MICROBIAL ANALYSIS OF LABORATORY PREPARED *SUKUTI* AND *SUKUTI* SOLD IN DHARAN

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Microbial Analysis of Laboratory Prepared *Sukuti* and *Sukuti* Sold in Dharan

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Approval Letter

This *dissertation* entitled *Microbial Analysis of Laboratory Prepared Sukuti and Sukuti Sold in Dharan* presented by Anup Chapagain has been accepted as the partial fulfillment of the requirements for the B. Tech. degree in Food Technology.

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_____

(Anup Chapagain)

Abstract

In the present study, microbiological analysis of 10 smoked buffalo *sukuti* samples (A, B, C, D, E, F, G, H, I, J) from market and non-smoked laboratory based buffalo *sukuti* prepared by taking cured, non-cured and spiced *sukuti* of both lean and fat meat *sukuti* were optimized. Its quality and sensory characteristics were evaluated in terms of appearance, flavor, texture, taste and overall acceptability.

Average TPC, TC and total *Salmonella-Shigella* in market *sukuti* were found to be $17.1x10^5$ cfu/g, $1.89x10^2$ cfu/g and $23.4x10^2$ cfu/g respectively. Average TPC, TC and total *Salmonella-Shigella* counts were found to be $11.1x10^4$ cfu/g, $9x10^1$ cfu/g and $7.3x10^2$ cfu/g in lean meat samples. Similarly, average TPC, TC and total *Salmonella-Shigella* counts in fat meat *sukuti* was found to be $27x10^4$ cfu/g, $11.7x10^1$ cfu/g and $10x10^2$ cfu/g. LSD showed significant difference between samples C, F and J; samples LC (Lean Cured), LS (Lean Spiced) and LNC (Lean not Cured) were found to be significantly different from each other whereas samples FS (Fat Spiced) was significantly different with FC (Fat Cured) and FNC (Fat not Cured) at 5 % level of significance. Sample F was found to be superior considering overall acceptability as TPC, TC and total *Salmonella-Shigella* counts were lower in sample F and had higher degree of acceptability on sensory score. Similarly, the mean sensory scores found higher degree of acceptability on the sample LC along with lower microbial counts. On sensory evaluation, the mean sensory scores for the sample FS were found to be highest; whereas, TPC, TC and total *Salmonella-Shigella* counts were lower in sample FC. From which it can be concluded that processing of *sukuti* requires hygienic environment, GMP and storage.

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List of Abbreviation

Abbreviations	Full form
ANOVA	Analysis of Variance
A_w	Water activity
FC	Fat cured
FS	Fat spiced
FNC	Fat not spiced
TC	Total Coliform
TPC	Total plate count
AOAC	Association of Official Analytical Chemist
LSD	Least Significant Difference
LNC	Lean not cured
LS	Lean spice
MOAD	Ministry of Agricultural and Livestock Development
LC	Lean cured

Part I

Introduction

1.1 General introduction

Meat is a part of the daily diet for human. Dry meat is widely accepted around the world. In Nepalese context, *sukuti* is traditionally prepared dry meat. It is mostly prepared by sun drying and smoking. The nature and level of microbial contamination in meat have important consequence in relation to public health, storage life and the type of spoilage of meat (Gracey and Collins, 1994). The most important pathogens associated with meat include *Salmonella, Staphylococcus aureus, Escherichia coli, Clostridium perfringens, Campylobacter jejuni, Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila* (Koutsoumanis and Sofos, 2004).

The prevalence and levels of bacteria on meat carcasses depend on a number of factors including the origin of the animal, sanitation procedures and hygienic practices employed during handling and processing and conditions of storage. Extremely high numbers of microorganisms are found in the animal's intestinal content, and it is expected that some will find their way to the surface of the carcass during the dressing operations. Microorganisms reach the carcass via butcher's hands, tools, clothing, water etc. The number can be proliferated during cutting and distribution (Khedkar *et al.*, 2003; Wilson *et al.*, 1981).

Meats contain an abundance of all nutrients required for the growth of bacteria, yeasts and molds and adequate quantity of these constituents exist in fresh meats in an available form (Jay, 1996). Fresh meats such as beef, pork and lamb, as well as poultry, seafood and processed meats have high pH values. During bleeding, skinning and cutting, the main sources of micro-organisms are the hide, hooves, hair and intestinal tract. Knives, cloths, air, hands and clothing of the workers can serve as intermediate sources of contaminants. Contamination can come from carts, containers, other contaminated meat and personnel (Fraser *et al.*, 1996). Bacteria of many genera are found in meat, among which some of the more important are *Pseudomonas, Acinetobacter, Moraxella, Alcaligenes, Micrococcus, Streptococcus, Sarcina,*

Leuconostoc, Lactobacillus, Proteus, Flavobacterium, Bacillus, Clostridium, Escherichia, Campylobacter, Salmonella and *Streptomyces* (Fraser *et al.*, 1996).

1.2 Statement of the problem

There are very few researches being carried out in *sukuti* in the world whereas in context to Nepal for instance, not much work has been done on many important and relevant areas. Only, few works have been initiated on its traditional knowledge and traditional preparation and its usage. Since, the researchers might not have initiated the research so far.

Beside these, there are huge laps and gaps in processing and production concerning safety and quality issues. It is hoped from R and D that the improved safety and quality of *sukuti* will increase demand and market areas as the business barriers will be eliminated and minimized the cost.

So, there is adequate reason to believe that the study on screening and characterization of *sukuti* borne microbes as well as its optimization can proof the better implication and its future prospective.

1.3 Objectives

1.3.1 General objective

The general objective of this work is microbial analysis of *sukuti* prepared in laboratory and *sukuti* sold in Dharan.

1.3.2 Specific objectives

The following are the specific objectives of this work:

1. Microbial analysis of raw buffalo meat and various *sukuti* samles from different place of Dharan.
2. Preparation of various *sukuti* samples in the laboratory and their microbial analysis.
3. Evaluation of sensory properties of laboratory prepared *sukuti* and *sukuti* from different places of Dharan.

4. Proximate analysis of final products.

1.4 Significance of the study

Nepal is one of the poor country in the world but rich in diversity of nature, culture and food culture. Different ethnic groups of Nepal produced variety of food by application of indigenous knowledge (IK) passed from fore-fathers and still being passed from generation to generation. The IK can be innovated and developed in the technology for future perspective. Through this, Nepal not only gains reputation but also economic status can be boost up. So, the local innovation of indigenous food such as *sukuti,* a dry meat and its commercialization may be beneficial in future too. For this optimization of *sukuti* is must.

Last but not least, the successful findings of this thesis will definitely help in the increase in consumer's safety and nutritional status of public and its positive consequences and also contribute positive impact for healthy citizens, country and world. This study can make real sense and match with the motto "Healthy and wealthy people and country". Thus, this work has great significance in the innovation and development of technology for the commercialization of Nepalese dry meat "*sukuti"*.

1.5 Limitations of the work

1. Shelf life of the product was not studied due to time constraint.
2. Instrumental texture analysis was not carried out.

Part II

Literature review

2.1 Meat

Meat, the flesh or other edible parts of animals (usually domesticated cattle, swine, and sheep) used for food, including not only the muscles and fat but also the tendons and ligaments. Meat is valued as a complete protein food containing all the amino acids necessary for the human body. The fat of meat, which varies widely with the species, quality, and cut, is a valuable source of energy and also influences the flavor, juiciness, and tenderness of the lean (Britannica, 2019).

In the broadest sense, meat is the edible postmortem component originating from live animals. On the other hand, meat is defined as those tissues exclusively originating from an animal's carcass-a proportion amounting to about one-half to three-fourths of the animal's live weight (Hui *et al.*, 2001).

2.1.1 Preservation of meat

The origin of methods for preserving foods in times of plenty for use in times of food scarcity is buried in antiquity. In Asia and the far north of Europe, freezing and icing of fresh foods for preservation have been practiced since time immemorial. It is difficult to say which of the early methods of food preservation was the first to be devised but it was probably drying either in the sun or by a fire; an early observation would have been the good keeping qualities of dry food, e.g. nuts, cereals and grains (Mahato, 1997).

2.1.2 Principles of meat preservation by drying and dehydration

The pressure of water vapor will be constant in the closed space around the material. This pressure is lower or equal to vapor pressure above pure water at the same temperature. The numerical expression of this is the water activity (a_w):-a water pressure above the material water pressure above t w = h the pure water (Zukal and Incze, 2010).

During drying, water is removed as vapor. Thus, the activity of microorganisms decreases because the portion of water they can utilize decreases, and this in turn means the shelf life of the product increases, the mass and volume of product decreases, the texture will be harder, aroma compounds develop during longer processes, mainly in no heated products. By means of these changes, meat products can be manufactured, stressing and improving the favorable texture of raw meat and developing products with extraordinary attributes (Zukal and Incze, 2010).

The manufacture of fermented meat products such as, raw hams or dry sausages, is an example, where drying is one processing amongst several others. To have an extended shelf life, fermented products need to lose moisture during their fermentation, they are dehydrated or "dried" to a certain extend. Drying and fermentation must go hand in hand to achieve the desired flavor and shelf life. The drying of such products is mostly done in controlled chambers with exact temperature and humidity parameters. Drying under natural conditions is increasingly rare (Heinz and Hautzinger, 2007).

In contrast to simply drying, most preparation of dried foods is more correctly referred to as dehydration in which there is a least some degree of control over air temperature and movement, and control of humidity (Cassens, 1994).

2.1.3 Technology of dried meat

2.1.3.1 Hot air drying of meat

Convention drying process of food products is extensively employed as a preservation technique but oven drying is the simplest and faster than the sun drying (Mishra *et al.*, 2013). Solar drying is considered as the best due to its low cost and a smaller number of microorganisms as compared to oven drying process (Talib *et al.*, 2014). Hot air convectional drying is a process where drying is achieved by circulating hot air in closed cabinets. In this process, heat is transferred from hot air to solid surface (Lewicki, 2004) which is thus transferred from the surface to the interior by conduction and shrinkage is excessive, around 80% (Ratti, 2001).

Hot-air drying is not suitable for uncooked meat or for cooked chops and steaks. For products as large as chops and steaks it is too slow, causes surface hardening and gives product with poor rehydration and acceptability (Price and Schweigert, 1971). The temperature used for the drying of meat can be 60-70ºC as stated by Tornberg (2005) that the degradation of connective tissue starts in that temperature range.

2.1.3.2 Smoke drying of meat

Smoking extended the shelf life and changed the sensory properties of the meats. The procedures of smoking have been gradually improved to suit the requirements of people in different regions of the world in respect to shelf life and sensory properties.

Traditionally, smoking was carried out over several days in a brick oven with smoldering oak, hickory or hardwood sawdust and hot ash piled on the floor. It is now more common to use an insulated steel cabinet enclosing a heat-exchanger system. The cuts to be smoked are hung on racks and placed in the cabinet and the temperature raised to approximately 32°C for 30 min. It is important to ensure that the temperature does not rise above 37°C or the fat may melt (Gracey *et al.*, 1999).

In modern automatic smokehouses, the draft is forced by mechanical equipment and shaped according to a computer program adjusted to the kind of smoked goods. The temperature of the smoke affects the sensory properties and the preservative effect, and controls the rate of the process. Cold smoking takes place in the range of 12 – 25°C and warm smoking at 23 – 45°C. In hot smoking, since thermal denaturation of the meat proteins is required, the smoke temperature during various stages of the process ranges from about 50°C to 90°C (Sikorski and Kol, 2010).

