RECIPE OPTIMIZATION OF BUFFALO MEATBALL

by

Saroj Ghimire

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

2018

Recipe optimization of buffalo meatball

A dissertation submitted to the Department of Food Technology, Central Campus of Technology, Tribhuvan University, in partial fulfillment for the degree of B.Tech. in Food Technology

by

Saroj Ghimire

Department of Food Technology

Central Campus of Technology

Institute of Science and Technology

Tribhuvan University, Nepal

January, 2018

Tribhuvan University

Institute of Science and Technology

Department of Food Technology

Central Campus of Technology, Dharan

Approval Letter

This *dissertation* entitled *Recipe Optimization of Buffalo Meatball* presented by Saroj Ghimire has been accepted as the partial fulfillment of the requirement for the B. Tech. degree in Food Technology

<u>Dissertation Committee</u>

1. **Head of the Department** ____

   (Mr. Basant Kumar Rai, Assoc. Prof.)

2. **External Examiner** ____

   (Mr. Birendra Kumar Yadav, Asst. Prof.)

3. **Supervisor** ____

   (Mr. Devraj Acharya, Asst. Prof.)

4. **Internal Examiner** ____

   (Mr. Arjun Ghimire, Asst. Prof.)

March, 2018

Acknowledgements

I express my deep sense of gratitude to my guide Mr. Devraj Acharya, Asst. Prof., Central Campus of Technology, Dharan for his excellent guidance, encouragement and inspirations throughout the work.

I am also grateful to Prof. Dr. Dhan Bahadur Karki (Campus chief, Central Campus of Technology), Associate Professor Basanta Kumar Rai (HOD, Department of Food technology) and Associate Professor Geeta Bhattarai (Chairperson, Central Department of Food Technology) for their generosity and co-operation in providing an opportunity and facilities to perform this work successfully. My sincere thanks to all my friends Pankaj Dahal, Ajit Kumar Sah, Gajendra Bohora, Prajwal Pokharel, Nabin Parajuli, Niseel Manandhar, Nikesh Niroula, Bibek Adhikari, Saurav Sanjel, Anusmriti Lamsal, Abishikha Regmi, Bibita Joshi, Sanjana Shrestha and Juniors Prabesh Bhattarai, Manish Dahal, Sagar Parajuli, Diwas Acharya and all who assisted me directly or indirectly, throughout the work. Thanks to all the laboratory and library staffs of Central Campus of Technology, Dharan for their kind co-operation. Finally, I am highly indebted to my parents and family members for their constant encouragement, love, inspiration and moral support without whom this work has not been completed successfully.

Date of Submission: March, 2018

Saroj Ghimire

Abstract

The present work was undertaken to develop ready to eat, a convenience fast food product from buffalo meat. Meatballs were prepared from buffalo meat incorporated with soy flour and corn starch mixing with other ingredients such as monosodium glutamate (MSG), phosphates and other spices. The formulations were prepared by varying soy flour and corn flour incorporation while keeping amount of other ingredients constant in all the formulations. Meatballs prepared from each formulation was subjected to sensory evaluation and best product was obtained in terms of appearance, flavor, texture and tenderness, juiciness and overall palatability. Chemical analysis of the best sample obtained from sensory evaluation was also performed.

From the sensory evaluation, the product prepared with 80% meat, 10% soy flour and 10% corn starch was found to be significantly ($P < 0.05$) superior at 5 % level of significance in terms of flavor, tenderness, juiciness and overall palatability. Chemical composition of significantly best meatball was found to have 55% moisture content, 3.2% crude fat, 20.58% crude protein, 2.7% crude ash, 0.2% crude fiber and 18.32 % carbohydrate on dry basis. Physico-chemical and sensory analysis of meatball samples showed that with increase in percentage of soy flour and corn starch, processing yield as well as water holding capacity (WHC) of the product increases. The processing yield, and WHC of the optimized product were found to be 104.23% and 59.62%. The product with 60% meat, 20% soy flour and 20% corn starch and with 73.33% meat, 6.67% soy flour and 20% corn starch according to solutions shown by RSM was found to be optimized recipe. Processing yield and WHC of optimized samples was 118.904%, 65.529% and 106.680%, 61.207 for solution 1 and solution 2 shown by RSM respectively.

Contents

List of Tables

Lists of figures

Lists of Abbreviations

Abbreviations	Full form
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemist
CCT	Central Campus of Technology
CRA	Corn Refiners Association
FAO	Food and Agricultural Organization
KJ	Kilo joule
LSD	Least significant difference
NHMRC	National Health and Medical Research Council
RSM	Response surface methodology
USAID	United State Agency for International Development
WHC	Water holding capacity

Part I

Introduction

1.1 General introduction

Meat has been the source of nutrients to humans since their very conception. With different kinds of meat available, the advancing human civilization has added diversity to meat products, as newer methods of consuming meats were developed (Hoogenkamp, 1997).

Among the meat products, Buffalo meat is known to be a part of the human diet with a favorable effect on vitality and incidence of diseases as demonstrated by some comparative trials between buffaloes and cattle or other species (De Mendoza *et al.*, 2005a). Buffalo meat is known by various names in different countries. It is known as *buffen*, or *buff* in India and Nepal. Buffalo meat has gained importance in the recent years because of its domestic needs and export potential (Kandeepan *et al.*, 2009c).

Buffalo meat is well in many of the physicochemical, nutritional, functional properties and palatability attributes. Furthermore, its utility in meat processing is on increase because of higher content of lean meat and less fat. Buffalo meat is getting popular worldwide because it has some inherent properties with respect to attributes such as lower inter muscular fat, cholesterol, calories, higher units of essential amino acids, biological value and iron content (Anjaneyulu *et al.*, 1990).

Processing of buffalo meat aids to produce value added, variety and convenience meat products to meet life style requirements. It offers better utilization of different-carcasses, cuts and edible byproducts. It facilitates incorporation of non-meat ingredients for quality and economy. Value added products are further processed products with increasing convenience to consumer through decreasing preparation time, minimizing preparation steps. It facilitates the use of specific parts, creation of products with different flavours and increases the shelf life of products. Value added products could be broadly classified based on processing, variety/convenience and function (Anjaneyulu *et al.*, 2007).

Meatballs are classified as finely comminuted meat products, sometimes referred to as meat emulsions (Hsu and Chung, 1999). A meatball is ground meat rolled into a small ball, sometimes along with other ingredients, such as bread crumbs, minced onion, eggs, butter, and seasoning. Meatballs are cooked by frying, baking, steaming, or braising in sauce. There are many types of meatballs using different types of meats and spices. The term is sometimes extended to meatless versions based on vegetables or fish (Esposito, 2013).

Various types of factors can affect the quality of meatballs, either in terms of nutritional value or overall acceptability of the meatballs over its consumers. Only those meatballs with high nutritional value, good textural properties, acceptable flavor and taste profiles will be preferred by consumers. Studies have shown that textures appears to be the most important characteristics of meatballs and consumers prefers harder texture (Hsu and Chung, 1998a). Soy protein isolate and corn starch concentrate were reported to be able to improve textural properties and to suppress lipid oxidation of meatballs as compared toasted bread-crumb which was traditionally used in the production of meatballs (Ulu, 2004)

Rheological, structural and nutritional properties of the processed comminuted meat products depend heavily on the fat in the formulation and method of cooking. Fat plays a pivotal role in the formation of stable emulsion and imparts a better texture, juiciness and flavour to the comminuted meat products (Kumar and Sharma, 2004). Method of cooking determines it's compositional, processing determinants and sensory attributes especially appearance and color and juiciness of the meat product. Minced meat is used for the preparation of a variety of products, such as patties, meatballs and kebabs. The minced meat is mixed with condiments and spices. It is shaped and then cooked by frying or baking (Gujral *et al.*, 2002).

Non-meat ingredients, such as soya protein, egg, cereal flours, starch, whey protein and fat, play a significant role in the modification of functional properties, such as emulsification, water- and fat-binding capacity and textural properties (El-Magoli *et al.*, 1996) Particularly, non-meat proteins and carbohydrates are often used to enhance the texture of meat products (Hongsprabhas and Barbut, 1999). In the meat industry, soya protein is the most widely used vegetable protein, due to its biological value, its properties as an emulsifier and stabilizer and its capacity to increase water holding capacity and improve the texture of final product (Macedo-Silva *et al.*, 2001).

1.2 Statement of problem

Meatball is getting popularity nowadays all over the world. In context of Nepal meatball is getting popularity day by day. Various kinds of meatball are already present in food market, but their formulation and processing are not standardized and technical. Lack of research and development is the main problems for the promotion. The main problem is cost optimization, recipe optimization for the marketing of meatballs. Meatball with higher proportion of meat is costly so cost and recipe optimization is of great importance now a days.

1.3 Objectives

1.3.1 General objectives

The objective of the dissertation work is,

- To study the effect of variation of soy flour and cornstarch in the preparation and on the quality of buff meatball.

1.3.2 Specific objectives

The following aspects of the work is to be carried out

- To prepare soy flour and corn starch incorporated buff meat balls.
- To analyze the processing yield of all the samples according to RSM.
- To analyze the WHC of all the samples according to RSM.
- To perform sensory evaluation of the samples.
- To perform proximate composition analysis of the optimized product.

1.4 Significance of the study

Buff meat is easily available and cost effective in context of Nepal. Minced meat is used for the production of meatball. The minced meat is mixed with condiments and spices. It is shaped and then cooked by frying or baking. The main problems for the shelf life of meat ball is due to lipid oxidation and rancidity. So non-meat ingredients, such as soya protein, cereal flours, corn flour plays a significant role in the modification of functional properties, such as emulsification, water- and fat-binding capacity, textural properties and also helps to prevents lipid oxidation and rancidity. Soy flour and corn starch incorporation in meat for the preparation of meatball also helps in the cost minimization of meatball without much loss in the nutritional value of meatball so a detailed study of the product is very important.

Present work helps to develop low cost product which will be sustainable in the market. New product development plays significant role giving completely different taste, aroma, flavor, and appearance. So it is, helpful for the development of nutritionally dense and quality meat product. Product with Consistent quality thus helps in its commercialization. Thus, giving the new product a sort of life style foods, which can be further accelerated when they become a part of the menu in fast food restaurants and companies. Present work can be taken as reference materials and will be useful for further research.

1.5 Limitations of the study

During the work the limitations found was,

- Shelf life and microbiological quality of the product was not studied.
- Binding properties of binding agent in different sample variation was not studied.
- Effect of frying temperature on the sensory quality of meatball was not studied.

Part II

Literature review

2.1 Historical background

The history of the meatball is obscure and early recipes are rare. Though many culinary inventions have been recorded decisively, no one is sure where the meatball originated (Cilantro, 2015). The meatball is a mysterious staple in food history, as no one really knows where and how the first meatball originated (Anon., 2016).

The most commonly accepted theory is that meatballs come from Persia. In Persia, there is a food called “Kofta” which has many variations of preparation but essentially means, “pounded meat”. Based on their shape, ingredients and preparation, most trust this is where our traditional meatball derived. In the earliest of times, meatballs were made from leftover meat that was pounded, chopped finely, shredded by hand or prepared in a way that could be rolled into a small ball. Since most meatballs were made from leftover meat, it’s safe to assume that some of the earliest recipes have not been recorded as they were prepared for the common folk. In the 1800’s, the Oxford English Dictionary defined them as “any combination of raw or cooked meat shaped into balls.” With such a broad definition, all cultures had room to create their very own staple recipe. Some of the earliest records of meatballs are in countries along the trade routes. It seemed everyone had their own version but the dish was essentially the same. Because of this, meatballs tie nations together. The main difference were the ingredients used. Regions played a big part in the components. For instance, China had an abundance of pork and therefore made plenty of pork meatballs while early ambitious Roman eaters enjoyed peacock, pheasant and rabbit meatballs (Anon., 2016).

