of semi-dry fermented beef meat sausage had lower overall acceptability scores (range 7.30-6.20) (Table 4-22) compared to those samples inoculated with *L. rhamnsus* which had overall acceptability scores (range 8.50-7.80) during the storage period at 4°C (Table 4-23).

Table 4-22 Sensory evaluation of semi-dry fermented beef meat sausages control during the cold storage at 4°C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	7.80±0.02[a]	6.80±0.02[a]	7.80±0.03[a]	7.30±0.03[a]
10	7.70±0.03[b]	6.45±0.02[b]	7.75±0.02[b]	6.90±0.02[b]
20	7.45±0.02[c]	6.30±0.03[c]	7.40±0.02[c]	6.50±0.03[c]
30	7.35±0.02[d]	6.10±0.02[d]	6.50±0.03[d]	6.30±0.03[d]
40	6.90±0.02[e]	5.70±0.02[e]	6.40±0.02[e]	6.25±0.02[e]
45	6.70±0.03[f]	5.60±0.02[f]	6.10±0.02[f]	6.20±0.02[f]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

The best sensory characteristics regarding in color, flavor, texture and overall acceptability scores were found in the samples of semi-dry fermented beef meat sausage inoculated with *L. paracasei* and *L. rhamnsus* during the cold storage period at 4°C **(**Table 4-20 and 4-23).

Table 4-23. Sensory evaluation of semi-dry fermented beef meat sausages inculated with *L. rhamnsus* during refrigerated storage at 4°C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	8.40±0.03[a]	8.00±0.02[a]	8.13±0.01[a]	8.50±0.02[a]
10	8.20±0.02[b]	7.97±0.02[a]	8.00±0.02[b]	8.35±0.02[b]
20	8.00±0.03[c]	7.85±0.03[b]	7.92±0.02[c]	8.30±0.02[c]
30	7.95±0.02[d]	7.76±0.02[c]	7.87±0.02[d]	8.20±0.03[d]
40	7.87±0.02[e]	7.58±0.02[d]	7.66±0.02[e]	7.85±0.02[e]
45	7.84±0.01[e]	7.40±0.02[e]	7.53±0.03[f]	7.80±0.03[f]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

4.4.1.8. Overall acceptability based on camel meat

During the cold storage period at 4°C, the scores of overall acceptability significantly decreased ($p \leq 0.05$) in all samples of semi-dry fermented camel meat sausage (Figure 4-7, 4-8 and 4-9). Control samples had lower overall acceptability scores (range 6.6- 5.80) compared to those samples inoculated with *Lactobacillus casei* and *Lactobacillus paracasei* which scores (range 7.31-6.10 and 7.51-6.40) respectively during the cold storage period at 4°C.

Control samples of semi-dry fermented camel meat sausage had lower overall acceptability scores (range 7.25- 6.00) (Table 4-24) compared to those samples inoculated with *L. rhamnsus* which scores (range 8.45-7.75) during the cold storage period at 4°C (Table 4-25).

Table 4-24. Sensory evaluation of semi-dry fermented camel meat sausages control during the cold storage at 4°C for 45 day

Storage/day	Color	Flavor	Texture	Overall acceptability
0	7.50±0.02[a]	6.70±0.02[a]	7.30±0.02[a]	7.25±0.03[a]
10	7.10±0.02[b]	6.40±0.03[b]	6.70±0.02[b]	6.85±0.02[b]
20	6.85±0.03[c]	6.25±0.03[c]	6.50±0.03[c]	6.40±0.02[c]
30	6.60±0.02[d]	6.00±0.03[d]	6.40±0.02[d]	6.20±0.03[d]
40	6.30±0.03[e]	5.60±0.02[e]	6.25±0.02[e]	6.10±0.02[e]
45	6.10±0.02[f]	5.50±0.03[f]	5.90±0.02[f]	6.00±0.02[f]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

The best overall acceptability scores were found for semi-dry camel meat fermented sausage inoculated with *Lactobacillus paracasei* and *L. rhamnsus* during the cold storage period at 4°C **(**Figure 4-8 and Table 4-25**).**

Table 4-25 Sensory evaluation of semi-dry fermented camel meat sausages inoculated with *L. rhamnsus* during the cold storage at 4°C for 45 days

Storage/day	Color	Flavor	Texture	Overall acceptability
0	8.35±0.03[a]	7.95±0.02[a]	8.10±0.02[a]	8.45±0.02[a]
10	8.15±0.02[b]	7.90±0.02[b]	7.90±0.03[b]	8.30±0.03[b]
20	7.95±0.02[c]	7.75±0.02[c]	7.85±0.02[c]	8.25±0.02[c]
30	7.80±0.02[d]	7.70±0.03[d]	7.80±0.02[d]	8.15±0.02[d]
40	7.75±0.03[e]	7.55±0.02[e]	7.60±0.02[e]	7.80±0.02[e]
45	7.55±0.02[f]	7.10±0.02[f]	7.45±0.02[f]	7.75±0.02[f]

*Values are means of three replications ± standard deviation (SD).