The chief bacteriostatic and bactericidal substance in wood smoke is formaldehyde. The combination of heat and smoke usually causes a significant reduction in the surface bacterial population. In addition, a physical barrier is provided by superficial dehydration, coagulation of protein and the absorption of resinous substances (Gracey *et al.*, 1999). The disadvantage of this method is that

intensive smoking has a negative influence on the quality, especially in the case of prolonged storage in which concentrated smoke compounds develop increasingly unpleasant tarry flavors (FAO, 1990).

2.1.3.3 Sun drying

Sun drying is the oldest and widely used method of drying by many peasant farmers in recent times (Kuponiyi *et al.*, 1984; Talib *et al.*, 2014). It is a longer process and exposes meat to an extensive contamination by microorganisms and dirt (Gailani, 1988) This contamination can be avoided by drying of meat in mechanical dryers. The sun-dried samples have higher functional properties, acceptability level, proteins and lower fat contents as compared to oven-dried samples. Sun drying of the meat samples is recommended provided it is done under hygienic conditions (Ayanwale *et al.*, 2007). Combinations of sodium chloride and sub inhibitory levels of anti-microbial agents are highly effective in controlling/inhibiting microbial growth during sun-drying process (Talib *et al.*, 2014).

2.1.3.4 Solar drying

In solar drying, the products to be dried are laid out. In this closed system, consisting of a solar collector and a meat drying chamber, without direct exposure of the meat to the environment, meat drying is more hygienic as there is no secondary contamination of the products through rain, dust, insects, rodents or birds. The products are dried by hot air only (Hartog *et al.*, 2006).The basic principles employed in a solar dryer are:

Converting light to heat: Any black on the inside of a solar dryer will improve the effectiveness of turning light into heat.

Trapping heat: Isolating the air inside the dryer from the air outside the dryer makes an important difference. Using a clear solid, like a plastic bag or a glass cover, will allow light to enter, but once the light is absorbed and converted to heat, a plastic bag or glass cover will trap the heat inside. This makes it possible to reach similar temperatures on cold and windy days as on hot days.

For moving the heat to the food. Both the natural convection dryer and the forced convection dryer use the convection of the heated air to move the heat to the food (JIN, 2012). Recent efforts to improve sun-drying have led to solar drying. It utilizes the sun as the heating source, but specially designed dehydrator increases the temperature and air current to accelerate the drying time.(Susan, 1993).

2.1.3.5 Microwave drying

Microwave drying is a faster method because of volumetric heating. Here the microwave energy absorbed by the food material is converted into heat. Microwave heating produces significant advantages over conventional drying in reducing time and improving food quality. Microwave dried meat products have better rehydration property and lower a_w and better microbial quality than hot air-dried meat products. Higher microwave radiations increase the outward flux of vapor preventing the collapse of tissue structure and increasing the rehydration capacity of the dried products (Mishra *et al.*, 2017).

2.2 Preparation of dried meat (*Sukuti*) in Nepal

Dried meat products have a history of more than a thousand years in China. During the Sung Dynasty already 200 types of dried meat products, based on red meat, poultry and fish were known (Acharya, 2014). As indigenous food, *sukuti* preparation is being carried in Nepal from long time ago.

2.2.1 Process of making dried meat

a) Slaughtering of buffalo

Local butchers slaughter the buffaloes in open ground by traditional method. In general, the buffaloes are stunned by direct blow in the skull using a pole axe, then bleeded with sticking the major arteries of the neck immediately. Jhatka (deheading with heavy knife) method is rarely used. The most objectionable thing is that there is lack of slaughter hygiene and no provision of modern slaughtering. So, meat often becomes contaminated with dust, mud, etc., leading to entry of different types of spoilage and pathogenic microorganisms in the meat. The buffaloes themselves may

be suffering from various zoonotic diseases, which are very dangerous for human health.

b) Cutting and trimming

In general, hind quarter portion is taken for the preparation of *sukuti*. However, forequarter and sirloin parts can also be used. Bones, fat and other undesirable portions are removed.

c) Strip preparation

There is no any fixed standard dimension for meat strips. In general, they are stripped in the dimensions of approximately (250 × 20 × 20 mm) (Acharya, 2014).

d) Curing

Curing is a method of preservation by treating with salt and sodium nitrate (and nitrite), which serves to inhibit the growth of pathogenic organisms while salt tolerant bacteria develop. During the pickling process the nitrate is converted into nitrite, which combines with the muscle protein, myoglobin, to form the red colored nitrosomyoglobin which is characteristic of pickled meat products (Anonymous, 2005). There are several methods of curing:

Dry Curing

The most common salting/curing process for dried whole muscle meats is dry curing; often this class of products is referred to as “dry cured” whole muscle meats. The first step in the dry curing process is mixing the meat pieces with the salt and the other curing mixture.

During dry curing, moisture migrates from inside the tissue to the surface due to osmotic pressure with the higher salt concentration at the surface. The salt and cure migrate into the tissue due to osmotic pressure. This is a relatively slow process and temperatures must be below 40°F (4.4°C) to minimize microbial growth until the salt percentage is high enough to inhibit many of the spoilage and pathogenic microbes.

Pickle Curing

The principles of pickle curing are the same as with dry curing, except that a liquid brine is used where pickle curing employ a concentrated liquid brine (10-20% salt) in which the meat pieces are immersed for some time to allow for uniform salt distribution in the tissue. This brine can also contain the cure, spices and, possibly, a starter culture, or these ingredients are added after the salting stage.

Injection Curing

Although not common for whole muscle shelf-stable meat products, this process injects the curing ingredients directly into the meat muscle by random injection or artery injection directly into the blood vessels. This artery injection process is most often used on very large bone-in hams.

2.2.2 Drying process and storage

Traditionally these meat strips were hung over a fireplace of the kitchen where the strips dried due to the mild heat and smoke produced during cooking the meal. Sometimes air-and sun drying may be used. A typical sun-dried product requires a drying time of 3-10 days. In the rural areas, some people still used perunga (a bag prepared by weaving bamboo strips) as a packaging material. Perungo (along with the product) is often hung in the andiron for storage. However, perungo is less suitable than plastic packaging (Acharya, 2014).

2.3 Buffalo as a source of meat

Buffaloes (*Bubalus bubalis*) are large-ruminant animals that play an important role in the lives of millions of human beings as a source of milk, meat, draught power, transportation, and on-farm manure in several developing countries of Asia, including India. Buffalo meat is almost similar to beef in terms of composition, quality, and organoleptic characteristics and has an added advantage of less fat, cholesterol, and calories. Buffalo meat also has superior processing characteristics and is suitable for development of value-added meat products. Buffalo production makes an important contribution to economic development, rural livelihood, poverty alleviation, and

meets the fast-growing demand for animal protein requirement (Kiran and Naveena, 2014).

2.3.1 Zoological position and domestication of buffalo

Buffalo, like cattle, belong to even-toed (artyodactyl) hoofed (ungulate) animals of the suborder Ruminantia, subfamily Bovinae, and tribe Bovini. There are two major types of wild buffalo, *Bos arnee*, the Asiatic buffalo, and *Syncerus caffer*, the African buffalo. It is generally accepted that the domesticated water buffalo, *Bubalus bubalis*, originated from *Bos arnee*, the wild buffalo whose habitat was the northeastern region of India (Khan, 2002). Buffalo includes two subspecies: the river type (*Bubalus bubalis bubalis*; 2n = 50) and the swamp type (*Bubalus bubalis carabensis*; 2n = 48), which were domesticated approximately 5,000 years ago (CIRB, 2014).

The swamp buffaloes are found in South-east China, Burma, Assam, Laos, Vietnam, Thailand, Malaysia, Indonesia, Philippines, etc., and prefer marsh lands which wallow in mud. Other river buffaloes prefer clean water and generally found in India, Pakistan, Bangladesh, Nepal, Srilanka, etc. (Banerjee, 1992).

2.3.2 Production of buffalo meat in Nepal

Buffalo is the main source of milk and meat in Nepal. Also, it is useful as manure and draft power for soil fertility. It is the second largest group of livestock in terms of animal mass units in Nepal. But from the economic point of view, it is more valuable than cattle in Nepal (Anonymous, 2011/12). The Statistics of buffalo meat production is shown in Table 2.1

Table 2.1 Statistics of buffalo meat production

Year	No. of buffalo	Net meat Production/Mt
2012/13	52,41,873	1,75,132
2013/14	5,178,612	1,73,906
2014/15	51,67,737	1,74,012
2015/16	51,68,809	1.75,005

Source:(MOAD, 2015/16)

2.3.3 Chemical composition of buffalo meat

Moisture percentage of 74.04 to 77.75% has been reported for fresh buffalo meat. Buffalo meat showed a protein percentage of 17.33 to 23.3% (Naveena and Kiran, 2014).

Among all of the red meats, buffalo meat has been reported to have the lowest concentration of total lipids (1.37 g/100 g). Buffalo meat from 2-year-old male calves showed a fat percentage of 1.0 to 3.5. The relatively low-fat content in buffalo meat is attributed to poor marbling. Buffalo meat has less fat and saturated fat than beef. Low cholesterol content and energy value (6.8 Kcal/g dry matter) of buffalo meat was also reported by Anjaneyulu *et al* (1985). Palmitic, stearic, oleic, and linoleic acids were reported to be predominant fatty acids in the phospholipids of buffalo meat (Rao and Kowale, 1991). Water buffalo meat was also reported to contain a greater

concentration of conjugated linoleic acid (1.83 mg/g fatty acid methyl esters) compared with meat from zebu-type cattle (1.47 mg/g fatty acid methyl esters; (Naveena and Kiran, 2014). Chemical composition of buffalo meat is shown in Table 2.2.

Table 2.2 Chemical composition of buffalo meat (Nutrient composition and Meat quality characteristics)

Nutrient composition (value per 100g raw, lean meat)	
Water, g	76.30
Protein, g	20.39
Total lipids, g	1.37
Ash, g	0.98
Energy, kcal	173
Saturated fatty acids, g	0.460
Monounsaturated fatty acids, g	0.420
Polyunsaturated fatty acids, g	0.270
Cholesterol, g	46
Meat quality characteristics	
Ultimate pH	5.56
Water holding capacity, %	15.33
Collagen content, mg/g tissue	0.67
Collagen solubility, %	45.5

Source: (Naveena and Kiran, 2014)

2.4 Quality problems of dried meat

The quality and stability of traditional dried meats, as for other dried foods, depends on the extent of a_w depression and water removal which vary with the degree of roasting, smoking, sun-drying, salting and fermentation during processing (Labuza, 1971).

2.4.1 Microbial activity

Contamination of sterile animal muscle used as food is a direct consequence of slaughtering and dressing of animal carcasses. Wide ranges of microorganisms from different sources are introduced onto moist muscle surfaces that are rich in nutrients. It is argued that only a small portion (10%) of these microorganisms is capable of survival and proliferation during storage, distribution, and retail sales of meats. Additionally, an even a smaller portion will eventually predominate and cause spoilage (Cohen *et al.*, 2007).

A decrease in water activity increases the osmotic stress to microbial cells because the cell always tries to maintain a slightly lower internal osmolality (i.e., water activity). This causes an influx of water into the cell to maintain surface integrity. It is the disruption of this process by solutes that leads to cell damage and death (Halwai, 2004).

The minimal water activity is the limit below which microorganisms or group of microorganisms can no longer reproduce. There is a critical water activity below which no microorganisms can grow. For most of the foods this is in the 0.60-0.70 water activity range. Pathogenic bacteria cannot grow below a water activity of 0.85-0.86, whereas yeast and molds are more tolerant of a reduced a_w of 0.80, but usually no growth occurs below a_w of about 0.60. During storage special care has to be taken to prevent dried meat from becoming wet, resulting in rapid growth of bacteria and molds. The most troublesome group of microorganisms in dried foods is the molds, with the *Aspergillus glacus* group being the most notorious at low water activity values (FAO, 1990).