Like many other foods, the invention of a kitchen tool revived the traditional recipe and made meatballs exciting and sought after for all. Specifically what was critical for meatballs was meat grinder. In the global history of food and cooking, meat was rare and was reserved primarily for the rich. As a precious commodity, we can assume that no part of meat was ever wasted and the meatball, invented long before there were sophisticated grinders or cookbooks jammed with recipes, was a way to get the nutrition from meat for another day's food. The variety of meat used for meatballs would be determined by geography. The world would have to wait for the invention of the meat grinder to begin the transition into using fresh meat. In the US Patent Office we find a patent issued to E. Wade in 1829 for a rudimentary grinder. In 1845 a second invention was recorded, this using a spiral feed and rotating cutting knives. This made it possible for the average person to buy ground meat, not mince the leftovers (Landrigan, 2016).

2.2 Buffalo meat

2.2.1 Meat quality

The major attractive features of buffalo meat are red color, reduced fat and cholesterol with poor marbling, low connective tissue, desirable texture, high protein, water-holding capacity, myofibrillar fragmentation index, and emulsifying capacity (Kandeepan *et al.*, 2013a). It is to be noted that buffalo meat is similar in tenderness to beef and has the added advantage of reduced cholesterol content (Paleari *et al.*, 1998). Buffalo meat quality was often studied in comparison with cattle meat (beef), and lots of similarities were reported for various meat quality characteristics and sensory attributes between these two meats. Palatability characteristics, shear force values, and taste panel scores of buffalo meat and beef obtained from identical age groups have been reported as almost similar (Neath *et al*., 2007a). Buffalo meat is stated to have physicochemical, biochemical, and technological properties comparable to those of beef. Post-mortem muscle pH ranging from 5.50 to 5.70 has been reported in fresh buffalo meat cubes and ground buffalo meat patties (Naveena *et al.*, 2004b).

Myoglobin content of fresh buffalo meat varied from 2.7 to 9.4 mg/g depending on the type of the muscle and animal age, and meat becomes darker with increasing age (Valin *et al.*, 1984). Different authors have reported the redness scores (a* value) ranging from 12.0 to 20.0 for fresh and frozen buffalo meat of different age groups (Tateo *et al.*, 2007). Dry-aged buffalo meat was reported to become darker faster than bovine meat, discouraging consumer purchase (Dosi *et al.*, 2006). Buffalo meat cubes and ground buffalo meat was reported to have a water-holding capacity ranging from 23.73 to 39.76% (Irurueta *et al.*, 2008) and 25.3 to 40.20% (Naveena *et al.*, 2011) respectively. Sarcoplasmic and myofibrillar protein concentration of 5.12 and 8.2% were recorded in buffalo meat (Kandeepan *et al.*, 2009b). Hydroxyproline content of 0.12% was recorded in young buffaloes. Muscles from young buffaloes of 1 to 2 years showed less collagen content (0.91

to 1.71 g/100 g) compared with old buffaloes of 12 years of age (1.16 to 2.23 g/100 g. Collagen solubility of 45.5% was observed in spent buffalo meat chunks.

Muscle fiber diameter ranging from 35.32 mm , 60.76 mm and 41.72 mm (Naveena *et al.*, 2004a) was reported for fresh buffalo meat. Myofibrillar fragmentation index (MFI) was reported to be 87.5 in 6-year-old male Murrah buffaloes. To understand the toughness of buffalo meat from old animals, scanning and transmission electron microscopy has been performed depicting the muscle fibers, connective tissue layers, and z-disk. Researchers have attempted to improve the tenderness of meat produced from old/spent buffaloes using plant proteases and chemicals (Naveena *et al.*, 2011). Use of different concentrations of sodium chloride and food grade polyphosphates are reported to improve pH, water-holding capacity, emulsion stability (ES), and emulsifying capacity (EC) in ground buffalo meat. Quality of ground buffalo meat was also reported to be improved by pre-blending with sodium ascorbate. Although buffalo meat from older animals is considered darker in color, tough in texture, and poor in flavor, this is not true with respect to meat from young buffaloes that are reared and fed for early slaughter (Kondaiah *et al.*, 1985a). The meat quality characteristics of buffalo meat is shown in Table 2.1.

Table 2.1 Meat quality characteristics

Parameters	Value
Ultimate pH	5.56
Water-holding capacity, %	> 50
Collagen content, mg/g tissue	0.67
Collagen solubility, %	45.5
Sarcomere length, µ	1.65
Myoglobin content, mg/g meat	4.0-6.0

Source: Faustman *et al.* (2010); Naveena *et al.* (2011); Muchenje *et al.* (2009); Valin *et al.* (1984); Kim and Lee (2003).

2.2.2 Functional properties and polyphosphates

The pH, water holding capacity(WHC), emulsifying capacity(EC) and Emulsion stability(ES) of buffalo meat play major role in processing of meat products and which depends on handling and processing conditions (Anjaneyulu *et al.*, 1994). Carcass and head meat have better functional properties than tripe and heart meat for use in processed meat products (Kondaiah *et al.*, 1986b). The popularity of food additives is based on their diverse functionality, nutritive value and economics. Polyphosphates are used widely in processing of meat products because they occur naturally in foods consumed by man. Incorporation of 2% salt and 0.3 to 0.5% sodium tri polyphosphate or tetra sodium pyrophosphates or blend of phosphates to buffalo meat improve the pH, WHC, EC, ES, extraction of salt soluble proteins and product yield (Kondaiah *et al.*, 1985b). Use of food grade polyphosphates in the formulation of meat products improved their quality due to increase in ph as well as specific effect of polyphosphates over and above the pH effect in meat system (Anjaneyulu *et al.*, 1990). They also prevent oxidative rancidity in muscles foods due to their sequestering/chelating ability on metal ions. Physico chemical parameters of buffalo meat, beef, mutton and goat meat were evaluated. Further, the EC of salt soluble proteins from muscles of buffalo was reported higher and more efficient than that of sheep and goat. Buffalo meat is stated to have physico chemical, biochemical and technological properties comparable to those of beef (Turgut, 1984).

2.2.3 Palatability of buffalo meat

Despite nutritional excellence, palatability of buffalo meat is the deciding factor for consumer acceptance. Although buffalo meat from older animals is considered darker in color, tough and poor in flavor, this is not true in respect of meat of young buffaloes that are reared and fed for early slaughter (Ognjanovic, 1974). Meat from buffalo calves was brighter in color than beef but the amount of meat pigments did not differ. The myoglobin content varied from 2.7 to 9.4 mg/g depending upon the age and meat becomes dark with increasing age (Marinova *et al.*, 1985). Visual evaluation of LD muscle cross section of spent buffaloes indicated darker meat color for males than females (Kondaiah *et al.*, 1981). Age of slaughter (20 to 34 months) and feeding regimes (four rations) did not influence the flavor and tenderness of meat (Charles, 1982). Corned beef made from buffalo meat was better in appearance due to the white color of fat (Karvir, 1985).

2.3 Physicochemical characteristics of buffalo meat

Buffalo meat is known to be a part of the human diet with a favorable effect on vitality and incidence of diseases as demonstrated by some comparative trials between buffaloes and cattle or other species (De Mendoza *et al.*, 2005b). Composition, physicochemical, nutritional and functional properties, and sensory attributes of buffalo meat are comparable with beef (Anjaneyulu *et al.*, 2007). 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% (Syed Ziauddin *et al.*, 1994b). 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 (Kesava Rao and Kowale, 1991). The relatively low fat content in buffalo meat is attributed to poor marbling. Buffalo meat has less fat and saturated fat than beef. The energy value for buffalo meat was found to be 57.22% less than beef. Low cholesterol content and energy value (6.8 Kcal/g dry matter) of buffalo meat was also reported by (Sengar *et al.*, 1985). Palmitic, stearic, oleic, and linoleic acids were reported to be predominant fatty acids in the phospholipids of buffalo meat. Buffalo calves have shown to produce meat with the most favorable (n-6)/(n-3) ratio (7.00) compared with the bovine calves and the buffalo cows (Dimov *et al.*, 2012) . Buffalo meat has an advantage of having low fat and cholesterol compared with beef and is rated superior to beef by a few researchers (Valin *et al.*, 1984). 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) (De Mendoza *et al.*, 2005b).

2.3.1 pH

The basic and most important parameter in determining the quality of the meat is pH. It is highly related to other meat processing parameters like water holding capacity and emulsifying capacity. Higher the pH more will be the water holding capacity and emulsifying capacity. Stress related aspects in carcasses cause Pale, Soft and Exudative meat with sudden fall in pH after slaughter, while Dark, Firm and Dry meat is observed in carcasses having very high pH due to depleted glycogen level in the carcass (Kandeepan *et al.*, 2013b).

Although processing characteristics of high pH meat is desirable, it is subjected to early spoilage due to higher microbial growth owing to high moisture and water activity present in the meat. The normal ultimate pH of buffalo meat varies from 5.4 to 5.6 (Kandeepan and Biswas, 2007).The pH of the meat from intensively reared young males was 5.57, which did not differ from spent male buffalo meat (Kandeepan *et al.*, 2009a). The lower ultimate pH in spent female buffalo meat might be due to the response of female buffaloes to transport stress than males (Jedlicka *et al.*, 1980). The meat obtained from young male buffaloes fed with high protein diet showed a pH of 5.54 (Anjaneyulu *et al.*, 1985). The ultimate pH of the muscle was higher in male than female. A pH of 5.69 in meat chunks and 5.64 in ground meat were observed in meat obtained from spent female Murrah buffaloes of 10 years age (Kandeepan *et al.*, 2013b). Neath *et al.*(2007b) indicated that postmortem pH decline of buffalo meat was slower than that of beef, which was confirmed by lactic acid concentrations, but was not explained by glycogen content. In addition, there was no significant difference in the ratio of slow to fast type muscle fibers in buffalo and cattle, indicating that myosin heavy chain type was not responsible for the difference in pH decline and tenderness between the buffalo meat and beef. The study demonstrated that the tenderness of water buffalo meat was superior to that of Brahman beef, which may have been due to the difference in pH decline and the subsequent effect on muscle protease activity.

2.3.2 Moisture

The moisture present in meat determines the binding ability of the meat to some of the binders and fillers added in making processed products. It has high correlation with the fat content of the meat. It is also related with the shelf stability of the processed products since it has relationship with the water activity of the meat for the microbial growth. Moisture content of the meat has direct relationship with juiciness of the processed meat products, which is one of the important sensory attribute. Young male Murrah buffaloes showed a moisture percentage of 74.04-77.75 (Kandeepan and Biswas, 2007).The meat obtained from high protein diet fed young male buffaloes showed moisture content of 76.36% (Anjaneyulu *et al.*, 1985). Whereas, spent female Murrah buffaloes showed a moisture percentage of 76.51-79.69 (Syed Ziauddin *et al.*, 1994b). Some authors did not find any significant difference in the moisture content between young and old animals (Joksimovic and Ognjanovic, 1977). The major changes in the percentage of chemical composition of the body of an undernourished animal were the loss of fat and protein and gain in proportion of water (Syed Ziauddin *et al.*, 1994b). The meat from intensively reared young male buffaloes showed a higher moisture content than the meat from spent male and female buffaloes

(Kandeepan *et al.*, 2009a). The moisture content of buffalo meat decreases as the age of the animal increases which is probably associated with an increase in fat content (Lawrie, 1998).

2.3.3 Protein

Meat is praised for its high protein content which makes it obligatory in balanced diet. The amount of protein especially the myofibrillar fractions are the basic supportive element through which meat emulsion is formed. The protein source open up their lipophilic and hydrophilic structures to bind with the water and lipid leading to emulsion formation by the addition of other non-meat ingredients into them. The sarcoplasmic fractions contribute to the color of the processed products while the connective tissue proteins contribute to the texture of the meat products. The protein content of the meat is highly related to the water holding capacity, emulsifying capacity and better nutritional quality of the meat. A higher protein content of 20.53% was recorded in meat obtained from young male buffaloes fed with high protein diet (Anjaneyulu *et al.*, 1985). Young male buffaloes showed a protein percentage of 17.33-23.3 (Kesava Rao *et al.*, 1985). Whereas, spent female Murrah buffaloes showed a protein percentage of 17.81-20.08 (Syed Ziauddin *et al.*, 1994a). Meat from males had markedly higher protein content than females (Mohan *et al.*, 1987). Intensive feeding of young male buffaloes with a high protein diet did not result in a significant difference in protein content of the meat compared to semi extensively reared spent male and female buffaloes (Kandeepan *et al.*, 2009c).