**Means with different superscript letter within column represent significant differences ($p \leq 0.05$).

Chapter5

Conclusion and recommendation

5.1. Conclusion

It is concluded that the fermentation process with *L. casei, L. paracasei* and *L. rhamnsus* of the low fat content semi-dry fermented sausage processed from beef and camel meat led to the dominance of lactic acid bacteria on the microflora in the fermented sausage; it helped to improve the sensorial quality, safety and shelf life by inhibition the spoilage and the growth of pathogenic bacterial and kept them in appropriate condition within 45 days of the refrigerated storage at 4°C.

There was significant increase in aerobic plate count particularly lactic acid bacteria (predominant microorganism) but, drop in counts of yeast and molds. The protein, fat and ash contents were significantly increased in all semi-dry fermented sausages while the moisture content decrease in all samples. The lactic acid value increased in all samples results in drop in pH value. The best sensory evaluation in the color, flavor, texture and overall acceptability scores was obtained in the samples of semi-dry fermented sausage inoculated with *L. paracasei* and *L. rhamnsus*.

The use of *Lactobacillus casei, L. paracasei* and *L. rhamnsus in processing fermented sausages improved* the quality and nutritional value of food by presenting functional properties.

5.2. Recommendation

1- Recommend to study the use of probiotic as mixture and study the effect on quality and safety of the fermented sausages.

2- Recommend to study the use other strain of *Lactobacillus* or of *Bifidobacterium* in formulation of fermented sausages.

3- Recommend to study the effect of probiotics on pathogenic bacteria.

3- Recommend to use other source of plant oil like (olive, Walnut and sesame) oil as ingredient in formulation semi-dry fermented sausages.

4- Recommend to change the storage period and temperature and study effect these on safety and quality of the product.

5- Recommend to use other source of meat like sheep and buffalo in the formulation of semi-dry fermented sausages.

6- Recommend to study other method of manufacturing, for example, dry, smoking or cooking in the fermented sausages.

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چکیده

این مطالعه با هدف توسعه و ارزیابی سوسیس تخمیر شده بر اساس کاهش محتوای چربی اجرا شد. سوسیس‌های تخمیری پروبیوتیک محصولات گوشتی ایمن و سالمی هستند. در این تحقیق سوسیس‌های تخمیری نیمه خشک با استفاده از گوشت گاو و شتر در چهار نوع مختلف تولید شدند: بدون استارتر (کنترل)، تلقیح شده به وسیله لاکنوباسیلوس کازئی، پاراکازئی و رامنوسوس. تمام تیمارها از لحاظ ویژگی‌های فیزیکی و شیمیایی (پروتئین، رطوبت، چربی، خاکستر، اسید لاکتیک و pH.) ویژگی‌های میکروبیولوژیکی (شمارش کل باکتری‌های هوازی، شمارش کل کپک و مخمر و شمارش کل باکتری‌های لاکتیک اسید) و ارزیابی حسی (رنگ، عطر و طعم؛ بافت و پذیرش کلی) بعد از ۰، ۱۰، ۲۰، ۳۰، ۴۰ و ۴۵ روز نگهداری در یخچال در دمای ۴ °C آنالیز شدند. آنالیزهای میکروبی نشان داد که تعداد باکتری‌های لاکتیک اسید در سوسیس تخمیری نیمه خشک در طی نگهداری در سرما (۴ °C برای ۴۵ روز) در نمونه‌های تلقیح شده با *L. paracasei* و *Lactobacillus rhamnsus* و *Lactobacillus casei* به ترتیب به $\log CFU.\ g^{-1}$ (۷/۹۵، ۸/۲۸ و ۹/۲۴) می‌رسد. آنالیز شیمیایی سوسیس تخمیری نیمه خشک نشان داد که یک تفاوت معنی دار در محتوای رطوبت وجود دارد که در تمام نمونه‌ها در طی نگهداری در سرما کاهش پیدا می‌کند. اما تمام پارامتر های دیگر مثل پروتئین ،چربی و خاکستر افزایش پیدا می کنند. کاهش pH در تمام نمونه‌ها به علت تولید لاکتیک اسید به وسیله لاکتیک اسید باکتری‌ها در طی تخمیر است. ویژگی‌های فیزیکوشیمیایی، میکروبی و حسی سوسیس تخمیر شده تلقیح شده توسط *Lactobacillus paracasei* و *L. rhamnsus* بهتر ازسایر نمونه ها بودند. نهایت ، نتایج نشان داد که محصول تولید با قابلیت ماندکاردر دمای ۴ °C به مدت ۴۵ روز را دارا می باشد. ارزیابی حسی نشان داد که سوسیس تخمیری نیمه خشک که حاوی *Lactobacillus casei* و *L. paracasei* و *L. rhamnsus* است در مقایسه با نمونه کنترل برتر می باشد.

کلمات کلیدی: باکتری لاکتیک اسید ، تولید سوسیس تخمیری نیمه خشک، ویژگی‌های کیفی

پردیس کشاورزی و منابع طبیعی

دانشکده‌ی مهندسی و فناوری کشاورزی

گروه علوم و مهندسی صنایع غذایی

فرمولاسیون و تولید محصول گوشتی (سوسیس) شتر تخمیری کم چرب فراسودمند بوسیله باکتریهای لاکتوباسیلوس کازی، لاکتوباسیلوس پارا کازی ولاکتوباسیلوس رامنوسوس

نگارش

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رساله برای دریافت **درجه دکتری** در رشته‌ی

بیوتکنولوژی مواد غذایی

اسفند ماه ۱۳۹۶