2.4.2 Enzymatic and Non-enzymic-browning

Enzymatic browning is the discoloration that results when monophonic compounds of plants or shellfish, in the presence of atmospheric oxygen and polyphenol oxidase (PPO), are hydroxylated to o-diphenols, and the latter are oxidized to o-quinones. The quinones condense and react non-enzymatically with other phenolic compounds, amino acids, etc., to produce dark brown, black or red pigments of indeterminate structure (Ozdemir, 2019).

Among the chemical reactions capable of causing deterioration of dried meats, a non-enzymic browning reaction of aldehyde-amino condensation (i.e. Millard type) is of prime importance. These reactions are strongly water dependent and reach a maximum at 0.6-0.7 a_w (Loncin *et al.*, 1968). These reactions cause a wide range of defects including darkening, development of scorched off-flavors and odors, toughening in texture, loss of rehydratability, and loss of nutritive value, particularly by lysine destruction. Meat develops a reddish brown discoloration and simultaneously the initial fresh flavor diminishes and is replaced, in the early stages of storage, by a stale flavor followed, in later storage, by unpalatable, bitter, burnt flavors (Sharp, 1957).

2.4.3 Organoleptic quality

Organoleptic (sensory) quality is a paramount factor determining meat quality and it plays a major role in meat marketability (Mancini and Hunt, 2005). Drying of meat with 5% salt and 1% agar prevents the development of case hardening and improves the texture of the dehydrated product. Storage of dehydrated meat products at ambient temperature significantly decreases color, flavor and overall acceptability of the product as compared to storage at chilling temperature. Different packaging methods, packaging materials and days of storage have also significant effects on the sensory qualities of the products (Mishra *et al.*, 2017).

Meat drying by hot-smoking has adverse effects on appearance, odor, texture and taste. Besides processing effects, these attributes of organoleptic quality decline due non-enzymic browning and lipid oxidation as well as lipid-protein and protein-

protein interactions induced by intermediate products of lipid oxidation on storage during drying (Shahidi, 1998).

Consumers associate every food with a characteristic color and any deviation from this leads to poorer acceptability. Besides, color change may be directly related to the nutritive value and can be correlated with general quality of dehydrated meat (Obanu and Ledward, 1975). The flavor of meat develops largely through the cooking process. However, fresh meat contains non-volatile constituents that are essential flavor precursors and contribute to the basic taste of cooked meat. Linolenate oxidation is the origin of l-penten-3-ol which has a penetrating, grassy etheralodor (Shahidi, 1998).The overall notion of tenderness to the palate includes the impression of wetness produced by the rapid release of fluids during the first few chews. Tenderness and juiciness are closely related; the more tender the meat, the more quickly juices are released by chewing and the juicier the meat appears to be. Dried meat products with reasonable ambient shelf-life are all too dry and hard (Seow *et al.*, 1988).

2.4.4 Environmental and personal hygiene

Environmental hygiene and its implementation will depend on the area where the slaughterhouse/meat plant is situated. The precautions to be taken will be different if the site is in a town or in the country. The main principles of environmental hygiene will consist of proper fencing (public, dogs, etc.), pest control (rodents, insects), liquid and solid waste disposal (Dave and Ghaly, 2011).

Personal hygiene will usually be the main element in the term “hygiene”; the reason being obvious. Bacteria causing diseases or spoilage may be carried and transmitted to surfaces and food by workers handling the food products (Bolton *et al.*, 2002). Careful and frequent hand-washing will do much to reduce contamination. Hands washing before work starts, after using the toilets, after touching dirty objects/materials and after smoking and eating must be done properly. The clothing of slaughterhouse workers must be clean. The purpose is not to protect the worker against contamination but to protect the meat/food against contamination (Dave and Ghaly, 2011)

2.5 Sources of contamination

Suggested primary sources and routes of carcass and/or fresh meat contamination include the knife used during exsanguination, the hide, the gastrointestinal tract, employees, the processing environment, and lymph nodes (Bacon, 2005). Bacterial contamination of carcasses may occur at virtually every stage of slaughtering and processing. Processing hygiene, however, aims at holding the initial bacterial numbers on a level as low as possible, since this affects shelf-life as the occurrence of pathogenic bacteria (Upmann *et al.*, 1878).

Besides environmental sources, product re- or cross-contamination may result directly from product-to-product contact, or indirectly following repeated contact with common surfaces (e.g., guide bars and employee hands)(Bacon, 2005).The exterior of the animal harbors large numbers of many kinds of microorganism from soil, water and manure, as well as its natural surface flora. Molds, mainly *Cladosporium, Sporotrichum, Mucor*, etc; yeasts, mostly asporogenous and bacteria, mostly *Micrococcus, Bacillus, Clostridium, Escherichia, Salmonella*, etc may reach the surface of meats and grow there (Subba, 2010).

On dried cured meat the pressing process at the salting room, spore concentration in the air of salting, brining and smoking rooms and the activity in the sorting room were identified as important factors facilitating the contamination of products by fungi, specifically molds. Visual observation revealed that most of the dry-cured meat products contaminated with toxigenic molds were those that have cracks on the surface due to improper pressing activity. Penicillia were largely dominant, and the contamination levels have been found to be associated to the environmental conditions of ripening rooms (Asefa *et al.*, 2010).

2.6 Microorganisms of public health concern

2.6.1 Aerobic mesophilic bacteria

The initial bacterial level of meat fluctuates depending on species and other factors, but usually is around 10^2-10^3cfu/cm^{2}or g. The initial bacterial load is extremely

important to meat shelf life. Holding other factors constant, it is known that lower initial bacteria counts are associated with the longer shelf life of meat (Yang, 2012).

The total plate count (TPC) expressed as organism/g on fresh meat or a meat product sets a limit to its shelf life. Meat will spoil with TPC at 10^6/g because of off odors. Slime and discoloration appear at 10^8/g (Anonymous, 2003).

Almost all food poisoning bacteria and most spoilage causing bacteria are mesophiles. A high TPC resulting from severe contamination during slaughter or processing will shorten the shelf life even in ideal conditions. It also indicates poor hygiene so that contamination with food poisoning bacteria is likely (Narasimha and Heinz, 1991).

2.6.2 Coliforms

Members of total coliforms and fecal coliforms groups are referred to as indicator organisms since a quantization of their presence are used to indicate the potential presence of pathogens in foods. It is believed by some investigators that the higher the numbers of coliforms, the greater the possibility of pathogenic organisms being present. This indicator/pathogenic relationship however, is scientifically debatable and by no means accepted unanimously by the scientific community (Yannick *et al.*, 2013).

The finding of Escherichia coli higher than 10^2cfu/g indicates dangerous contamination of food. Maximum limit for the coliforms according to the EU microbiological standards of cut meat and retail sale and further processing is 5×10^3/g (Anonymous, 2003).

2.6.3 *Salmonella*

Salmonella in red and white meat is a worldwide problem. Food borne *Salmonella* infection results from the ingestion of large numbers of the organism, which then multiply within the small intestine. Almost all members of the *Salmonella* genus are potentially pathogenic. *Salmonella* species are common inhabitants of the intestinal

tracts of many animals, especially cattle and during slaughter and dressing processes, they can easily contaminate food via fecal contamination (Robert, 1982).

Salmonella belongs to the Enterobacteriaceae family and is gram-negative, facultatively anaerobic, non-spore forming and rod shaped, and motile forms have peritrichous flagella. They can ferment glucose while producing acid and sometimes gas. There are more than 2,600 serovars of Salmonella. In the EU, *S. enteritidis* and *S. typhimurium* are the serovars most frequently associated with human illness. Human *S. enteritidis* cases are most commonly associated with the consumption of contaminated eggs and poultry meat, while *S. typhimurium* cases are mostly associated with the consumption of contaminated pig, poultry and bovine meat. *Salmonella* can cause gastroenteritis, enteric fever and sepsis. The infection dose can vary from 20 to 106 cells depending on the serotype, food, and host vulnerability (age and health state). Very low infection doses have been observed (< 100 cells) in water and fatty foods or foods with buffer capacities. The incubation period lies between 16-72 hours and the illness can take from 2 to 7 days. The pathogen can multiply in a wide range of temperature (5.2-46.2)ºC and pH (3.8-9.5). *Salmonella* growth is significantly affected by the a_w value that promote growth optimally at ca. 0.99 and inhibit growth below 0.94 in broth (Stollewerk, 2012).

2.6.4 Staphylococcus aureus

Meat is contaminated with *Staphylococcus aureus* by handling and by sneezing or coughing. Minute amounts of toxin will cause illness, which starts within 1-8 h of eating poisoned food. It does not produce off-odors or spoilage so it cannot be easily checked (Narasimha and Heinz, 1991).

Counts of 10^5/g or less wouldn't be expected to result in enterotoxin production. Minor and have shown that counts must be 107-108/g for detectable enterotoxin production. The greatest amount is produced at the optimum temperature for growth i.e. (35-37) ºC. 5×10^3cfu/g is the maximum limit for *S. aureus* on EU microbiological standards of cut meat and retail sale (Anonymous, 2003).

2.6.5 *Clostridium botulinum*

As clostridia are part of the normal intestinal flora of animals there is a possibility that *Cl. Botulinum* may be present. Human botulism is almost invariably caused by food which has been inadequately preserved, stored for some time and then consumed cold or without sufficient heating (Robert, 1982).

Botulism, the most serious form of food poisoning, results from consuming food containing toxin of *Clostridium botulinum* Types A, B, E and F are the main causes in man. The spores, apart from type E are heat resistant and can withstand cooking procedures apart from steam under pressure. The toxins however, can be easily destroyed by heating (Hersom and Hulland, 1980).

2.6.6 *Listeria monocytogenes*

Listeria can grow and survive in between wide pH (4.39 - 9.4) and temperature ranges (-0.4 to 45 ºC) and at relatively low aw levels (> 0.92) in broth, when other parameters are at optimum. The pathogen was furthermore described to be able to grow in the presence of nitrite and in up to 12 % NaCl (% w/w) (Stringer & Pin, 2005). *L. monocytogenes* is psychotropic and able to grow in food under various conditions: its growth limit in food with a neutral pH and with high content of nutrients stabilizes at 0 ºC. Depending on ambient conditions, the growth limiting aw value vary and has been described to lie at 0.93 in meat products (Stollewerk, 2012).

2.6.7 Yeasts and molds

They often manifest themselves in foods of low pH, low moisture, high salt or sugar content and can utilize organic acids, proteins and lipids. They spoil by causing off-color and flavor in meat products (Refai, 1979).

If insufficient oxygen is present, they use acid in the food and so increase the pH Current evidence suggests that mycotoxins do not present a major health hazard (Shapton and Shapton, 1991).

2.7 Pathogenic microorganisms in dry-cured meat products

Raw meat is highly perishable due to its pH near 7, its aw > 0.97 and its highly nutritive nature, representing optimum conditions for the growth of most bacteria. After some time of refrigerated storage in air, microflora of fresh meat largely consists of gram-negative, oxidase-positive rods, particularly psychrotrophic pseudomonads and psychrotrophic enterobacteriaceae, while gram-positive organisms including LAB usually occur only in small numbers (Gill, 1982; Lucke, 1998).

High levels of hygiene during meat processing are crucial as long as raw material contamination, for example through gastrointestinal tract, feet, hides, or skins of slaughtered animals, is still the primary source of contamination. The contaminating microbiota includes technologically important microorganisms but also spoilage and pathogenic bacteria (Garriga and Aymerich, 2007), of which *L. monocytogenes* and *Salmonella* are the most commonly linked to food-borne illness outbreaks derived from RTE food products (Moore, 2004).