2.3.4 Fat

The meat fat is responsible for the species specific flavor present in the meat products. The amount of fat contained in a meat product also determines the juiciness of the meat product. The ruminant fat are saturated and not easily dispersed in meat emulsion resulting in poor appearance of the product with fat droplets visible on the product. This result in a phenomenon called mouth coating while the product is consumed. Intact males contained less fat. Palmitic, stearic, oleic and linoleic acids were the four predominant fatty acids in the phospholipids of buffalo meat (Kesava Rao and Kowale, 1991). High protein feeding in young male buffaloes recorded a fat content of 1.50% in the meat. Buffalo meat from 2 years old male calves showed a fat percentage of 1-3.5. The intramuscular fat percentage varies between the muscles. The low level of intramuscular fat could be due to poor marbling reported in buffaloes (FAO, 2005). An increased fat content in intensively fed bulls were observed (Sami *et al.*, 2004). Male animals are leaner than females. The meat from females and steers contained a higher fat content than from bulls. Meat from spent female buffaloes had a higher fat content compared to the other groups. Fat is the last tissue to mature and older animals tend to be fatter (Warriss, 2000).

2.3.5 Meat pigments

The amount of pigment present in the meat decides the color of the product. Buffalo meat products are darker in color owing to their higher myoglobin content compared to other livestock species. The meat obtained from intact males was lighter in color. The myoglobin content varied from 2.7 to 9.4 mg/g depending upon the type of the muscle and age and meat becomes darker with increasing age (Valin *et al.*, 1984). A slight variation in myoglobin was observed in the meat from spent male and female buffaloes. The meat pigment concentration of spent male buffalo meat was higher than young males, which was attributed to greater content of haeme pigment and myoglobin (Mamino and Horn, 1996). The heme pigment concentration in meat samples of bulls was 3.59 to 3.99 mg/g (Maltin et al., 1998). The total meat pigment obtained from spent female buffalo was 0.25%. The meat pigment content from younger buffaloes was lower than spent male and female buffaloes. A slight variation in myoglobin concentration was observed in the meat from spent male and female buffaloes (Kandeepan *et al.*, 2009c).

2.3.6 Salt soluble protein (SSP)

The myofibrillar proteins can be extracted well in the presence of salts which form the basis for emulsion based and restructured meat products. The binding and emulsifying ability of the protein molecules are greatly improved after their extraction with salt resulting in good emulsion stability. About 35% of meat protein was salt soluble. The higher amount of SSP or extractable proteins would result in greater emulsifying capacity of the muscle (Turgut, 1984). The percent water soluble protein and SSP of buffalo thigh meat, tripe and heart were 4.08, 4.35; 2.87, 6.30 and 4.40 and 4.53 respectively (Kondaiah *et al.*, 1986a).

Sarcoplasmic and myofibrillar protein concentration of 5.12% and 7.19% were recorded in meat from high energy diet fed male buffalo calves. Spent female buffalo meat showed a SSP of 8.2%. Spent male buffalo meat had higher salt soluble protein compared to the meat from young male and spent female buffaloes. Salt soluble protein was related to the water holding capacity and moisture content of the meat in each group. Meat with higher salt soluble protein can retain more water to improve the cohesiveness and binding strength of the product during processing (Swan and Boles, 2006).

2.3.7 Collagen content

Among the connective tissue protein, collagen content of the meat is highly responsible for the texture of the product due to the connective tissue content. But collagen is heat labile and higher amount of collagen in meat may cause structural deformities in sausage production. The collagen content was 10-13% of total protein. Connective tissue in the buffalo meat had a bigger contribution to toughness. The total concentration of connective tissue components were not closely related to the scores for muscle fiber tenderness. Chronological age was related to the collagen content in the muscle. The collagen content increased with advancing age of the male Murrah buffaloes (Yadava and Singh, 1985).

A hydroxyproline content of 0.12% was recorded in high protein diet fed young male buffaloes. The muscles from young buffaloes of 1 to 2 years showed less collagen (0.91 to 1.71 g/100 g) than from 12 year old buffaloes (1.16 to 2.23 g/100 g). Some authors did not find any differences in the amount of connective tissue in young and aged animals. As animals get older the collagen cross links are stabilized. After cooking the collagen cross links weaken but do not break, so contributing to the toughness of meat from old animals. Collagen content of meat from intensively reared young male buffaloes was lower than other two groups. Collagen content of meat from spent female buffalo was markedly higher compared to spent male buffalo meat. The result suggests that the meat from spent male and female buffaloes could be tougher. An age related increase in pyridinoline content of intramuscular collagen and cross link formation influenced by sex contributed to the toughness of meat in spent groups (Bosselmann *et al.*, 1995).

2.3.8 Collagen solubility

The soluble fraction of collagen present in meat is responsible for the extent of tenderness of the product due to connective tissue presence. Higher the soluble fractions, less is its contribution to product toughness. As animals get older the collagen cross links were stabilized and the collagen was much less soluble (Warriss, 2000). Soluble collagen percentage was related to the contribution of connective tissue to toughness as assessed by sensory panel. Chronological age was related to percent soluble collagen in muscle. The soluble collagen decreases (13.5 to 3.6) as the age of the animal increases. A collagen solubility of 6.58% was observed in meat chunks from spent female Murrah buffaloes. The collagen solubility of meat in young male buffaloes was higher than that of other two groups. As animals get older the collagen cross links become stabilized and the collagen is much less soluble. The collagen of spent male buffalo meat was slightly less soluble than the collagen of spent female buffalo meat. This was attributed to the highly stabilized cross links induced by the work (draught) done by spent/old male buffaloes (Kandeepan *et al.*, 2009c).

2.3.9 Muscle fiber diameter

The size of muscle fibers in meat determines the texture of the processed products with increasing toughness and coarse texture with increasing thickness of myofibrils. Measurement of muscle fiber diameter could be useful for selection of animals with tender meat. A significant relationship was observed between muscle fiber diameter and tenderness. Large muscle fibers are generally indicative of less tender beef. The meat obtained from high protein diet fed young male buffaloes showed a muscle fiber diameter of 35.32μm. A much less diameter of muscle fibers were observed in young animals. A muscle fiber diameter of 49.2 to 49.7 μm was observed in raw beef from 18 month old bulls. In concentrate fed male Murrah buffaloes muscle fiber diameter of 80 μm was observed (Palka, 2003).

As the age and slaughter weight increased there was an increase in muscle fiber diameter. A muscle fiber diameter of 60.76 μm was recorded in meat chunks from spent female Murrah buffaloes .Fiber diameter was positively correlated to shear values but negatively correlated to tenderness and sarcomere length of the muscle. The fiber diameter of spent male buffalo meat was larger than that of the young males but lower in comparison to spent female buffalo meat. An increase in age of river buffaloes was associated with increasing muscle fiber diameter. Fiber diameter was positively correlated to shear values but negatively correlated to tenderness and sarcomere length of the muscle (Ragab *et al.*, 1966).

2.3.10 Sarcomere length

Sarcomere length has some important effect on meat quality. Shorter the sarcomere length, the meat is tough and has low WHC. The sarcomere length was positively correlated to lean texture. A sarcomere length of 2.3 μm was observed in raw beef from 18 month old bulls. Young buffalo bulls showed a sarcomere length of 1.73 to 1.88 μm. The longer the sarcomeres more tender the meat (Seideman *et al.*, 1987). Males had shorter sarcomere length than females in turkeys. However, the variation in sarcomere length had no impact on tenderness of beef from Charolais sires. The sarcomere length of buffalo meat was higher in young males compared to spent male and females. Sarcomere length decreases with advancing age and increases the toughness of meat. Spent male buffalo meat had lower sarcomere lengths than that of spent female buffalo meat. This might give rise to the tenderness variation due to sarcomere length in spent male and female buffaloes (Maher *et al.*, 2004)

2.3.11 Shear force value

Shear force values provide basic information on tenderness, WHC and texture of the meat. Determined the difference in tenderness and some characteristics of water buffalo meat and beef during postmortem aging. It was observed that the buffalo meat had lower shear force values compared to beef for Longissimus thoracis (LT) and semimembranosus (SM) muscles, which was supported by a difference in troponin T degradation. The meat obtained from young male buffaloes fed with high protein diet showed a shear force value of 4.03 kg/1.25 cm core. Shear force values for low fat ground buffalo meat patties prepared from spent female Murrah buffalo meat was found to be 0.37 kg/cm^2 (Suman and Sharma, 2003). The buffalo meat obtained from young males showed a lower shear force value than the other groups. Intensive feeding decreased the shear force value of the meat. The meat from spent female buffaloes showed higher shear force values compared to the spent male buffaloes. Tenderness was also higher in young bulls followed by steers and then cows. The shear force value was highly related to the muscle fiber diameter and collagen content of the buffalo meat (Shiba *et al.*, 2004). Buffalo meat nutrient composition is shown in Table 2.2.

Table 2.2 Buffalo meat nutrient composition

Nutrient	Value per 100 g 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
Iron, mg	1.61

Source: Kim and Lee (2003); Muchenje *et al.* (2009); Faustman *et al.* (2010); Naveena *et al.* (2011); Valin *et al.* (1984).

2.4 Functional characteristics

2.4.1 Water holding capacity (WHC)

Among the functional parameters, the inherent ability of the meat to hold its own water and its ability to bind with water added to it separately or as a constituent present in non-meat additives in a product formulation is the most important factor in deciding suitability of the meat for processing into products. It is directly related to emulsion stability and juiciness of the meat products. A water holding capacity of 20.61 ml/100g was recorded in meat obtained from young male buffaloes fed with high protein diet. The meat from young buffalo males of 2 years age showed WHC 13.50 ml/100 g. The meat from entire males had higher water holding capacity than castrates. Whereas, the meat samples from spent female Murrah buffaloes showed a WHC of 16.67 ml in ground meat (Anjaneyulu *et al.*, 1985). The water holding capacity of young male buffalo meat did not differ from the spent male buffalo meat. A slightly lower water holding capacity in castrates compared to entire males has been shown to be due to higher protein denaturation in castrates. Meat from intensively reared young male buffaloes had higher water holding capacity than the meat from spent female buffaloes. The WHC and salt soluble protein contents have been reported to be not related (Kandeepan *et al.*, 2009b).

2.4.2 Emulsifying capacity (EC)

The amount of myofibrillar proteins present in meat and their ability to emulsify added fat is an important criterion for emulsion stability and better product characteristics in terms of binding and texture. Emulsifying capacity at pH 7.0 was greater than at normal pH and pH 5.0, although the amount of salt soluble protein was not always greater. pH was more important in emulsifying capacity than was percent of salt soluble protein extracted from meat tissues . Emulsifying capacity of 99 ml oil meat was recorded in chilled meat from 2 year old buffalo male calves. Spent female buffalo meat showed an emulsifying capacity of 130.8 ml oil meat. The emulsifying capacity of the meat from young male buffaloes was lower than spent male buffaloes but not spent female buffaloes. The greater emulsifying capacity of the meat from spent males compared to the other two groups was attributed to the highly significant increased salt soluble protein content (Anjaneyulu *et al.*, 1989).