Instruments and surfaces in processing plants and human handling (infected personnel or healthy carriers) can further easily contribute to cross-contaminations (Garriga and Aymerich, 2007). Salmonella contamination of meat was similarly reported to be often provoked by cross-contamination via ambient and contaminated equipment (Stollewerk, 2012).

Within Europe, in Mediterranean countries (mainly Spain, France and Italy) the traditional hams are more frequently prepared out of hams containing femur bone (bone-in hams), which are dry salted, non-smoked and submitted to an ageing period from six months to two years. Products elaborated in Central Europe is characterized by brine immersion and vacuum tumbling, subsequent drying or smoking and ageing for 3 to 12 months (Arnau *et al.*, 2007). While dry salting achieves a better osmotic dehydration, brine immersion provides less consumption of salt. Smoking involves the typical smoked color and flavor and has antibacterial and fungicide properties (Flores, 1997). Therefore, smoking inhibits growth of surface bacteria and molds, to which products are more susceptible in the cold damp climates found in Central Europe (Flores, 1997).

Part III

Materials and methods

3.1 Materials

3.1.1 Meat

Four kg of buffalo meat (2 kg lean meat and 2 kg meat containing fat) of 3.5 years old buffalo was collected in clean plastic bags after 2 hours of slaughtering and after collecting meat samples were transported to the Laboratory at Central Campus of Technology, Dharan in ice-box within half an hour; for making cured, non-cured and spiced *sukuti*.

3.1.2 Sukuti

250g of samples (smoked) were collected in clean plastic bags from each ten different areas identified as hot spots of *sukuti* markets of Dharan sub-metropolitan city. The analysis were carried out in triplicate.

3.2 Methods

Fresh buffalo meat both lean and fat meat was washed with water and trimming was done on a clean table with the help of clean and sharp knife, and clean chopping board. During trimming, connective tissues were separated from the meat. The washed and trimmed meat was sliced with a sharp knife so as to get meat strips of thickness about 1cm × 1cm. Length of the meat strip was approx. 10-15 cm. After slicing, the strips were divided into lean meat strips and fat containing meat strips. After the division, all the strips were subjected to further preparation. Recipes required for *sukuti* formulation, curing mixture formulation and spices mixtures are shown in Table 3.1, 3.2 and 3.3 respectively.

Table 3.1 Recipes required for *sukuti* formulation

Material	Amount (kg)
Meat	100
Curing mixtures	1.650
Spices mixtures	0.350
Vinegar (1%)	300 ml

Table 3.2 Recipes of curing mixture formulation

Materials	Amount (%)
Salt	97.45
Sodium nitrate	1.0
Sodium nitrite	0.5
Sugar	1.025
Ascorbic acid	0.025

Table 3.3 Recipes of spices mixtures

Material	Amount (g in 1 kg)
Red chilli powder	440
Black pepper	350
MSG	50
Cumin powder	100
Coriander powder	100
Cinnamon powder	50
Nutmeg	10

3.2.1 Experimental Procedure

Flow sheet for laboratory preparation of *sukuti* is shown in Fig. 3.1.

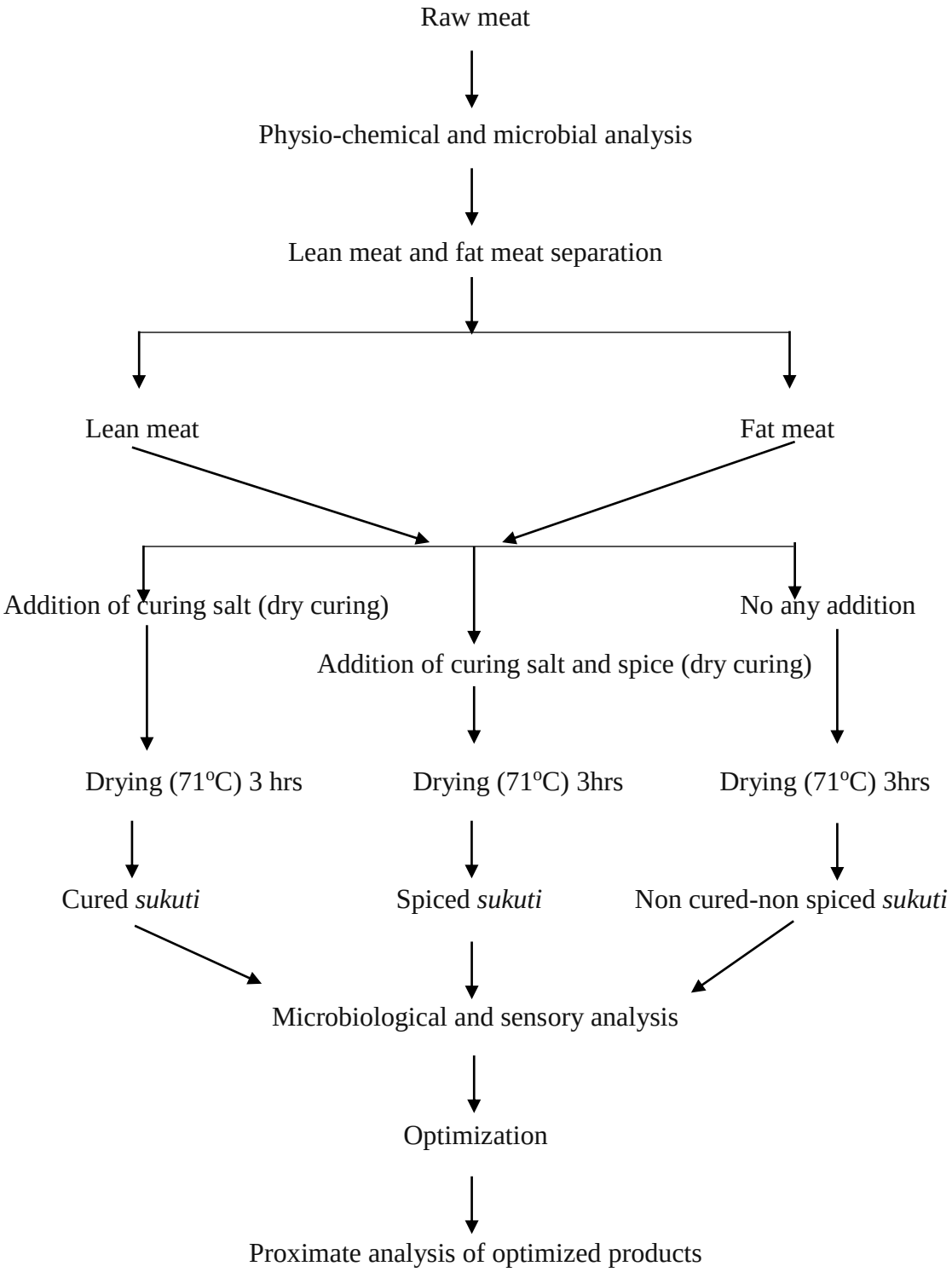

Fig 3.1 Flow sheet for laboratory preparation of *sukuti*

3.2.2 Analytical procedure

3.2.2.1 pH

It was determined using the method cited in Buyukyavuz (2014). Approximately 5 g of minced meat was homogenized with 50 ml distilled water. The pH value was determined by hand pH meter (HANNA instrument, made in Mauritius).

3.2.2.2 Moisture content

It was determined by hot-air oven method as per AOAC (2005). 10 g of minced meat was spread over the petri-dish and placed in hot air oven previously set at103 ±2^{o}C. The decrease in weight of the plate was noted every hour until the two consecutive weights differ only by ± 5 mg.

3.2.2.3 Crude fat

It was determined by Soxhlet extraction method cited in AOAC (2005). 5-8 g of sample was taken and packed in thimble. Then the fat was extracted using petroleum ether solvent. The temperature was maintained at 35-40^{o}C.

3.2.2.4 Crude protein

It was determined by Micro-Kjeldhal method as described in AOAC (2005). 2 g sample was digested with conc. H_2SO_4 and the nitrogen content estimated by Kjeldhal method was multiplied by conversion factor 6.25 to obtain protein content.

3.2.2.5 Ash content

The ash content was determined according to AOAC (2005). 10 g of sample was taken in crucible and the sample was charred over a low Bunsen flame to volatilize as much of organic matter. The crucible was then transferred to a muffle furnace set at 500 ^{o}C for 3-4 hrs.

3.2.2.6 Water holding capacity

Water holding capacity of meat was determined by filter press method according (Hamm, 1960). 0.5 g of meat sample was taken and pressed in between the flexi-plates with filter papers and held for 5 min. The ratio of meat film area and the total

area of meat sample was calculated. A ratio of > 0.5 is regarded as good and < 0.4 as poor.

3.2.3 Isolation of bacteria

Samples were serially diluted up to 10^{-7} dilution according to (KC and Rai, 2000). The bacterial isolates were isolated according to (Shah *et al.*, 2013).

Identification of microorganism

The microbiological analysis was carried out. For these, all the isolates were isolated and total plate count was done according to (AOAC, 1998) and (Shah *et al.*, 2013); from *sukuti* being manufactured in the different cottage industries in Dharan by spread plate technique. Coliform was isolated using EMB agar medium. *Salmonella* spp was isolated using *Salmonella-Shigella* Agar (SS agar) medium.

All the isolates will be enumerated according to (Shah *et al.*, 2013). The isolates were sub-cultured on to their selective media for the maintenance of pure cultures. After culturing, all the isolates was subjected to colony characterization as mentioned in (Ananthanarayan and Paniker, 2000; Fraser *et al.*, 1996), Gram staining and bio-chemical tests such as gelatin liquefaction test, IMViC test, catalase test, oxidase test and urease test was carried out according to (Shah *et al.*, 2013).

3.2.4 Sensory analysis

The prepared *sukuti* samples were cut into pieces of equal length about 2 cm and were evaluated in terms of appearance, flavor, texture and overall acceptability on nine-point hedonic scale as per Ranganna (2007). The panelist was given instruction to give '9' points to extremely liked one and '1' points to the extremely disliked sample. The coded samples were randomly presented to 10 panelists.

3.2.5 Statistical method

The analyses were carried out in triplicate. Data were statistically processed by Analysis of Variance (ANOVA) using Genstat programming (Genstat Discovery Edition 3, 2010). Means of the data were compared using LSD (Least Significant Difference) method at 5% level of significance.

Part IV

Result and discussion

Ten samples were collected from market and its microbial and sensory evaluation was done. Along with this sample six different samples of *sukuti* were prepared which were from lean meat and meat with fat content too. Through sensory evaluation market sample and sample prepared at lab were optimized. The best obtained products through sensory evaluation were subjected to physico-chemical analysis.

4.1 The physico-chemical composition of raw meat

The buffalo meat used for preparation of *sukuti* was analyzed for its physical and chemical composition. The chemical compositions of the meat used for sukuti preparation are presented in Table 4.1.

Table 4.1 Physico-chemical composition of raw meat

Parameters	Values
pH	5.66±0.15
Moisture (%)(wb)	76.4±0.4
Protein (%)(db)	19.67±0.15
Fat (%)(db)	2.93±0.41
Ash content (%)(db)	1.09±0.07
Water holding capacity	0.526±0.005
Cooking loss %	24±1.2

*The values in the Table 4.1 are the means of triplicates. Figure in the parentheses is the standard deviation.

The pH, moisture content, were similar to the result obtained by Kandeepan and Biswas (2007) which were 5.6, 76.9 %, 20.3 %, 1.4 %, & 1.2 % respectively. The

analysis showed that the meat used was of good quality in terms of water holding capacity i.e. 0.52. According to (Subba, 2010), a ratio of > 0.5 is regarded as good and < 0.4 as poor.

A large number of factors affect carcass traits and meat quality. These include: the animal itself, including breed or breed crosses, age, frame size, sex, age, and weight at slaughter, diet, management (production system, exercise, weather etc.), stress, pre-slaughter condition and slaughtering (Uriarte *et al.*, 2006).

4.2 Microbiological quality of raw meat

Samples of meat from market were analyzed for numeration of Total Plate Count (TPC), Total Coliforms (TC) and *Salmonella-Shigella*. The average values are shown in Table 4.2.

Table 4.2 Average microbiological values of buffalo raw meat.

Microbiological parameters	Values (cfu/g)
Total Plate Count (average)	$26x10^7$
Total Coliforms (average)	$31x10^3$
Salmonella-Shigella (average)	$19x10^3$

Taking the reference of microbial standards of Europe and United States (Appendix B) the total plate count was found to be higher than the inspected German quality meat standards referred for cutting and packaging plant which is less than $5.0x10^6$ cfu/g. It was also greater than the Oregon state microbiological standard for fresh meat i.e. $5x10^6$ cfu/g. The total coliform count of the analyzed sample was also found beyond the EU microbiological standard of cut meat for retail sale and further processing i.e. $5x10^3$ cfu/g.

In the previous study of (Munankami, 1998) total Coliform count was $1.3x10^5$ in the buffalo meat purchased from the local market of Dharan have been reported.

Salmonella was also reported to be present in the meat. (Bhaskar, 2006) reported that, all the samples (100%) of different places of Dharan were found to be *Shigella* positive. (Prasai, 2000) has reported the total Coliform count of buffalo meat of different places of Kathmandu in the range $1.6x10^5$ to $1.1x10^6$cfu/g.

Raw meat containing large numbers of bacteria does not present a health hazard but it should be viewed as having been produced unhygienically or poorly stored or contaminated during processing and it poses particular risk if it is eaten raw (Brown and Parker, 1982).

4.3 Microbiological quality of *sukuti* from market

Ten different samples of sukuti were collected from different places of Dharan were analyzed for numeration of Total Plate Count (TPC), Total Coliforms (TC) and *Salmonella-Shigella*. Average TPC in *sukuti* was found to be $17.1x10^5$ cfu/g. Similarly, Average Total Coliform was found to be $1.89x10^2$ cfu/g and average total Salmonella-Shigella were both found to be positive with total count $23.4x10^2$ cfu/g. as shown in Table 4.3.

This result supports the finding that microbial growth is inhibited at a low a_w reported by (Gould and Christian, 1998). Similarly, (Leistner, 1987). reported that many food spoilage bacteria are unable to multiply at an a_w value below 0.95 and that growth of most microorganisms is retarded or inhibited below a_w at 0.90 because of which TPC was found lower than raw meat in the present study. According to the Russian sanitary and epidemiological regulation the TPC in dried meat with permeable limit was given as $1x10^4$ cfu/g where present study exceeds the microbial criteria. The total plate counts of all jerky samples increased with storage (Nam *et al.*, 2016) which was the cause of TPC presence on *sukuti*.

The average total coliform counts ($1.89x10^2$cfu/g) which did not exceed the permissible value of (2.7 log10 cfu/g) as reported on (Lawan *et al.*, 2011). Maximum limit for the coliforms according to the EU Microbiological standards of cut meat and retail sale and further processing is 5×10^3 cfu/g (Anonymous, 2003) as *sukuti* was found to have lower coliform counts. Presence of high coliform counts from the

samples indicates poor hygienic practice, possible fecal contamination and poor handling of meat at meat retail points and possible potential presence of highly pathogenic microorganism such as *Salmonella,* and *E. coli* and this is of public health concern (Lawan *et al.*, 2011).

For growth, Salmonella requires a water activity of at least 0.93. However, it can survive and persist for months at much lower water activities, as low as a_w=0.18 as reported by (Kotzekidou, 1998). When Salmonella adapts to low-moisture environments, it becomes more resistant to heat, making it a serious problem in dried foods such as beef jerky and spices (Buege *et al.*, 2006). The number of Salmonella cells needed to cause disease may be very low in some cases (<10 cells), depending on the serotype and the type of food, with high-fat foods sometimes resulting in lower infectious doses (Li *et al.*, 2013). When compared at similar levels of a_w, growth of Salmonella in bacteriological media appears to depend on the type of solute used to control a_w. Thus, the natural chemical composition of the dry product would also affect the degree of Salmonella survival in environments of low a_w (Marshall *et al.*, 1971). Microbiological analysis of *sukuti* from different place of Dharan is shown in Table 4.3.

Table 4.3 Microbiological analysis of *sukuti* from different place of Dharan

Samples	TPC cfu/g	TC cfu/g	Total *Salmonella-Shigella* cfu/g
A	$22x10^5$	$8x10^2$	$6x10^2$
B	$31x10^4$	$11x10^1$	$8x10^2$
C	$41x10^5$	$14x10^1$	$10x10^3$
D	$39x10^5$	$15x10^1$	$13x10^2$
E	$22x10^4$	$7x10^1$	$4x10^2$
F	$24x10^4$	$8x10^1$	$2x10^2$
G	$19x10^5$	$2x10^2$	$5x10^2$
H	$37x10^4$	$12x10^1$	$2x10^2$
I	$29x10^4$	$11x10^1$	$4x10^2$
J	$36x10^5$	$11x10^1$	$9x10^3$
Average	$17.1x10^5$	$18.9x10^1$	$23.4x10^2$

[A, B, C, D, E, F, G, H, I and J are the *sukuti* samples collected from different locations of Dharan]

4.4 Sensory evaluation of *sukuti* from market

Sensory evaluation was carried out for color, texture, taste, and overall acceptability by semi trained panelists using 9-point hedonic scale. The statistical analysis (two-way ANOVA (no blocking) was done. ANOVA was carried out using LSD at 5% level of significance. There was significant difference for most of the sensory attributes viz., color, texture, taste, and overall acceptability at $P < 0.05$. The result of the sensory evaluation is shown in Appendix C. Ten samples of *sukuti* were collected

from different place of Dharan. The samples were subjected to sensory evaluation. The mean of sensory score given to each quality attribute of each sample was calculated and a bar diagram was plotted.

4.4.1 Texture

Mean sensory score for texture of market sample is shown in Fig. 4.1.

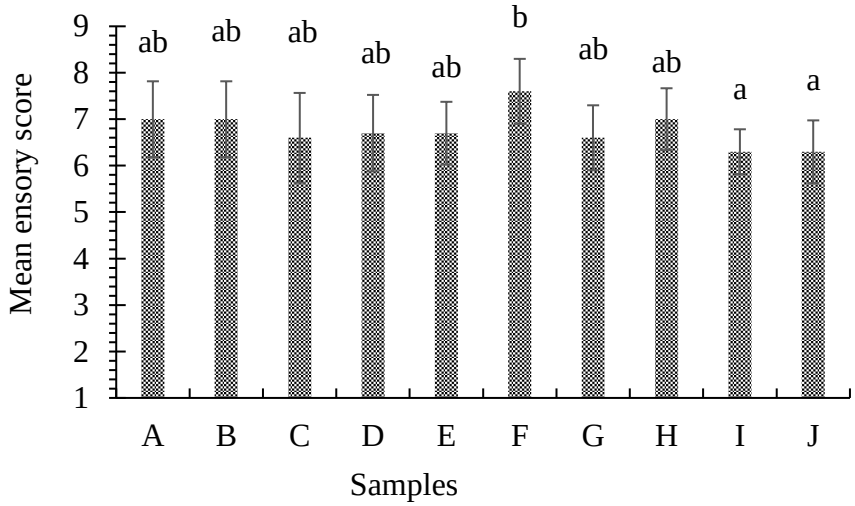

Fig: 4.1 Mean sensory score for texture of market sample

[A, B, C, D, E, F, G, H, I and J are the *sukuti* samples collected from different locations of Dharan]

The values in the Fig. 4.1 are the mean sensory scores. The similar alphabet above the bar diagram indicate not significantly different ($p > 0.05$).

The mean sensory score for texture ranged from 6.3 ± 0.674 to 7.6 ± 0.699 with J as the lowest and F as the highest. The statistical analysis showed that the sample J and F were significantly different with each other at 5 % level of significance. Similarly, the samples A, B, C, D, E, G and H; I and J were not significantly different with each other in terms of texture.(Subba, 2010) also reported that there is muscle to muscle variation in tenderness. In general, the muscles which are more exercised (high movement) are tough. Tenderness varies even from one anatomical cut to another within the same carcass.

4.4.2 Appearance

Mean sensory score for color of different sample ranged from 5.9 ± 0.87 to 7.4 ± 0.0.51. LSD showed that sample B, C, D, E and G; A,F, H and I were not significantly different to one another while other sample A, D, J were significantly different from each other at 5% level of significance. Sample J had lowest mean score whereas sample F and H had highest mean score. Mean sensory score for color of market sample is shown in Fig. 4.2.

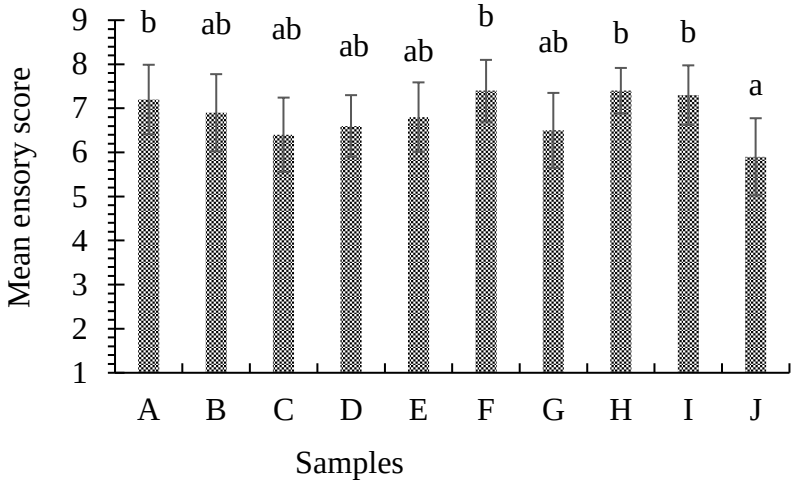

Fig.4.2 Mean sensory score for Appearance of market sample

[A, B, C, D, E, F, G, H, I and J are the *sukuti* samples collected from different locations of Dharan]

The values in the figure are the mean sensory score for color. Values on top of the bar bearing similar alphabet are not significantly different at 5 % level of significance. Vertical error bar represents standard deviation of scores. According to Mancini and Hunt (2005) the color of red meat can change because of exposure to various ingredients such as vinegar or salt. They also mentioned that the color of red meat can change over time as the pigments bind oxygen then ultimately become oxidized to brown or grey.

4.4.3 Taste

Mean sensory score for taste ranged from 4.9 ± 0.51 to 7 ± 0.51. LSD showed that E, F and J, was significantly different at 5% level of significance. Sample F had highest mean score and sample J had lowest. Mean sensory score for taste of market sample is shown in Fig. 4.3.