2.4.3 Myofibrillar fragmentation index

The amount of myofibrils in meat that gets fragmented by application of mechanical forces determines the texture of the meat product. More the fragmentation of myofibrils, tender will be product texture. The Myofibrillar Fragmentation Index (MFI) is a measure of myofibrillar protein degradation. This was highly related to shear force and sensory tenderness ratings and negatively correlated to lean color. Longissimus tenderness was highly and positively correlated with MFI and indicates the amount of myofibrillar proteolysis that has occurred. The MFI was observed to be 87.5 in six year old male Murrah buffaloes. The buffalo meat from young males had a higher myofibrillar fragmentation index compared to the meat from spent male and female buffaloes. Animal age has been shown to have more influence on tenderness attributes than sex of the animal. MFI was negatively correlated with the shear force value of the buffalo meat. The MFI of spent female buffalo meat was lower than the other two groups, which indicates more toughness (Kulkarni *et al.*, 1993)

2.5 Nutritional value of buffalo meat

2.5.1 Energy

A 100 g serving of separable buffalo lean meat provides 456 KJ of energy. Buffalo meat is a great source of numerous vitamins and minerals. It is high in B12, potassium, iron, zinc, copper and selenium, while lower in sodium, calories and cholesterol. Buffalo red meats are: An excellent source of high biological value protein, niacin, vitamin B6, iron, zinc and phosphorus, A source of long-chain omega-3 polyunsaturated fats, riboflavin, pantothenic acid, selenium and possibly also vitamin D. Mostly low in fat and sodium, Sources of a range of endogenous antioxidants and other bioactive substances including taurine, carnitine, carnosine, ubiquinone, glutathione and creatine (NHMRC, 2006).

2.5.2 Protein and amino acids

Raw red muscle meat contains around 20-25 g protein/100 g. Cooked red meat contains 28-36 g/100 g, because the water content decreases and nutrients become more concentrated during cooking. The protein is highly digestible, around 94% compared to the digestibility of 78% in beans and 86% in whole wheat (Bhutta, 1999). Protein from meat provides all essential amino acids (lysine, threonine, methionine, phenylalanine, tryptophan, leucine, isoleucine, valine) and has no limiting amino acids. Protein Digestibility Corrected Amino Acid Score (PDCAAS) is a method of evaluating the protein quality, with a maximum possible score of 1.0. Animal meats like beef have a score of approximately 0.9, compared to values of 0.5-0.7 for most plant foods. The amino acid glutamic acid/glutamine is present in meat in the highest amounts (16.5%), followed by arginine, alanine, and aspartic acid (Schaafsma, 2000).

Amino acid composition (% protein) of separable lean raw buffalo meat is shown in Table 2.3

Table 2.3 Amino acid composition (% protein) of separable lean raw buffalo meat

Nutrient	Value per 100 g
Threonine, g	0.890
Isoleucine, g	0.911
Leucine, g	1.679
Lysine, g	1.686
Methionine, g	0.513
Phenylalanine, g	0.809
Tyrosine, g	0.695
Valine, g	0.978
Arginine, g	1.282
Histidine, g	0.573
Alanine, g	1.228
Aspartic acid, g	1.875
Glutamic acid, g	3.150
Glycine, g	1.039
Proline, g	0.863

Source: USDA National Nutrient Database for Standard Reference (2007)

2.5.3 Fat and fatty acids

There is a wide variation in the amount of total separable fat between the different cuts. The gross composition values show that there generally appears to be less separable fat in the untrimmed raw retail samples. This trend to lower fat cuts has been due to three factors: selective breeding and feeding practices designed to increase the carcass lean to fat ratio; meat classification and marketing systems designed to favor leaner products; and modern butchery techniques such as seaming out whole muscles and trimming away intermuscular fat . Most consumers today prepare and consume their meat after trimming external fat, and the most recent nutritional analyses show that all trimmed lean red meats are relatively low in fat (<7%) and have moderate cholesterol content, with the exception of mince meats . An important contributor to the leanness of muscle meat in buffalo is that almost all animals are pasture (grass) fed for most of their lives, although some are given short periods of grain finishing before slaughter (Higgs, 2000).

Much of the discussion about the fat content of red meat focuses on the saturated fat content. However, the amount of saturated fat in buffalo meat is actually less than the total amount of unsaturated fats on a per edible portion basis. Saturated fatty acids comprise, on average, 40% of total fatty acids in the lean component and 48% in the fat component of red meat. In buff meat and veal, approximately half of the saturated fatty acid in both the lean and fat component of red meat is palmitic acid (16:0) and about a third is stearic acid (18:0). In lamb and mutton the proportions of these two fatty acids is more similar. There is little variation between cuts in the proportion of fatty acids. Polyunsaturated fatty acids (PUFA) range from 11% to 29% of total fatty acids. Pasture fed buffalo is better source of omega-3 fats than grain feed buffalo, and this explains the better fatty acid ratio in red meat, where there is extensive grain feeding. Buffalo meat also have more omega-3 fatty acids than either chicken or pork, although fish is still a better source than any of the red meats. The recent revision of the recommended dietary intakes recommended a daily adequate intake of long-chain omega-3 fats (DHA, EPA and DPA) of 160 mg for men and 90 mg for women, with higher targets of 610 mg and 430 mg respectively to reduce the risk of long term chronic disease. Buff meat, which has more than 60 mg EPA + DHA per serving of red meat, can be described as a good source of long chain n-3 polyunsaturated fats (Marmer *et al.*, 1984). Fatty acid and cholesterol content per 100 g of buffalo meat is shown in Table 2.4.

Table 2.4 Fatty acid and cholesterol content per 100 g of buffalo meat

Nutrients	Values per 100 g
Fatty acids, total saturated, g	0.690
Fatty acids, total mono unsaturated, g	0.720
Fatty acids, total polyunsaturated, g	0.190
Cholesterol, mg	62

Source: USDA National Nutrient Database for Standard Reference (2007)

2.5.4 Vitamins

As with other animal foods, red meat is an excellent source of bioavailable vitamin B12, providing over two thirds of the daily requirement in a 100 g serve. Up to 25% RDI of riboflavin, niacin, vitamin B6 and pantothenic acid can also be provided by 100g of red meat, but compared to pork it is a relatively poor source of thiamin. Liver is an excellent source of vitamin A and folate, but the levels in lean meat tissue are low. For all these vitamins, older animals tend to have higher concentrations, so the levels in buff are generally higher than those in veal, and mutton has more than lamb. Levels of vitamin D in meat are low and difficult to measure and have often not been included in food composition data previously. However recent assays of meat in New Zealand have reported levels of 0.10 μg Vitamin D3/100 g and 0.45 μg 25-OHD3/100 g in buff and levels of 0.04 and 0.93 μg/100 g 4 respectively in lamb. Given the higher biological activity of the 25-OH vitamin D, this means that 100 g of cooked buff could provide 12% of the estimated adequate intake of 10 μg/d for a 51-70 year old (NHMRC, 2006). Vitamin content per 100 g is shown in Table 2.5.

Table 2.5 Vitamin content per 100 g

Vitamins	Values per 100 g
Vitamin C, mg	0.0
Riboflavin, mg	0.094
Niacin, g	1.910
Vitamin A, IU	0

Source: USDA National Nutrient Database for Standard Reference (2007)

2.5.5 Minerals

Buff meat are among the richest sources of the minerals iron and zinc, with 100 g providing at least one quarter of daily adult requirements. The iron in meat is mostly haem-iron which is well absorbed, and meat protein also appears to enhance the absorption of iron from meat. Similarly, absorption of zinc from a diet high in animal protein is greater than from plant foods, and the requirements for zinc may be as much as 50% higher for vegetarians. Red meats are also good sources of selenium, providing over 20% RDI per 100 g serve, although it is likely that selenium values in meat will be affected by where animals feed and the time of the year of sampling. Lean meat is low in sodium with a potassium/sodium ratio of greater than five. The copper content in raw lean cuts range from 0.055 to 0.190 mg/100 g in beef and veal, 0.090 to 0.140 mg/100 g in lamb, and 0.190 to 0.240 mg/100 g in mutton, all higher than values reported in British meat (NHMRC, 2006).

Minerals content per 100 g is shown in Table 2.6.

Table 2.6 Minerals content per 100 g

Minerals	Values per 100 g
Calcium, mg	6
Iron, mg	2.60
Magnesium, mg	25
Phosphorus, mg	187
Potassium, mg	343
Sodium, mg	54
Zinc, mg	2.80
Copper, mg	0.090
Manganese, mg	0.007
Selenium, µg	27.0

Source: USDA National Nutrient Database for Standard Reference (2007)

2.5.6 Moisture

Moisture less occurred unevenly among the different buff portions, with smaller portions producing the greatest percentage moisture loss. Due to the moisture loss, the concentration of many of the nutrients increased during cooking despite partial destruction of certain heat liable nutrient loss in the drained juices. The edible portion of buff contains about 71-72% moisture (Kandeepan and Biswas, 2007).

2.6 Sensory attributes

The physical, chemical and functional quality of meat is highly related to its sensory characteristics. The sensory attributes of meat products vary with characteristic change in their constitution in meat. Appearance, flavour and juiciness scores did not differ between groups. The tenderness and connective tissue residue scores of cooked meat chunks differed among young male, spent male and spent female buffalo groups. Beef from more mature animals repeatedly had been found less tender than beef from younger animals (Smith *et al.*, 1982). The decrease in tenderness score was attributed to decreased activation of the μ-calpain in older animals. Observed that tenderness increased with postmortem aging of buffalo meat. The connective tissue residue scores were highly related to the tenderness of the meat. The higher amount of connective tissue in older animals resulted in decreased tenderness of meat (Morgan *et al.*, 1993).

2.7 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 (Anon., 2011/12). Statistics of buffalo meat production in year 2009, 2010 and 2011 is shown in Table 2.7

Table 2.7 Statistics of buffalo meat production

	2009	2010	2011
Population (in 000' numbers)	4680.49	4836.98	4993.65
Slaughtered (in 000’ numbers)	712.60	737.00	763.00
Production (in 000’ tones)	156.62	162.21	167.86

Source: Food and Agricultural Organization (FAO)

In most of the countries, buffaloes were mainly reared as drought animal and milk animal. The concentration of buffaloes is more in tropical and developing countries where grain are staple food for human consumption (Anon., 2011/12)

2.8 Soy flour

Soy flour, derived from ground soybeans, boosts protein, brings moisture to baked goods, and provides the basis for some soymilks and textured vegetable protein. This versatile ingredient improves taste and texture of many common foods and often reduces the fat absorbed in fried foods. The taste of soy flour varies from a “beany” flavor to a sweet and mild flavor, depending on how it is processed (Anon., 2017).

Soy flour is a great source of high quality soy protein, dietary fiber and important bio-active components, such as isoflavones. This versatile ingredient provides a good source of iron, B vitamins and potassium. Important bio-active components found naturally in soybeans are being studied in relation to relieving menopausal symptoms, such as hot flashes, maintaining healthy bones, and preventing prostate, breast cancers, and colorectal cancer. The content and profile of bio-active components varies from product to product, depending upon how much soy protein is in the food and how the soy protein is processed (Anon., 2017).

Defatted soy flour is a processed product made from finely ground defatted soy meal and contains less than 1 percent oil. It is the base ingredient in soy protein isolate, soy protein concentrate and textured soy protein, and contains 50% protein by weight. Defatted soy flour is used as an ingredient to enrich other cereal products, such as corn, wheat and rice. It may be used by commercial process or in the field to enrich foods locally. It is currently an ingredient in soy-fortified cornmeal, corn soy blend (CSB) and wheat soy blend (WSB), which are used in emergency and development settings. Soy flour is a good source of plant-based protein that is highly digestible. It can be fortified with a variety of vitamins and minerals. Soy flour can be used in the same way as rice, wheat or corn flour to make breads, complementary foods for children, cereals, porridges, cookies, muffins, pastries, cakes, noodles, naan, soups and sauces, snacks, beverages and tortillas. Soy flour is made from roasted soybeans that have been ground into a fine powder. Rich in high-quality protein and other nutrients, soy flour also adds a pleasant texture and flavor to a variety of products (Anon., 1998)

Two kinds of soy flour are available: Natural or full-fat soy flour contains the natural oils that are found in the soybean. Defatted soy flour has the oils removed during processing. Both kinds of soy flour will give a protein boost to recipes; however, defatted soy flour is even more concentrated in protein than full-fat soy flour. Like whole grain flours, both defatted and full-fat soy flour should be stored in the refrigerator or freezer. Soy contains complete protein with all the amino acids essential to human nutrition. It is also good source of dietary fiber, calcium, iron, magnesium and phosphorus. Soy flour is gluten free; this makes it an excellent substitute for individuals who are sensitive to gluten. Soy flour is derived from roasted soybeans finely grounded into a powder. It is a rich source of proteins, as well as iron, B vitamins and calcium, and it adds a pleasant texture and flavor to a variety of products. Defatted" soy flour provides a slightly higher percentage of protein and calcium. Both forms of soy flour have health benefits. In addition to the excellent nutritional value of soy protein, scientists have found that consumption of soy protein can contribute to reducing the risk of heart disease by lowering blood cholesterol and increasing the flexibility of blood vessels (USAID, 2016).

A recent scientific study, “Soy fiber improves weight loss and lipid profile in overweight and obese adults”, found that those consuming soy fiber from soy flour saw significant improvements in BMI, body weight, and LDL cholesterol. Soyfoods are a healthy protein source because of the high quality of protein that contains all essential amino acids needed for growth. Soyfoods are a good source of essential fatty acids and contain no cholesterol and little or no saturated fat (USAID, 2016). This comparison of the protein content of several flours indicates the high protein content of soy flours in relation to wheat flours*:

Full-fat soy flour: 40 % protein

Low-fat soy flour: 52 % protein

Defatted soy flour: 55 % protein

Whole wheat flour: 16 % protein

Enriched white flour: 12 % protein

* approximately (Anon., 2017).

Nutritional value of soy flour is shown in Table 2.8

Table 2.8 Nutritional value of soy flour, nutrients per 100 g (by weight)

Nutrient	Full-fat, roasted	Defatted
Calories, KJ	441	329
Protein, g	34.80	47.00
Fat, g	21.90	1.20
Carbohydrate, g	33.70	38.40
Fiber, g	2.20	4.30
Calcium, mg	188.00	241.0
Iron, mg	5.80	9.20
Zinc, mg	3.50	2.40
Thiamin (B1), mg	41.00	7.00
Riboflavin, mg	94.00	25.00
Niacin, mg	3.29	2.61

Source: Composition of Foods: Legume and Legume Products. United States Department of Agriculture, Human Nutrition Information Service, Agriculture Handbook, Number 8-16. Revised December 1986.

2.9 Corn starch

Corn starch, corn flour or maize starch or maize is the starch derived from the corn (maize) grain (Anon, 1828). The starch is obtained from the endosperm of the kernel. Corn starch is a common food ingredient, used in thickening sauces or soups, and in making corn syrup and other sugars. It is versatile, easily modified, and finds many uses in industry as adhesives, in paper products, as an anti-sticking agent, and textile manufacturing. It has medical uses, such as to supply glucose for people with glycogen storage disease. Like many products in dust form, it can be hazardous in large quantities due to its flammability. When mixed with a fluid, cornstarch can rearrange itself into a non-Newtonian fluid. Cornstarch is used as a thickening agent in liquid-based foods (e.g., soup, sauces, gravies, custard), usually by mixing it with a lower-temperature liquid to form a paste or slurry. It is sometimes preferred over flour alone because it forms a translucent, rather than opaque mixture. As the starch is heated, the molecular chains unravel, allowing them to collide with other starch chains to form a mesh, thickening the liquid (Starch gelatinization). It is usually included as an anti-caking agent in powdered sugar (10X or confectioner's sugar). Meatballs with a thin outer layer of cornstarch allows increased oil absorption and crispness after the latter stages of frying (Moncel, 2017).

Corn starch, sometimes referred to as corn flour, is a carbohydrate extracted from the endosperm of corn. This white powdery substance is used for many culinary, household, and industrial purposes. In the kitchen, corn starch is most often used as a thickening agent for sauces, gravies, glazes, soups, casseroles, pies, and other desserts. Because corn starch is made from corn and only contains carbohydrates (no protein), it is a gluten free product. For this reason, corn starch is an excellent gluten-free alternative to flour thickeners in recipes. Corn starch can be mixed into cool or room temperature liquids and then heated to cause a thickening action. Corn starch is often preferred to flour as a thickener because the resulting gel is transparent, rather than opaque. Corn starch is also relatively flavorless compared to flour and provides roughly two times the thickening power. Corn starch can be substituted at half the volume of flour in any recipe that calls for flour as a thickening agent (CRA, 2006).

Corn starch can also be used to coat fruit in pies, tarts, and other desserts before baking. The thin layer of corn starch mixes with the fruits' juices and then thickens as it bakes. This prevents pies and other desserts from having a watery or runny texture. Corn starch is also used as an anti-caking agent. Shredded cheese is often coated with a thin dusting of corn starch to prevent it from clumping in the package. The corn starch will also help absorb moisture from condensation and prevent a slimy texture from developing (Moncel, 2017).

Nutritive value of corn starch is shown in Table 2.9.

Table 2.9 Nutritive value of corn starch.

Amount per serving

Calories 381 Serving size 100 g

Nutrient	% by weight
Dietary fiber	0.9
Ash	0.09
Sodium	0.03
Total Carbohydrate	91
Proteins	0.4
Fat	0.2
Sodium	0.03
Calcium	0.02
Iron	0.2
Phosphorous	0.1
Copper	0.2
Magnesium	0.06

Source: USDA Economic Research Service (2001).

2.10 Technology of meat balls

2.10.1 Ingredients

2.10.1.1 Meat

Meatballs can be made from all types of meat. It can be made either from one type of meat or combination of different types of meat such as all chicken, all buffalo meat and all fish meat and from combination of different meat at various proportion . In meatball most desired are the lean muscles having less connective tissue. Thus meat pieces of relatively uniform size, trimmed of connective tissues are given first priority but second quality meat having partially trimmed off connectives are also used. The different animal tissue will vary in moisture to protein ration, lean to fat ratio. Approximately 60% of total protein is myofibriller composed mainly of myosin and actin, which combine to Form actomyosin during onset of rigor-mortis. When choosing raw material particular attention should be paid to its origin, composition and pre- treatment. The composition and quality of meat differs with animal species. The differences affect the water holding capacity pH, color, flavor and tenderness of meatball (Hoogenkamp, 1997)

There is also a significant difference in meat quality between breeds of different species as well as same species. Like myoglobin concentration of same muscle may vary which affects the color of the final product. Similarly the proportion intramuscular fat may vary which also affects the flavor and juiciness of the final product. With increase in age the concentration of intramuscular fat increases meat becomes darker and tougher. thus with the change in meat quality tenderness juiciness and color of meat ball do change. Generally good nutrition increases the level of intramuscular fat thus affects the juiciness of the meat ball i.e. juiciness increases. Another aspect of nutrition on meat quality the composition of the forage can lead to flavor variation. The pH of the meat depends on the glycogen content and glycolytic activity resulting in increase or decrease in glycolytic activity. After slaughter glycogen of the muscle is converted into lactic acid fall in pH from an initial value of 6.3-7.3 to about 5.4-5.8 at rigor-mortis. Electrically stimulated muscles hasten the process of rigor and subsequently a quick drop of pH (Hoogenkamp, 1997).

When animals stress immediately prior to slaughter the muscle glycogen released into blood stream and after slaughter is rapidly broken down to lactic acid and produce pale soft and exudative meat. The meat has low pH, has reduced water-holding capacity whereas DFD meat caused by long-term stress before slaughter or starvation has normal or increased water holding capacity and pH of meat greater than 6. Both the DFD and PSE meat has poor meat flavor too. The meat with pH greater 6 usually has good water holding properties (i.e. less cooking loss) and retains it native color when heated at pH greater than 6 has fixation effects on color attributes (Hoogenkamp, 1997).

The pre-rigor meat (hot boned) mainly on account of high pH has good emulsifying and water holding properties. Thus is being used in some meat production such as sausages patties then chilled or frozen meat. Longer the period elapsing from slaughters to use, the poorer the binding quality (Anjaneyulu *et al.,* 1994)

The freezing causes certain damage to the muscle fibers and protein denatures which causes drip loss from meat. Also during thawing after freezing, there is a loss of characteristics. In addition, it has been found that hot boned meat tends to have its own antioxidative properties and thus taste and rancidity of hot meat is better than chilled meat. Post rigor storage/treatment (ageing) if meat is associated with both tenderization and increased water holding capacity as the myofibriller system decrease. Cold and thaw shortened meats are very tough, has low water holding capacity as demonstrated by the large amount of drip loss (Gerrard, 1976)

2.10.1.2 Seasonings

According to FDA, spices have been defined by the food and drug administration aromatic vegetable substances used for the seasoning of food. They are true to man and from them no portion of volatile oil or other flavoring principle has been removed. Seasoning is a comprehensive term applied to blends of spices, which may or may not contain other ingredients such as onion, garlic, MSG, salt and sugar. The various seasoned salts (onion salt, garlic salt and celery salt are seasoning salts as are chilli powder and curry powder). The meat industry uses a whole black pepper in several meat items. Very often some other form of spices is used with whole spices for flavor strength and uniformity of flavor, but whole spices usually do not compete with other form of spices (Anjaneyulu *et al.,* 2007).

2.10.1.3 Ground spices

Since the beginning of the recorded history ground spices have been used extensively to seasoned foods but more recently other forms of spicing have become well recognized Because of some desirable characteristics. In addition to ground spices the food processing Industry use considerable quantities of soluble spices and essential oils and to a lesser extent, aromatic chemical compounds. Of great importance are the ground spices which are used widely specially in meat, Bakery and caned products. Ground spices are available in wide range of particle sizes varying from cracked spices (pepper) to very finely milled spices averaging 10-50 microns. Many method of grinding spices are commonly used but the factor, which determines method for particular spices include grinding rate, Power requirement and the amount of heat generated and transferred to the ground spice. The amount of heat and aeration determine to a large extent, the loss of volatile constituents during the grinding operation. Some of the very oily spices such as nutmeg are difficult to grind to a fine mesh size with conventional grinding technique (Anjaneyulu *et al.*, 2007).

2.10.1.4 Soluble spices

Soluble spices are made by mixing spices extractives from one or number of spices with a soluble carrier such as sucrose, dextrose, salt or MSG. either the volatile oil from distillation or the oleoresin from solvent extraction or both are mixed with a soluble carrier in approximately the same concentrations as they occur in nature. Since the characteristics flavor comes from both volatile oil and oleoresin in most spices, a blend of both fractions will result in soluble spice with a truer flavor than when either is the sole source of spice flavor. Spice extractives also been mixed with non - soluble carrier such as dehydrated onion and garlic, other spices and occasionally with drying agent such as calcium silicate (Anjaneyulu *et al.*, 2007).

2.10.1.5 Spice oils

Spice oil include the essential oil and non- volatile fraction known as oleoresin. Essential oils are commonly used in pickle and to a limited extent to catsup. The food manufacturer has four form of spice flavor with which to season his products; ground spices, soluble spices, essential and aromatic chemicals (Anjaneyulu *et al.*, 2007).

2.10.1.6 Flavor

Ground spices are usually very good unless they are exceptionally old or have picked up off-flavor during storage. Ground spices are considered the most stable of these four forms of spice but exposure to air and light can cause considerable loss of flavor in storage, especially in very finely ground material. Soluble spices can be very good, providing the essential oil and or the oleoresin are of good quality. These can vary as much as a ground spices but since they are compounded, there is ample opportunities for standardizing the flavor. Soluble spices can be much more uniform than ground spices. For soluble spices sucrose, dextrose and MSG is better carrier than salt. Essential oil can be good but the addition of oleoresin usually improves the flavor, making it resemble the original spice flavor more closely. Aromatic chemicals, Poorest of the four type. They are used in occasion to extend essential oils as cinnamic aldehyde in oil of cassia. These react much the same as essential oil but often-cinnamic aldehyde will change to a mixture having a bitter almond taste (Anjaneyulu *et al.*, 2007).