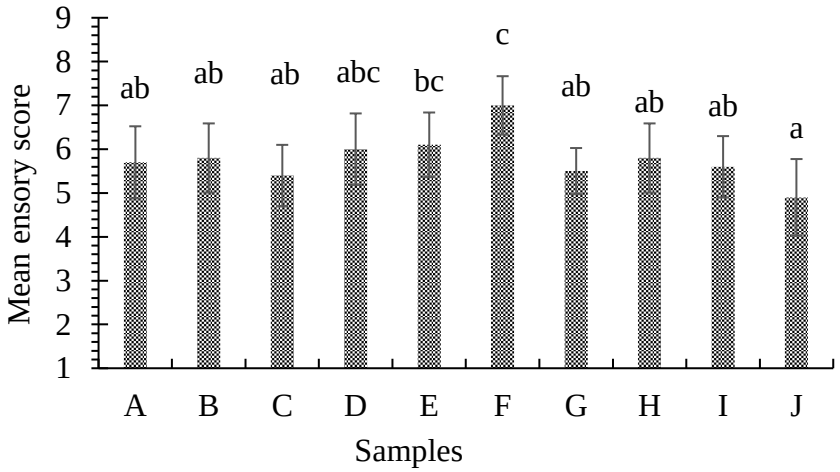

Fig.4.3 Mean sensory score for taste of market sample

[A, B, C, D, E, F, G, H, I and J are the *sukuti* samples collected from different locations of Dharan]

The values in the figure are the mean sensory score for taste. Values on the top of bar bearing similar alphabet are not significantly different at 5% level of significance. Vertical error bar represents standard deviation of scores.

4.4.4 Flavor

The mean sensory score for flavor of the samples A, B, C, D, E, F, G, H, I and J were found to be 6.9, 6.8, 6.4, 7.0, 6.5, 6.3, 6.5 & 6.1 respectively. LSD showed that C, E, G, H and I; A, B, D and F were not significantly different from one another. Through the analysis the sample F got highest mean sensory score and found to be best in terms of flavor. Mean sensory score for flavor of market sample is shown in Fig. 4.4.

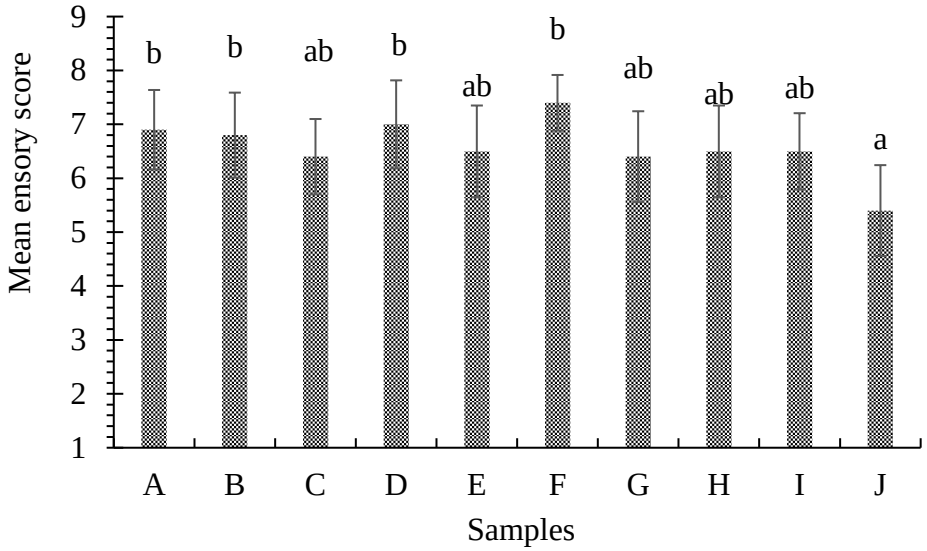

Fig.4.4 Mean sensory score for flavor of market sample

[A, B, C, D, E, F, G, H, I and J are the *sukuti* samples collected from different locations of Dharan]

4.4.5 Overall acceptability

Mean sensory score for overall acceptability sample ranged from 5.5 ± to 7.2. LSD showed significant difference between samples C, F and J but no significant difference between samples A, B, D, H and I; C and G. Sample F was found to be superior considering overall acceptability. Mean sensory score for overall acceptability of market sample is shown in Fig. 4.5.

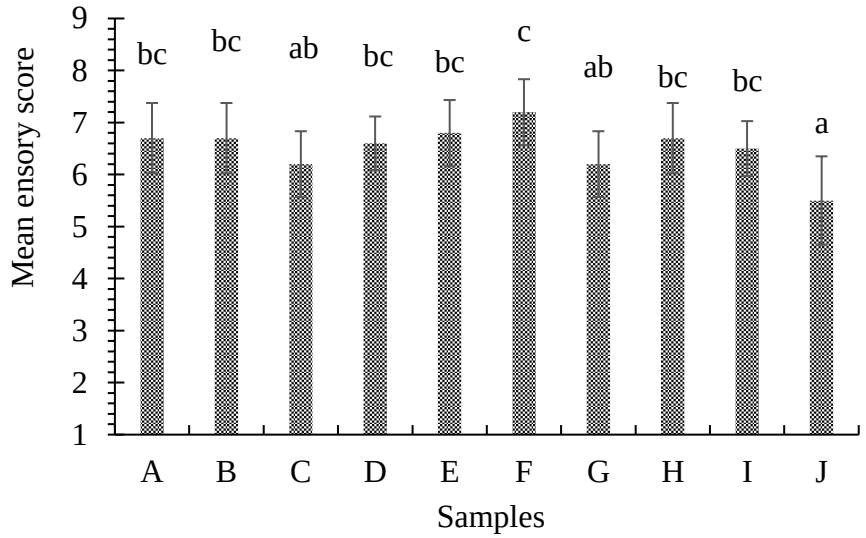

Fig.4.5 Mean sensory score for overall acceptability of market sample

[A, B, C, D, E, F, G, H, I and J are the *sukuti* samples collected from different locations of Dharan]

4.5 Microbiological quality of *sukuti* prepared from lean meat

Three samples of *sukuti* were prepared from buffalo meat available at Dharan market was analyzed for numeration of Total Plate Count (TPC), Total Coliforms (TC) and *Salmonella-Shigella*. Average TPC in *sukuti* was found to be $11.1x10^4$ cfu/g. Similarly, Average Total coliform was found to be $9x10^1$ cfu/gand *Salmonella-Shigella* were both found to be positive with total count $7.3x10^2$ cfu/g as shown Table 4.4.

In a study, it was reported that many food spoilage bacteria are unable to multiply at an a_w value below 0.95 and that growth of most microorganisms is retarded or inhibited below a_w at 0.90 (Leistner, 1987) because of which TPC was found lower than raw meat in the present study. According to the Russian sanitary and epidemiological regulation the TPC in dried meat with permeable limit was given as $1x10^4$cfu/g where present study exceeds the microbial criteria.

The effects of single as well as combined treatments significantly reduced the bacterial population as compared to control sample. The treatments of either curing followed with smoking or curing and antioxidant followed with smoking were found to be significantly different (Ahmad *et al.*, 2005).

Maximum limit for the Coliforms according to the EU Microbiological standards of cut meat and retail sale and further processing is 5×10^3cfu/g (Anonymous, 2003) as *sukuti* was found to have lower Coliform counts. For growth, *Salmonella* requires a water activity of at least 0.93. However, it can survive and persist for months at much lower water activities, as low as a_w=0.18 as reported by (Kotzekidou, 1998). In a study, it was found that the natural chemical composition of the dry product would also affect the degree of *Salmonella* survival in environments of low a_w as similar result was observed in present study where cured sample had lower total *Salmonella-Shigella* count than non-cured (Juven *et al.*, 1984).

Harrison *et al.* (2018) analyzed ground-beef jerky made with a commercial spice mixture with and without a curing mix containing salt and sodium nitrite. The authors found that ground-beef jerky made with a curing mix had greater destruction of bacteria than jerky made without it (Nummer *et al.*, 2004). The pH value of all treatments was significantly lower than that of the control sample at 24 h of curing time. (Leistner, 1987) reported that low pH can inhibit or delay the spoilage of various dried meat products by mold and microorganism growth which was the reason for lower TPC, TC and total *Salmonella-Shigella* count on cured *sukuti* than non-cured *sukuti* prepared at laboratory.

A study have recently examined antibotulinal activities of 90 fresh herbs and spices, and found that nutmeg (0.05%), sage (0.02%), and clove (0.05) extracts

possess antibotulinal activities in a model meat product without compromising its organoleptic properties (Cui *et al.*, 2010). In a study, it was found that several herbs, such as cumin, bay, black pepper, lemon, parsley or nutmeg had antimicrobial properties (Diez *et al.*, 2016) which was observed in the present study as result to lower TPC, TC and Total *Salmonella-Shigella* count on spiced *sukuti* than non-cured-non spiced *sukuti* prepared at laboratory. Microbiological analysis of *sukuti* prepared from lean meat is shown in Table 4.4.

Table 4.4 Microbiological analysis of *sukuti* prepared from lean meat

Samples	TPC cfu/g	TC cfu/g	Total *Salmonella-Shigella* cfu/g
LC	$11x10^3$	$7x10^1$	$8x10^1$
LS	$19x10^3$	$9x10^1$	$9x10^2$
LNC	$31x10^4$	$11x10^1$	$12x10^2$
Average	$11.1x10^4$	$9x10^1$	$7.3x10^2$

[LC, LS and LNC are lean cured, lean spiced and lean not cured respectively.]

4.6 Sensory evaluation of *sukuti* prepared from lean meat

The statistical analysis ANOVA (no blocking) was done. ANOVA was carried out using LSD at 5 % level of significance. There was significant difference for most of the sensory attributes viz., color, texture, taste, and overall acceptability at $P < 0.05$. Three samples of *sukuti* were collected from prepared from lean meat. The samples were subjected to sensory evaluation. The mean of sensory score given to each quality attribute of each sample was calculated and a bar diagram was plotted. Mean sensory score for *sukuti* prepared from lean meat is shown in Fig. 4.6.

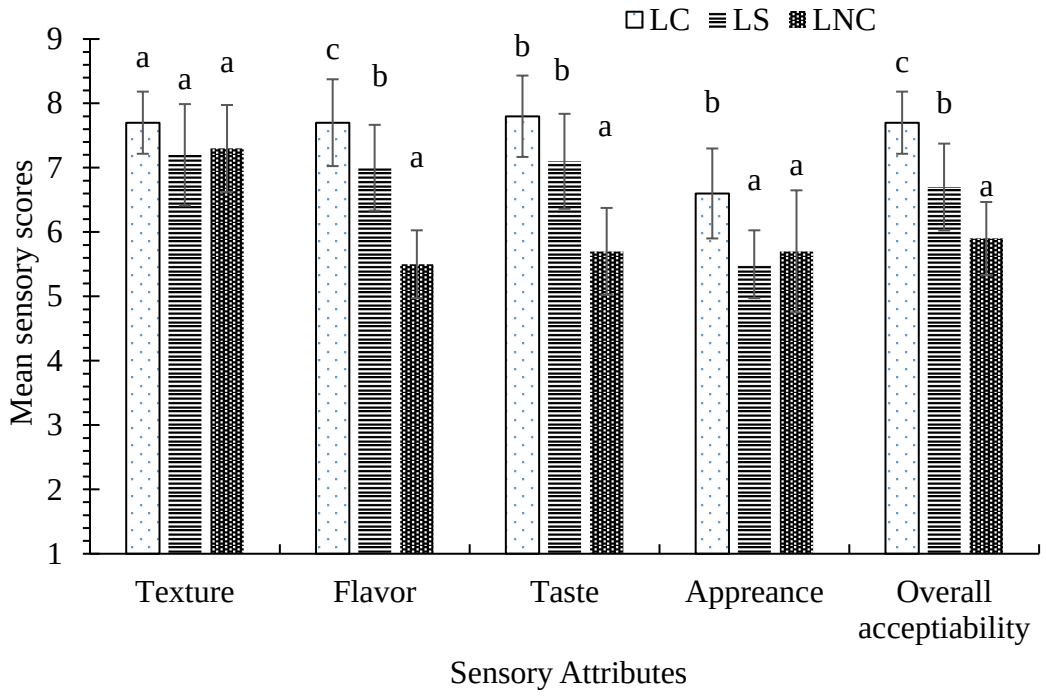

Fig.4.6 Mean sensory score for *sukuti* prepared from lean meat

Values on the top of bar bearing similar alphabet are not significantly different at 5 % level of significance. Vertical error bar represents standard deviation of scores.