2.10.1.7 Salt

Salt is a non-meat ingredients added to meatballs. Salt which decreases water activity reduces microbial growth in most instances increases self-life and improves flavor. In sufficient concentration, salt inhibits microbial growth as the result of the increasing osmotic pressure of the medium of the food, which is also reflected in lowering the water activity. Some bacteria are inhibited by concentration as low as 2% and these microorganisms are referred to as salt tolerant. Many of the micrococci and bacillus Species are examples. Salts serves as a preservatives by retarding bacterial growth thereby functioning as bacteriostatic rather than bactericidal agent. Bacteriostatic effectiveness is dependent on brine concentration in the comminuted meat products (Anjaneyulu *et al.*, 1989).

2.10.1.8 Phosphates

Food grade phosphates are used in additives in many phases of meat packing industries. Among their functions are moisture retention emulsification and sequestration role as Well as participation in the curing and preservation of meat color, flavor and tenderness. The phosphates used are sodium tri polyphosphates, for cured meats phosphates are usually added to the pickling solution, which is injected or soaked into the meat. Phosphates, which reduces moisture loss during processing and improve firmness (Food and food production encyclopedia) 3% fat are generally used for the processed meat product such as sausage (Anjaneyulu *et al.*, 1989).

2.10.1.8.1 Effects of polyphosphates on water retention capacity of meat

There appears to be more or less continuous relationships between water retaining capacity of a sample of meat and the solubility of actomyosin through treatment with appropriate salt solutions .In this respect, polyphosphates are especially solubilizers. The poly phosphates may be divided into several types according to extent and mode of polymerization of the phosphates residues. Pyrophosphate sand Tripoli phosphates have a dissociating effect similar to ATP on the actomyosin. In the presence of univalent cation as Na^+ or K^+ or divalent cations such as Mg^{++}. They are capable of breaking some of the bonds between actin and myosin filament formed at rigor and allow the space between the filaments to enlarge (Anjaneyulu *et al.*, 1989).

When the meat is aged, a slow increase in water retaining capacity occurs. Aged meat losses less juice alter freezing and thawing than meat frozen shortly after onset of rigor. The aging effects do not appear to be due to breakage of the bonds between the actin and myosin filaments (Anjaneyulu *et al.*, 1989).

2.10.1.9 Frying fats and oils

Deep fat frying is a process of cooking involving the direct transfer of heat from hot fat to cold food. Because of direct application of heat from the frying fat to the food, the cooking process is rapid. When cold food is dropped into hot shortening, several thing occur.

a. Heat continues to transfer even after the food is cooked and is removed from the kettle.
b. Moisture from the food starts to form steam, which is evaporated with a bubbling action that gradually subsides as the food becomes cooked.
c. A desirable browning or caramelization of the surface of the food takes place.
d. The food absorbs fat during the cooking process. Usually from 4% to 30% of the finished weight of the cooked fried food is absorbed fat. The amount of fat absorbed is affected by the time the food takes to cook, the surface area of the food, the finished moisture content of the product and the nature of food (Lawson, 1995).

Most food fry properly at a range of 163°C-191°C. Temperature close to 204°C ordinarily produce a browned surface before the inside is completely done. By the time the inside is properly cooked, the food is burned on the outside. Generally, frying should never be done above 204°C. Most foods can be fried at 177°C in food service operation (Lawson, 1995).

2.11 Quality attributes of meatballs

The buyer may define quality of foods as the composite of those characteristics that differentiate individual units a product and have significant in determining the degree of acceptability of the unit. Thus overall quality of the good product not should be, analyzed for its components attributes, each of which should be measured and controlled independently (Hsu and Chung, 1998a).

Meatballs, patties, sausage like meat products have their typical characteristic and basic knowledge of their important attributes is necessary for sensory evaluation. The important meat attributes to be assessed are appearance (color) flavor, juiciness, texture and tenderness. Knowledge of these attributes is of prime importance (Hsu and Chung, 1998b)

Warm meatballs (after frying) are used to evaluate the above attributes. The quality of the fried/cooked products varies markedly with the type of heat treatment and time of frying. Although flavor color and texture are all important quality attributes of cooked meats but it is well established that texture factor usually indicates the method of cooking is paramount importance and this quality (Hsu and Chung, 1998b).

2.11.1 Appearance

Surface structure and the overall shape of the comminuted meat have an important bearing on their appearance. Degree of doneness is appearance parameters seen in hamburger. Consumers relate color to determine doneness in cooked meat patties (Mancini and Hunt, 2005).

2.11.2 Flavor / aroma

Flavor is a complex sensation comprising mainly of odor and taste, odor being more important. It is sensed collectively by the oral and olfactory and senses. There are four basic tastes viz. sweet, salty, sour and bitter. For sound odor perception, the sample should be smelled first, followed by tasting. Of all the attributes, flavor has a profound effect on overall acceptability of meat product (Jha, 2010).

The flavor of meat and meat products is affected by many factors such as species, age, sex, pH, condition of storage, method of cooking and ingredient added in the processed added. Meat flavor, like aroma, is very difficult to evaluate and describe. It is hard to separate these two characteristic since many of the flavor properties are really the result of odor sensations. When the odor effect is reduced or removed, meat flavors are extremely difficult to distinguish. The flavor of the raw meat is weak, salty and blood like; the true meaty flavor develops during cooking. The nature and intensity of meat flavors depend in part on the type, length of time and temperature of cooking (Lawrie, 1985).

2.11.3 Texture and tenderness

Claus *et al.* (1989) Defined the texture as the attribute of substance resulting from a combination of physical properties perceived by the sense of touch, sight and hearing. The physical properties include size, shape, number, nature and continuation of constituent elements. Thus, texture encompasses all properties of food, which are perceived by kinesthetic and tactile senses in mouth example tenderness, density, granular structure, fragility, humidity etc.

Claus *et al.* (1989) Categorized the textural components of animal foods as

a. Mechanical characteristic which relate to the reaction of the food to stress example, hardness, brittleness, gumminess, chewiness, elasticity and cohesiveness etc.
b. Geometrical characteristics, which relate the shape, size and orientation of particles with meat e.g. Coarseness, grittiness, fibrous, cellular, etc and
c. Other characteristics which relate to moisture and fat perception of meat e.g. Greasiness, oiliness, watery, dry, moist, etc. The degree of tenderness may be evaluated as the number of chews required masticating the sample.

2.11.4 Juiciness

Meat juiciness is an attribute having two organoleptic components. The first one is impression during initial chews, because of rapid release of meat fluid, the second being the sustained juiciness due to stimulatory effect of fat on salivation. A good quality meat is juicier than poor quality due to higher content of intramuscular fat. Fresh frozen meat with high ultimate pH is quite juicy (Karmer and Twigg, 1973).

The degree of shrinkage on cooking is inversely proportional to the juiciness of meat. Juiciness and tenderness are closely related to meat attributes. Overall acceptability of a meat product is not the sum average of all the eating quality attributes. This is so because some attributes influence the overall acceptability of the product as compared to others. Juiciness in meat product is largely determined by combined effect of fat, moisture and Salt (Decker *et al.*, 1986).

2.11.4.1 Water binding in comminuted meat products.

The batters of comminuted meat products are complex colloidal suspension of meat and fat particles partially extended with solubilized proteins. Myosin is the primary constituents responsible for binding of water and fat particles. Manufacturing comminuted meat products with proper textural properties is related to the functionality of the muscle proteins in three dimensional matrixes. Formation of this matrix in sausage batter is due to interaction between protein-water, protein-protein and protein-lipid. Proteins are the major structural components in the system; they combine and develop the structure by binding water and fat. Various proteins are added to emulsion type sausage batter to balance the quality and quantity of protein. With processing, functionality, nutritional value and cost (Honikel, 1983).

2.11.4.2 Mechanism of water binding in comminuted meats

Proteins in the comminuted meats must bind water and fat and form a firm, elastic gel. The WHC of comminuted meat product is affected by pH, temperature, ionic strength, extent of muscular and connective tissue disruption and other factors. The amount of water held is affected by the comminution. At higher degree of comminution and tissue disruption, there is a greater amount of protein extraction, more protein- water interaction and an increased amount of water binding. Comminution process can be considered as effective, if the maximum amount of proteins is released from myofibrils. During comminution and physical disruption of muscular tissue at ionic strength above 0.6, intense fiber swelling was observed with myosin polymerization and solubilization. Efficient comminution of lean muscle tissue must disrupt membranes and sarcolemma release myofibrils and myofilaments and to accelerate swelling and extraction of myofibriller proteins .Extraction of myosin and actomyosin accelerated by the presence of sodium chloride and phosphates increase protein-water interaction and water binding (Honikel, 1983).

During comminution a local increase in temperature to 400°C and higher at edge of knife blades can decrease WHC of sausage batter .meat pre-blending increased the level of protein extraction and improved water and fat binding properties. grinding and comminution increases WHC of meat as a result of increasing the number of polar groups available for binding water molecules a decrease in water binding capacity of sausage batter is possible if the time after comminution and heat treatment is prolonged and the binding temperature is too high .The reason for WHC decreased is the change in pH. Because of fast microbial growth of lactobacilli and micrococcus that are predominant in sausage batter, water-binding capacity could decrease markedly. The pH of sausage batter can drop notably within a few hours as a result of accumulation of acids, especially if sugar was added (Honikel, 1983).

In comminuted meat, a lower level of water was released by pressure. The rapid drop in pH in pale soft and exudative (PSE) meat leads to a reduction of WHC; it is recognizable from the wet, watery cut surface of the meat. Consequently, PSE meat has a poor functionality specially water retention in CMP (Hsu and Yu, 1999).

2.11.4.3 Effect of sodium chloride and phosphates on water binding in comminuted meats

The physical and the chemical properties of the meat proteins are influenced by ionic strength. The studies of the influence of various salts showed that protein functionality is dependent on the balance of interactions between protein, water and salt. The capacity of the meat proteins to retain water is affected by the ionic strength of the medium. As the concentration of neutral electrolytes is reduced, the WHC is increased. The effect of sodium chloride on the WHC of meat is utilized in the manufacturing of sausage batters and during the curing of meat. Salt not only increases WHC but also liberates the proteins of the myofibrils (salt soluble proteins) they can function as emulsifier. The increase in WHC on the addition of NaCl is considered to be related to binding of chloride ions to the myofibriller and the sarcoplasmic proteins. Proteins can retain more water if chloride ions are bound to proteins (Kondaiah *et al.*, 1985a)

The effect of NaCl and pH on WHC can be explained by the changes in the electrical charges of the myofibriller and sarcoplasmic proteins. The ability of the meat to hold water is greatest during the few water after slaughter (hot meat), and it rapidly declines to the minimum level after 24 hr. Alkaline phosphates added to lean meat effectively increased the WHC in proportion to their ionic strength and increased the pH of the meat. Certain phosphates (diphosphates) have synergistic effects. Phosphates cause dissociation of actomyosin, increased solubility of myosin and as a result increase the extraction of proteins. Pyrophosphates formed by the breakdown of larger poly phosphates and NaCl influence the strength of binding of myosin heads to actin, which leads to the dissociation of actomyosin. The phosphates (tetra sodium pyrophosphate and sodium tri polyphosphate) increase the pH about 0.2 units, which accelerates the water retention. The effects of the phosphates on proteins include increased pH and ionic strength and inter action with proteins that cause dissociation of actomyosin by pyrophosphates (Kondaiah *et al.*, 1985a).

2.12 Basic operation of meatball making

2.12.1 Mincing/ grinding

Several methods of comminution have been employed in the processing of ground meat Products, which include mincing, chopping, slicing and flaking. Meat chunks are of variable sizes, shape, and with variable fat contents are ground to Form uniform cylinder of fat and lean. The worm or screw feed in the barrel of the grinder conveys the meat and presses it into the holes of the grinder plate. The size of the hole in the grinder plate determines the diameter, and the thickness determines the length of the cylindrical particle. Proper mixing of these particles is extremely important to obtain a uniform blend. Which is necessary requirement if the premixed or pre-batching technique is to be used. Mincing completely destroys the muscle structure, percent extractible protein increased with each step of muscle destruction during grinding and closer contact between meat surfaces added in reducing cooking losses and increases binding strength .Also fat are ground separately the particle size of ground meat varies from 3 mm to as small as 1mm (Hoogenkamp, 1997).

2.12.2 Mixing

Mixers mix the products to incorporate all of the ingredients. Cylinders of fat and lean meat obtained by grinding are tumbled in a mixer to give a uniform distribution of fat and lean particles. This can be used for sausage. Also mixing have been employed in an attempt to increase binding through enhance myofribilar protein as mixing and massaging Causes disruption of muscle fiber. The effect is still more if the massaging is done after conjunction of salt and phosphate. Also during mixing other ingredients like spices, seasoning, phosphate e.t.c can be added to obtain the desired texture and uniformity of composition (Theno *et al.*, 1978).

2.12.3 Frying

Deep fat frying is a process of cooking involving the direct transfer of heat from hot fat to Cold food. Because of direct application of heat from the frying fat to the food, the cooking process is rapid. When cold food is dropped into hot shortening, several thing occur

a. Heat continues to transfer even after the food is cooked and is removed from the kettle.
b. Moisture from the food starts to form steam, which is evaporated with a bubbling action that gradually subsides as the food becomes cooked.
c. A desirable browning or caramelization f the surface of the food takes place.
d. The food absorbs fat during the cooking process. Usually from 4% to 30% of the finished weight of the cooked fried food is absorbed fat. The amount of fat absorbed is affected by the time the food takes to cook, the surface area of the food, the finished moisture content of the product and the nature of food.

Most food fry properly at a range of 163°C - 191°C. Temperature close to 204°C ordinarily produce a browned surface before the inside is completely done. By the time the inside is properly cooked, the food is burned on the outside. Generally, frying should never be done above 204°C. Most foods can be fried at 177°C in food service operation (Lawson, 1995)

2.13 Physical properties of meat and meatball

Water binding capacity of meat refers to the ability of meat to retain its own inherent water or added water during such operations as pressing, heating, chewing and mincing. Meat is able to bind water because the highly polar water molecules are attracted to the muscle proteins by ionizable basic and acidic groups. The pH of the muscle just after death is about 7 and then decrease sharply until pH reaches 5.5 which is isoelectric pH of the meat and at this condition the water holding capacity is pretty low. The WHC can be measured in various ways and the WHC entirely does not however depend only on the method used but also on the condition of the treatment being given to the meat. 1n the slaughter house water binding is under stood as meaning the Juice holding capacity of meat, when it is being boiled or fried, we speak of cooking loss. When meat is being processed into meat product, WHC means the ability of the meat when in a comminuted and salted condition to bind its moisture content or the water added to it, so that there will no jelly deposit. When determining the juice holding capacity of fresh meat the most suitable method is to determine drip loss, whereas the WHC of heat treated meat is best determined by finding the cooking loss under the temperature-time conditions appropriate (Huang *et al.*, 2005).

Tenderness, juiciness, color, taste and shrinkage on cooking and drip on freezing, thawing all appear to be directly related to WHC of meat (Honikel, 1983).

2.14 Processing yield

Large quantities of juice can be lost when cooking meat because of shrinkage (up to 40% or more). This affects the cooking yield largely. At temperature of up to 60℃, shrinkage occurs transversely to the direction of the fibers and at higher temperature this also happen when shrinkage is along the fibers. The losses due to the shrinkage on cooking, however, will be greater- to an extent determined by such extraneous circumstance as method, time and temperature of cooking. The low frequency ultrasonic treatments on patties, meat rolls facilitates binding strength and thus decrease cooking loss and increase yield by disruption of tissues and thus increasing the extraction of salt soluble protein from grind meat. But ultrasound had no effect on the extractability of water soluble protein (Anjaneyulu *et al.*, 2007).

Part III

Materials and methods

3.1 Materials

3.1.1 Meat

Fresh buff meat was purchased from the local market of Dharan. The age of buffalo slaughtered for meat was about 12-15 yrs. Only meat from round parts was separated and trimmed. Further trimmings obtained lean meat with minimal connective tissue.

3.1.2 Soy flour

Soy flour was purchased from the local market of Dharan. It was manufactured by Pashupati Biscuit Industries Pvt. Ltd., Duhabi, Sunsari.

3.1.3 Corn starch

Corn starch packaged by Trishul Ancillary Products and Packaging, Birgunj-16, Nepal was purchased from the local market of Dharan.

3.1.4 Oils

Sun flower oil manufactured by Bagmati Oil Industries was purchased from the local market of Dharan.

3.1.5 Salt

Aayo iodised salt distribued by salt trading corporation, Kathmandu was purchased from the local market of Dharan.

3.1.5 Other ingredients

The MSG, phosphate (STPP), and chilli powder were weighed out as required. Garlic (fresh), ginger (fresh), onion (fresh), black pepper were finely grounded in a electric grinder and weighed. All ingredients were purchased from the local market.

3.2 Equipment

3.2.1 Meat mincer

National meat grinder

Model- MK- G 10 N

Matsusita Electric Industries Company limited, Japan

3.2.2 Plywood boards

A pair of locally made plywood boards of length 5″and breadth 3" with sunmica® laminate with Screw was used for the estimation of WHC.

3.2.3 Cutting knives

Stainless steel cutting knives from meat plant of central campus of technology (CCT) were used.

3.2.4 Chopping boards

Chopping boards from meat plant of CCT were used.

3.2.5 Frying pan and kitchen ware

Frying pan and kitchen ware of central campus of technology were used.

3.2.6 Stainless steel bowl and other equipment

Stainless steel bowl and other equipment of central campus of technology were used.

3.3 Methods of meatballs making

3.3.1 Recipe formulation

Different recipes are formulated. Recipe formulation was done by using Design Expert ®10. The variation made in the formulation was in the proportion of buff meat, soy flour and corn flour incorporation. The amount of other ingredients were kept constant for all the formulation of samples. The different recipe for meatballs are presented in Table 3.1.

Table 3.1 Different recipe of samples

S.N	Code	Raw meat (g)	Soy flour (g)	Corn starch (g)
1.	A	200	0	50
2.	B	225	25	0
3.	C	250	0	0
4.	D	200	25	25
5.	E	175	25	50
6.	F	175	50	25
7.	G	250	0	0
8.	H	200	0	50
9.	I	200	50	0
10.	J	150	50	50
11.	K	216.67	16.67	16.67
12.	L	225	0	25
13.	M	200	50	0

The total weight of meat ball batter is 250 g for all the formulation. The amount of other ingredients which were kept constant for all the samples is shown in the Table 3.2.

Table 3.2 Amount of other ingredients.

Ingredients	Amount in the formulation
Ice, g	25
Onion, g	5
Garlic, g	2.5
Ginger, g	5
Salt, g	5
MSG, mg	12
Phosphate, mg	5
Chili powder, mg	12
Black pepper, mg	5

3.3.2 Flowchart for preparation of meatball

Buffalo meat (Lean meat)

↓

Preliminary treatment

(Washing, trimming, cutting)

↓

Mincing

(Through 3&5 mm plate)

↓

Addition of Soy flour and corn flour according to recipe → Fine Chopping

(With or without addition of water, 10%)

↓

Addition of spices → Mixing uniformly

↓

Kneading and fisting

Till cohesive and good binding mass results

↓

Forming of meatballs

↓

Deep frying 175±10°C/4±1 min

↓

Cooling, Packaging and Storage

Fig. 3.1 Flowchart for preparation of meatball.

3.3.3 Mincing and mixing

The buff meat brought from the market was first washed, then trimmed to remove skin and separated with help of knife into different parts. Again the trimmings were done to separate connective tissue.

The lean buff meat was taken after washing the buff carcass. This lean meat part was used for making meatball according to recipe formulation. Every portion of meat was minced through meat mincer (3 mm plates) and meatball was formed according to formulation. The minced meat, soy flour, corn flour, phosphates and seasoning were weighed accurately and used for making meatball.

3.3.4 Fisting

The weighed minced meat, starch and seasonings with phosphate or without phosphate were mixed uniformly for making meatball. Then the batter was mixed properly and fisted manually for about 10-15 min so that the protein released from minced meat adhere all the ingredients together.

3.3.5 Frying

The meatballs on the wire mesh were deep-fried in frying pan containing oil under gas flame. The frying temperature determined by using digital thermometer was in the range l75±10°C.

3.3.6 Cooling

The products after frying were allowed to cool up to room temperature.

3.3.7 Packaging

From the sensory analysis at 5% level of significance the best formulation was analyzed and packaging was done. The most accepted formulation frying was packed in polythene and then vaccum-sealed and then proximate analysis were carried.

3.4 Sensory evaluation

The fried samples of the meatball were sensorically evaluated through hedonic rating test while still warm.

3.4.1 Hedonic rating

The coded sample of the meatballs were sensorically evaluated while still warm for appearance, flavor, texture, juiciness, taste and overall palatability on 9 point hedonic scale. The panelists were given instruction to give 9 points to extremely liked and 1 points to the extremely disliked point sample. The coded samples were randomly presented. For the above hedonic rating test, semi trained panelist of B.Tech 4th year and teachers of Central Campus of Technology were taken. Before sensory evaluation, instructions were given to panelists. They were asked to give maximum scores for its purple brown color. For texture and taste, they were instructed to give marks as they like. The various parameters tested were appearance, texture and tenderness, taste, flavor and overall palatability. The specimen card for sensory evaluation is shown in appendix A. The panelists were untrained. Differences in the quality were determined by statistical analysis according to Ranganna (1986).

3.4.2 Statistical analysis

The analyses were carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2013. All the data obtained in this experiment were analyzed for significance by Analysis of Variance (ANOVA) using the statistical program known as Genstat Release 12.1 (2009). From this, means were compared using Fisher protected LSD (Least Significance Difference) at 5% level of significance (Payne, 2007).

3.5 Chemical analysis

3.5.1 Moisture content

The moisture content was determined by using hot air oven method (Ranganna, 1986). 10 g of the grinded sample was spread over the petri-dish and placed in hot air oven previously set at103 ±20℃.

3.5.2 Crude fat

The crude fat of the raw buff meat, soy flour, corn flour and meatball was determined after extracting fat by Soxhlet extraction apparatus, using petroleum ether following the method cited in AOAC (2005).

3.5.3 Crude protein

Crude protein was determined by estimating nitrogen content in the sample and multiplied by Kjeldahl factor 6.25 according to Ranganna (1986).

3.5.4 Ash content

Ash content of the meat was determined according to K.C. and Rai (2007). 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 hr.

3.5.5 Crude fiber

The crude fiber content of the product was determined by recovering the ash free residue after sequential treatment sample with 1.25% sulphuric acid and 1.25% sodium hydroxide each under standard conditions. The ash that came along with the residue was removed by ashing in ashless filter paper (K.C. and Rai, 2007).

3.6 Processing yield

According to Kowale *et al.* (2008) for processing yield (%) of meatball, sample weight of meatballs before and after cooking was noted. The processing yield was calculated as percentage weight of cooked meatballs to weight of raw meatballs.

$$\text{Processing yield \%} = \frac{\text{Weight of meat ball after cooking}}{\text{Weight of meat ball before cooking}} \times 100\ \%$$

3.7 Water holding capacity

For determining the WHC, 500 mg weighed minced meat sample was placed between the centers of two weighed filter papers. The filter papers were kept over a rigid, flat surface and covered by PE sheet above and below it and pressed by 2.81 kg weight for 5 min. The meat flake after pressing was weighed. The filter paper was dried and weighed. WHC (%) is given as:

$$\text{WHC \%} = \frac{\text{Actual weight of meat flake}}{\text{Sample weight}} \text{ 100 \%}$$

Where, actual weight of meat flake = weight of meat flake after pressing + subtraction of weight of filter paper before and after pressing (Kowale *et al.*, 2008).