4.6.1 Texture

The mean sensory score for texture of the samples LC, LS and LNC were found to be 7.7, 7.2 and 7.3 respectively. LSD showed that there were not significantly different

among the samples at 5 % level of significance. According to (Ahmad *et al.*, 2005), the treatment of curing and smoking significantly improved the texture of controlled meat which was also observed in the present study.

4.6.2 Appearance

The mean sensory score for appearance of the samples LC, LS and LNC were found to be 6.6, 5.5 and 5.7 respectively. LSD showed that the sample LC was significantly different from other samples at 5 % level of significance in term of appearance. But the samples LS and LNC with each other were not significantly different at 5 % level of significance. According to (Ahmad *et al.*, 2005), Curing significantly improved the color of meat sample to a bright red. The sample LC in this case got the highest mean sensory score and found to be best sample.

4.6.3 Taste

Mean sensory score for taste ranged from 5.7 to 7.8. LSD showed that LNC was significant different from other samples at 5 % level of significance. Sample LC had highest mean score and sample LNC had lowest. The values in the Fig. 4.6 shows the mean sensory score for taste.

4.6.4 Flavor

Mean sensory score for flavor varies from 5.5 to 7.7. The analysis showed that the sample LC, LS and LNC were significantly different with each other samples at 5 % level of significance. The sample LC in this case got the highest mean score. Similar finding was reported as the flavor and overall acceptability of cured pork loin treated with refined salt scored the highest score (Choi *et al.*, 2016).

4.6.5 Overall acceptability

The mean sensory score for overall acceptability of the samples LC, LS and LNC were found to be 7.7, 6.7 and 5.9 respectively. LSD showed that the samples LC, LS and LNC were significantly different from each other at 5 % level of significance. The mean sensory scores for color, flavor, taste & texture of the sample LC were found to be highest; therefore, sample LC got the highest mean sensory score in terms

of overall acceptability. Also, the statistical analysis showed the higher degree of acceptability for sample LC.

The improvement of color, taste, flavor, tenderness, protein extractability and water holding capacity was attributed to the curing agents (Babdji *et al.*, 1982; Ockerman *et al.*, 1978). (Carballo *et al.*, 1995) reported that an increase in protein content, and hence in extracted protein, generally causes an increase in the number of locations in the polypeptide chains capable of interacting.

4.7 Microbiological quality of *sukuti* prepared from fat meat

Three samples of *sukuti* were prepared from fat containing buffalo meat available at Dharan market were analyzed for numeration of Total Plate Count (TPC), Total Coliforms (TC) and *Salmonella-Shigella*. Average TPC in *sukuti* was found to be $27x10^4$ cfu/g. Similarly, Average Total coliform was found to be $11.7x10^1$ cfu/g and Salmonella-Shigella were both found to be positive with total count $10x10^2$ cfu/g as shown in Table 4.5

This result supports the finding that microbial growth is inhibited at a low a_w reported by (Gould and Christian, 1998). The total plate counts of all jerky samples increased with storage (Nam *et al.*, 2016) which was the cause of TPC presence on *sukuti*. The number of *Salmonella* needed to cause disease may be very low in some cases (<10 cells), depending on the serotype and the type of food, with high-fat foods sometimes resulting in lower infectious doses (Li *et al.*, 2013). In a study, it was found that the natural chemical composition of the dry product would also affect the degree of *Salmonella* survival in environments of low a_w (Juven *et al.*, 1984).

In a study, the authors found that ground-beef jerky made with a curing mix had greater destruction of bacteria than jerky made without it (Nummer *et al.*, 2004). In several studies in the past decade confirm that the growth of both gram-positive and gram-negative food borne bacteria, yeast and mold can be inhibited by garlic, onion, cinnamon, cloves, thyme, sage, and other spices (Farahnaah *et al.*, 2016). In fact, many compounds isolated from spices have shown antimicrobial activity against

some of the most common microorganisms that affect the food quality and shelf life (Tajkarimi *et al.,* 2010) because of which cured and spiced samples were found to have lower microbial count than non-cured-non spiced samples. Microbiological analysis of *sukuti* prepared from fat meat is shown in Table 4.5.

Table 4.5 Microbiological analysis of *sukuti* prepared from fat meat

Samples	TPC cfu/g	TC cfu/g	Total *Salmonella-Shigella* cfu/g
FC	$21x10^4$	$9x10^1$	$7x10^2$
FS	$29x10^4$	$12x10^1$	$11x10^2$
FNC	$31x10^4$	$14x10^1$	$12x10^2$
Average	$27x10^4$	$11.7x10^1$	$10x10^2$

[FC, FS and FNC are fat cured, fat spiced and fat not cured respectively]

4.8 Sensory evaluation of *sukuti* prepared from fat meat

Three samples of *sukuti* were prepared from the meat containing higher fat percentage side. The samples were subjected to sensory evaluation. The mean of sensory score given to each quality attribute of each sample was calculated and a bar diagram was plotted. Mean sensory score for *sukuti* prepared from fat meat is shown in Fig. 4.7.

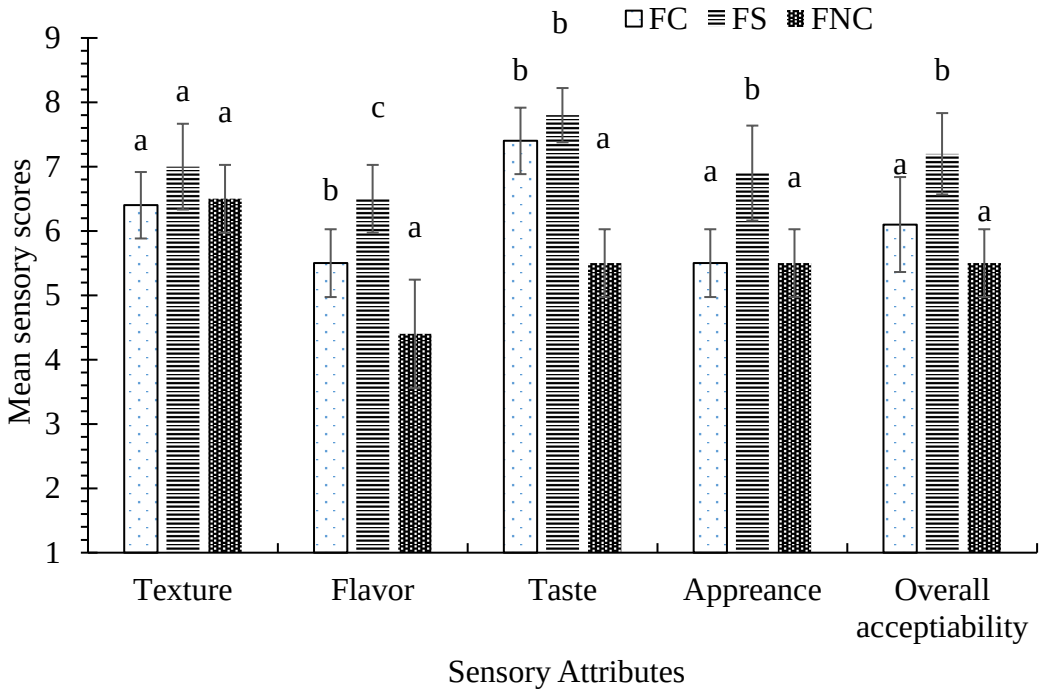

Fig.4.7 Mean sensory score for *sukuti* prepared from fat meat

Values on the top of bar bearing similar alphabet are not significantly different at 5 % level of significance. Vertical error bar represents standard deviation of scores.

4.8.1 Texture

The mean sensory score for texture of the samples FC, FS and FNC were found to be 7, 7 and 6.5 respectively. LSD showed that there were not significantly different among the samples at 5 % level of significance.

4.8.2 Appearance

The mean sensory score for appearance of the samples FC, FS and FNC were found to be 5.5, 6.6 and 6.7 respectively. LSD showed that the sample FNC was significantly different from other samples at 5 % level of significance in term of appearance. But the samples FS and FC were not significantly different to each other at 5 % level of significance. The sample FC in this case got the highest mean sensory score and found to be best sample.

4.8.3 Taste

Mean sensory score for taste ranged from 5.5 to 7.8. LSD showed that FS and FNC were significantly different at 5% level of significance where FC and FS was not significantly different with each other at 5% level of significance. Sample FS had highest mean score and sample FNC had lowest. The values in the Fig. 4.7 show the mean sensory score for taste.

4.8.4 Flavor

Mean sensory score for flavor varies from 4.4 to 6.5. The analysis showed that all the samples were significantly different with each other. The sample FS in this case got the highest mean score.

4.8.5 Overall acceptability

The mean sensory score for overall acceptability of the samples FC, FS and FNC were found to be 6.7, 7.2 and 5.5 respectively. LSD showed that the samples FS was significantly different with other samples at 5 % level of significance. The mean sensory scores for flavor, taste & texture of the sample FS were found to be highest; therefore, sample FS got the highest mean sensory score in terms of overall acceptability. Also, the statistical analysis showed the higher degree of acceptability for sample FS.

In a study, it was reported that tenderness and juiciness scored better in the control than other samples, probably because of the high fat content in beef jerky (Nam *et al.,* 2016). Similarly the amount and type of fat in meat influence two major components of meat quality, tenderness and flavor was reported by (Wood *et al.,* 2008). In general, the intramuscular fat (IMF) content was found to be correlated with meat tenderness (Wood *et al.,* 2003) this may be the reason due to which overall acceptability of fat meat s*ukuti* were found higher.

4.9 Optimization of *sukuti*

From microbial analysis sample E and F from market samples were found to have high degree of acceptability whereas sensory analysis found sample F with higher

degree of acceptability. In a study, it was found that raw meat containing large numbers of bacteria does not present a health hazard but it should be viewed as having been produced unhygienically or poorly stored or contaminated during processing and it poses particular risk if it is eaten raw (Brown and Parker, 1982) which may also be the reason of higher microbial count on other market samples.

Similarly, sample prepared on laboratory lean cured *sukuti* were found best on both microbial and sensory analysis where fat cured meat was found best on microbial quality and fat spiced sample was with higher degree of acceptability.

The number of *Salmonella* needed to cause disease may be very low in some cases (<10 cells), depending on the serotype and the type of food, with high-fat foods sometimes resulting in lower infectious doses (Li *et al.*, 2013). According to these reports, spices possess a very wide spectrum of activity against Gram-positive and Gram-negative bacteria, yeasts and molds (Tajkarimi *et al.*, 2010).

4.10 Proximate analysis of optimized products

The proximate analysis of the optimized products i.e., sample F (sample from market), LC (lean meat cured *sukuti*) and FS (fat meat spiced *sukuti*) is presented in Table 4.6.

Table 4.6 Proximate analysis of the optimized products and control samples

Samples	Parameter*			
	Moisture (%)(wb)	Protein (%)(db)	Fat (%)(db)	Ash (%)(db)
F	$8.333^{a} \pm 0.07$	$82.57^{b} \pm 0.27$	$3.24^{a} \pm 0.055$	$4.51^{a} \pm 0.015$
LC	$8.753^{a} \pm 0.116$	$82.35^{b} \pm 0.13$	$3.33^{a} \pm 0.025$	$4.55^{a} \pm 0.01$
FS	$9.403^{b} \pm 0.525$	$79.39^{a} \pm 0.20$	$6.77^{b} \pm 0.215$	$4.76^{b} \pm 0.055$

*The values in the Table 4.6 are the means of triplicates. Figure in the parentheses is the standard deviation.

Part V

Conclusions and recommendations

5.1 Conclusion

Conclusively, microbial qualities of different types of *sukuti* both brought from market and prepared in the laboratory were evaluated along with their sensory evaluation. Among them, *sukuti* which were bought from the market were randomly collected and samples prepared in the laboratory were from lean and fat meat of buffalo with different variations during their processing.

Following conclusions can be drawn from the research work:

a. The microbial load of raw buffalo meat was found to be higher than that of EU standards.
b. The TPC, TC and Total Salmonella-Shigella on market *sukuti* was found higher than permeable limit.
c. The average TPC of both *sukuti* sample prepared from lean and fat meat were found to be just above the standard whereas TC was found to be lower than permeable limit
d. On the sensory evaluation of different samples from market, Sample F showed higher degree of acceptability whereas on laboratory prepared samples, lean cured and fat spiced showed higher degree of acceptability.
e. All the findings of the study suggest about the unhygienic and unscientific method of handling, lack of sanitation and knowledge of microorganisms resulting higher number of contaminations.

5.2 Recommendations

The *sukuti* samples of Dharan were found to contain high counts of microorganisms in comparison to laboratory prepared *sukuti*. The hygiene quality was unsatisfactory. The findings imply that people of these areas need to be careful about the quality. To improve the bacteriological quality of the raw meat some well-known, simple techniques that can be recommended are:

a. For research, I would recommend for further study on antibiotic resistance of microorganism detected on dried meat.

b. For producers, I would recommend to make use of multi-hurdle treatments on reduction of pathogenic microorganism in *sukuti*.

Part VI

Summary

Sukuti was optimized with microbial analysis and sensory evaluation of various samples of smoked from different place of Dharan and different samples prepared at laboratory by taking cured, non-cured and spiced *sukuti* of both lean and fat meat. *Sukuti* to evaluate its quality by AOAC (2005) and sensory characteristics were evaluated on nine-point hedonic scale as per Ranganna (2007).

TPC in buffalo meat was found to be $26x10^7$cfu/g. Similarly, Total coliform and Total *Salmonella-Shigella* were found to be $31x10^3$ and $19x10^3$ respectively whereas average TPC, TC and total *Salmonella-Shigella* in market *sukuti* were found to be $17.1x10^5$ cfu/g, $1.89x\ 10^2$ cfu/g and $23.4x10^2$ cfu/g respectively. LSD showed significant difference between samples C, F and J but no significant difference between samples A, B, D, H and I; C and G. Sample F was found to be superior considering overall acceptability.

Average TPC, TC and total *Salmonella-Shigella* counts were found to be $11.1x10^4$ cfu/g, $9x10^1$ cfu/g and $7.3x10^2$ cfu/g in lean meat samples. LSD showed that the samples LC, LS and LNC were significantly different from each other at 5 % level of significance. Sample LC got the highest mean sensory score in terms of overall acceptability.

Similarly, average TPC, TC and total *Salmonella-Shigella* counts in fat meat *sukuti* was found to be $27x10^4$ cfu/g, $11.7x10^1$ cfu/g and $10x10^2$ cfu/g. LSD showed that the samples FS was significantly different with other samples at 5 % level of significance. Sample FS got the highest mean sensory score in terms of overall acceptability.

Overall, it was found that raw meat containing large numbers of bacteria which were reduced during processing. The present study concludes that market *sukuti* contain higher microbial load than those prepared in laboratory which may be due to the reason reported by (Brown and Parker, 1982) as meat product has been produced unhygienically or poorly stored or contaminated during processing which it poses particular risk. From which it can be concluded that processing of *sukuti* requires hygienic environment and storage.

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Appendices

Appendix A

Specimen card for sensory evaluation

Hedonic rating test

Name of Panelist……

Date…….……

Product: Dried meat (*Sukuti*)

Please taste the following samples and check how much you prefer for each of the samples. Give the points for your degree of preferences for each parameter for each sample.

Parameters	Sample A	Sample B	Sample C	Sample D
Appearance				
Color				
Flavor				
Tenderness				
Overall acceptability				

Give points as follows:

Like extremely – 9 Like slightly – 6 Dislike moderately – 3

Like very much – 8 Neither like nor dislike – 5 Dislike very much – 2

Like moderately – 7 Dislike slightly - 4 Dislike extremely – 1

Comments……

…….……

……...

…….

Signature

Appendix B

Microbiological Standards

1. Guidelines for Total Plate Count in Meat and Meat Products

Product TPC Max Fresh Meat

TPC $_{max}$ Cut and Packaging meat 5xlog 6/sq.cm or g

TPC max Separated Meat 5xlog 6/ g

2. Inspected German Quality

Meat ≤ log 4/ g or sq.cm. in freshly slaughtered meat ≤ 5xlog6/g or sq. cm. in cutting and packaging plant

Danish Quality Assurance Warranty ≤ log 4/ sq. cm. in freshly slaughtered meat

3. EU microbiological standards of cut meat for retail sale and further processing

Bacteria	Counts
Coliform bacteria	5 x log 3/g 5 x log 2/g n=5, c=2
Staph. aureus	5 x log 3/g 5 x log 2/g n=5, c=2
Salmonella	not detectable in 1g n=5, c=0

4. Oregon State Microbiological Standard

Total Plate Count:	max. 5 x 106/g
E. Coli.:	max. 50/g

(Note: M = maximum limit; beyond which meat is not acceptable, and applies the following: M = 10m while counting in solid medium M = 30m while counting in liquid medium m = limit, at and under which meat is acceptable n = number of replicates c = number of replicates, the count of which lies between m and M.)

Appendix C

Table C.1 ANOVA for moisture content analysis of optimized products

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	1.74380	0.87190	8.85	0.016
Residual	6	0.59100	0.09850		
Total	8	2.33480			

Table C.2 ANOVA for protein content analysis of optimized products

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	18.98607	9.49303	211.79	<.001
Residual	6	0.26893	0.04482		
Total	8	19.25500			

Table C.3 ANOVA for fat content analysis of optimized products

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	24.37327	12.18663	731.69	<.001
Residual	6	0.09993	0.01666		
Total	8	24.47320			

Table C.4 ANOVA for ash content analysis of optimized products

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Samples	2	0.104422	0.052211	45.62	<.001
Residual	6	0.006867	0.001144		
Total	8	0.111289			

Appendix D

Table D.1 Analysis of Variance for texture in optimization of market sample

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	9	13.5600	1.5067	2.59	0.011
Panelist	9	2.5600	0.2844	0.49	0.877
Residual	81	47.0400	0.5807		
Total	99	63.1600			

Table D.2 Analysis of Variance for appreance in optimization of market sample

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	9	22.2400	2.4711	3.97	<.001
Panelist	9	2.8400	0.3156	0.51	0.865
Residual	81	50.3600	0.6217		
Total	99	75.4400			

Table D.3 Analysis of Variance for taste in optimization of market sample

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	9	26.7600	2.9733	5.10	<.001
Panelist	9	3.1600	0.3511	0.60	0.792
Residual	81	47.2400	0.5832		
Total	99	77.1600			

Table D.4 Analysis of Variance for flavor in optimization of market sample

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	9	24.7600	2.7511	4.47	<.001
Panelist	9	3.7600	0.4178	0.68	0.726
Residual	81	49.8400	0.6153		
Total	99	78.3600			

Table D.5 Analysis of Variance for overall acceptability of market sample

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	9	18.8900	2.0989	5.28	<.001
Panelist	9	5.8900	0.6544	1.65	0.116
Residual	81	32.2100	0.3977		
Total	99	56.9900			

Appendix E

Table E.1 Analysis of Variance for texture in optimization of lean meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	1.4000	0.7000	1.47	0.257
Panelist	9	3.2000	0.3556	0.74	0.666
Residual	18	8.6000	0.4778		
Total	29	13.2000			

Table E.2 Analysis of Variance for appreance in optimization of lean meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	6.8667	3.4333	4.96	0.019
Panelist	9	2.5333	0.2815	0.41	0.915
Residual	18	12.4667	0.6926		
Total	29	21.8667			

Table E.3 Analysis of Variance for taste in optimization of lean meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	22.8667	11.4333	17.44	<.001
Panelist	9	0.8000	0.0889	0.14	0.998
Residual	18	11.8000	0.6556		
Total	29	35.4667			

Table E.4 Analysis of Variance for flavor in optimization of lean meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat_type	2	25.2667	12.6333	33.77	<.001
Panelist	9	3.8667	0.4296	1.15	0.381
Residual	18	6.7333	0.3741		
Total	29	35.8667			

Table E.5 Analysis of Variance for overall acceptability of lean meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat_type	2	16.2667	8.1333	20.72	<.001
Panelist	9	2.0333	0.2259	0.58	0.800
Residual	18	7.0667	0.3926		
Total	29	25.3667			

Appendix F

Table F.1 Analysis of Variance for texture in optimization of fat meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	2.0667	1.0333	3.53	0.051
Panelist	9	3.6333	0.4037	1.38	0.267
Residual	18	5.2667	0.2926		
Total	29	10.9667			

Table F.2 Analysis of Variance for appreance in optimization of fat meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	13.0667	6.5333	16.96	<.001
Panelist	9	2.9667	0.3296	0.86	0.578
Residual	18	6.9333	0.3852		
Total	29	22.9667			

Table F.3 Analysis of Variance for taste in optimization of fat meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	30.2000	15.1000	52.95	<.001
Panelist	9	1.3667	0.1519	0.53	0.832
Residual	18	5.1333	0.2852		
Total	29	36.7000			

Table F.4 Analysis of Variance for flavor in optimization of fat meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat_type	2	22.0667	11.0333	30.09	<.001
Panelist	9	4.8000	0.5333	1.45	0.238
Residual	18	6.6000	0.3667		
Total	29	33.4667			

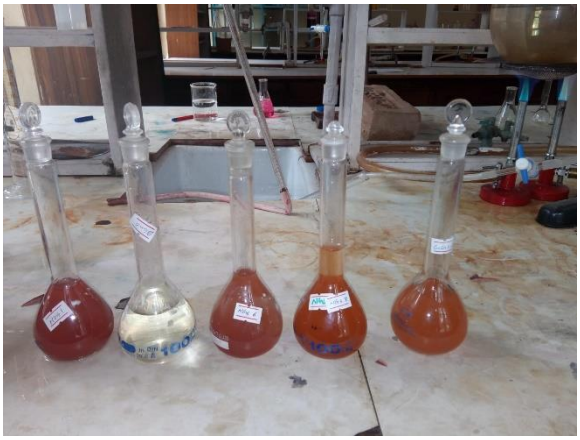
Table F.5 Analysis of Variance for overall acceptability in optimization of fat meat *sukuti*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Meat type	2	14.8667	7.4333	23.07	<.001
Panelist	9	5.2000	0.5778	1.79	0.139
Residual	18	5.8000	0.3222		
Total	29	25.8667			

Photo Gallery

P1: Samples for fat analysis

P2: Fat analysis



P3: Samples for protein analysis

P4: Protein analysis

P5: Microbiological Work

P6: Sample Preparation

P7: Microbiological count