3.8 Fat and jelly separations

According to Kowale *et al.* (2008) for fat and jelly separation (%) of meatball, pre weighed can were filled with the samples (fried meatball). The cans were closed and heated for 35 in boiling water bath. After cooling in running tap water the can were stored at 4°C for 24 hr. after warming up the cans in water bath at 45°C for 1 hr. The fluid fat and jelly calculated as a percent of the original weight of the meatball.

$$\text{Fat and jelly \%} = \frac{\text{Weight of the fluid (fat and jelly)}}{\text{Original weight of sample}} \times 100\ \%$$

Part IV

Results and discussion

Buff meatball incorporated with soy flour and corn starch according to formulation was prepared in the lab of CCT. For each different formulation calculated and weighed amount of buff meat, soy flour and corn starch was mixed and chopped in a bowl chopper. Necessary spices were also weighed and mixed in the chopper with the batter. Different formulations were prepared according to Design Expert version®10. Ten different samples were subjected to sensory evaluation. The optimized product according to data of sensory evaluation was obtained and proximate composition analysis of that sample was performed. Processing yield and WHC of all the thirteen samples were also analyzed.

4.1 Analysis of raw materials

In the preparation of soy flour and corn starch incorporated buff meatball, buffalo meat, soy flour and corn starch is the major raw materials. They were analyzed for their composition. pH and WHC of raw buffalo meat was 5.5 (0.1) and 0.51 (0.0058). The proximate composition of raw buffalo meat is presented in the Table 4.1

Table 4.1 Proximate composition of raw buffalo meat

Parameter	Composition (% dry basis)
Moisture content	76.27 (0.351)
Crude protein	22.33 (0.404)
Crude fat	0.77 (0.251)
Ash	1.1 (0.264)

The values in the table are arithmetic mean of triplicate samples. Figure in the parentheses indicates standard deviation.

From the proximate analysis of meat, moisture content, protein, fat, ash content, WHC and pH were found to be 76.27%, 22.33%, 0.77%, 1.1%, 0.51 and 5.5 respectively. The values so obtained for moisture content, crude protein, crude fat and ash content were slightly different then the result obtained by Kandeepan and Biswas (2007) which were 76.9%, 20.3 %, 1.4%, and 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.*).

The chemical composition of soy flour is presented in the Table 4.2

Table 4. 2 Chemical composition of defatted soy flour

Parameter	Composition (% dry basis)
Moisture content	7.47 (0.152)
Crude fat	0.5 (0.2)
Crude protein	50.41 (0.301)
Crude fiber	3.23 (0.305)
Ash content	5.7 (0.1)
Carbohydrate	32.34 (0.45)

The values in the table are arithmetic mean of triplicate samples. Figure in the parentheses indicates standard deviation.

From the proximate analysis of defatted soy flour, moisture content, protein, fat, fiber, ash content and carbohydrate were found to be 7.47%, 50.41%, 0.5%, 3.23%, 5.7% and 32.34% respectively. The values obtained above were slightly different than that of the value presented by (USAID, 2016) which may be due to species, physiological maturity, climatic conditions of growth of soybean production etc.

The chemical composition of corn starch is presented in the Table 4.3

Table 4. 3 Chemical composition of corn starch

Parameters	Corn starch (% dry basis)
Moisture content	10.17 (0.152)
Crude fat	0.52 (0.191)
Crude protein	0.54 (0.152)
Crude fiber	0.69 (0.070)
Ash content	0.13 (0.057)
Carbohydrate	88.44 (0.208)

The values in the table are arithmetic mean of triplicate samples. Figure in the parentheses indicates standard deviation.

From the proximate analysis of corn flour, moisture content, protein, fat, fiber, carbohydrate and ash content were found to be 10.17%, 0.54%, 0.52%, 0.69%, 88.44% and 0.13% respectively. The values obtained above were slightly different then that data presented by (CRA, 2006). And this may be due to difference in variety of corn, processing factors, climatic condition during growth of corn etc.

4.2 Sensory evaluation of different formulation

Ten different samples of varying proportion of minced buffalo meat, soy flour and corn starch was taken and coded as A, B, C, D, E, F, G, H, I and J respectively. The frying temperature and time of the samples was kept constant *viz.* 175±10℃ and 4±1 min respectively. The necessary spices and condiments for the preparation of meat ball was taken constant for all the formulation. Then the formulations having different proportion of minced meat, soy flour and corn starch was subjected to sensory evaluation.

4.2.1 Effect of formulations on appearance (Color)

The mean sensory score for the appearance of the samples A, B, C, D, E, F, G, H, I and J were found to be 5.3, 5.7, 7, 7.6, 6.5, 7.4, 6.5, 6.7, 6.4 and 6.7 respectively as shown in Fig.4.1. The mean score was found to be highest for sample C (7), D (7.6) and F (7.4).

On the basis of superiority at 5% level of significance following conclusion can be drawn:

[CDF] > [HJ] > [EGI] > [B] > [A]

In the statistical analysis at 5% level of significance samples C, D and F weren't significantly different with each other and found to be superior on the basis of appearance of buff meatball.

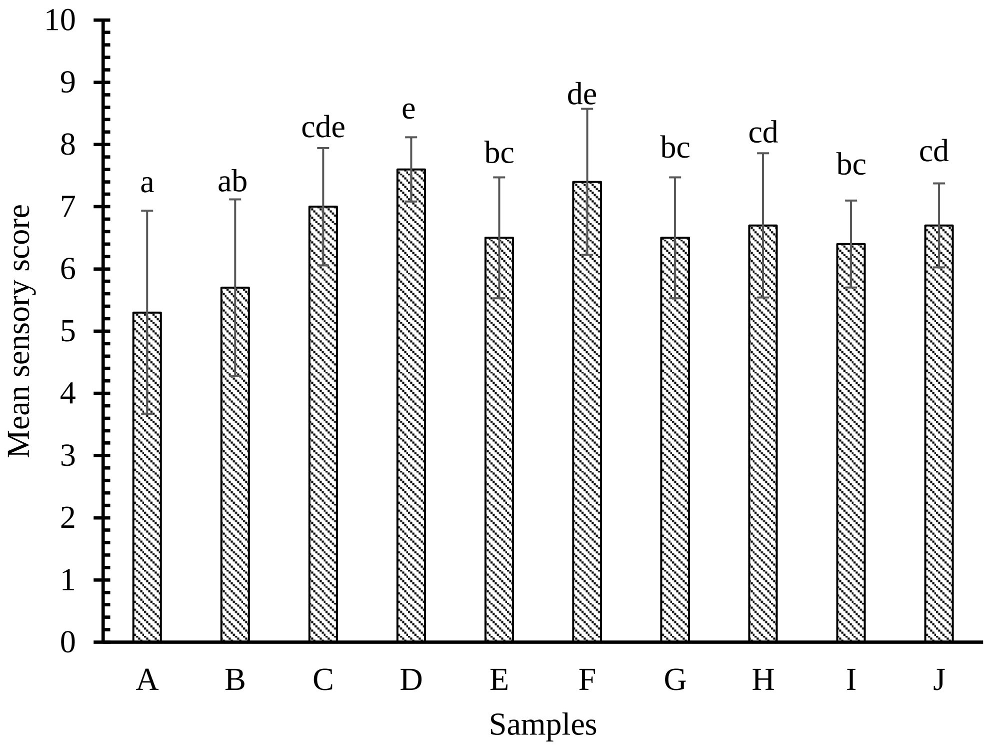

Fig. 4.1 Mean sensory scores for appearance of buff meatball

Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelists.

The apparent color is affected by the amount of water in or on the fresh meat. Proteins in meat with a low pH (<5.4) do not bind water very tightly. This free water in the tissues reflects light in many directions, or scatters it. So, it makes the meat appear very light compared to higher pH meat in which water is more tightly bound. The color of red meat can also change because of exposure to various ingredients such as vinegar & salt. The proportion of incorporated soy flour and corn starch can change the color on the product. And finally, the presence or absence of oxygen in the surrounding environment will also have an impact on the color of the meat (Mancini and Hunt, 2005).

4.2.2 Effect of formulations on flavor and aroma

The mean sensory score for the flavor of the samples A, B, C, D, E, F, G, H , I, and J were found to be 6.1, 6.3, 6.7, 7.9, 6.3, 6.8, 6.6, 6.6, 6.7 and 6.3 respectively as shown in Fig.4.2. The mean score was found to be highest for sample D (7.9).

On the basis of superiority at 5% level of significance following conclusion can be drawn:

[D] > [BCEFGHIJ] > [A]

In the statistical analysis at 5% level of significance sample D was significantly different with other samples and found to be superior on the basis of flavor and aroma of buff meatball.

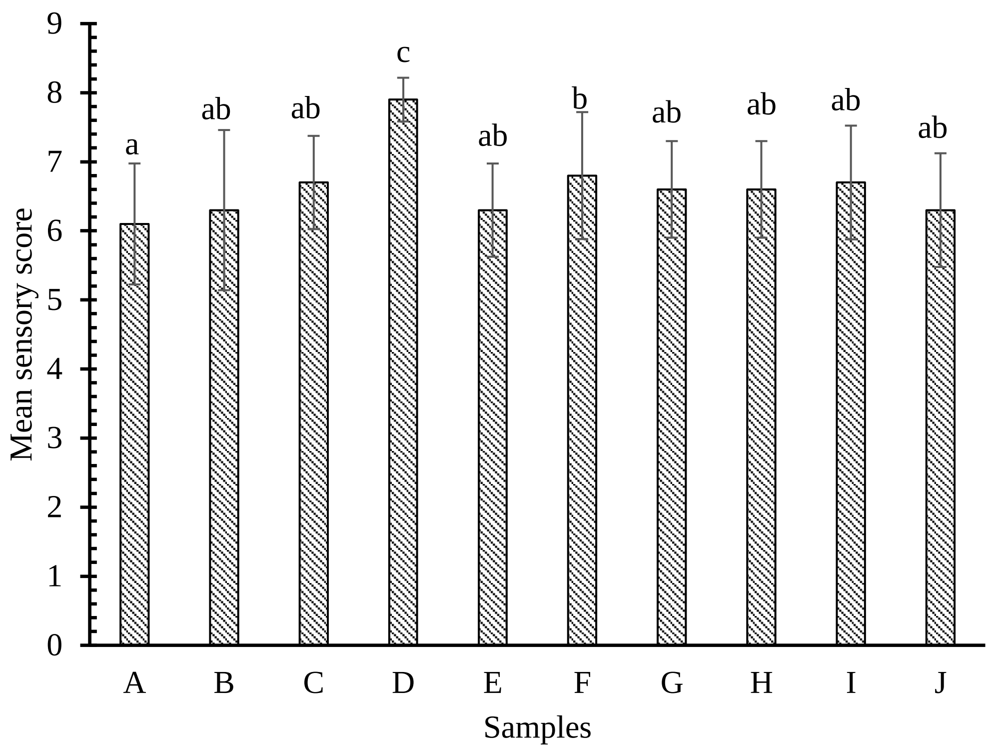

Fig. 4.2 Mean sensory scores for flavor and aroma of buff meatball

Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelist.

Flavor is a complex sensation comprising mainly of odour and taste, odour being more important (Jha, 2010). Flavor is a complex sensation of volatile components and is marked when meat is subjected to cooking (Macleod and and Seyyedain, 1981). Fat and fat soluble precursors have been shown to be implemented in accounting for species differences and contributing to meat flavor (Lawrie, 1985).

4.2.3 Effect of formulation on texture and tenderness

The mean sensory score for the texture and tenderness of the samples A, B, C, D, E, F, G, H, I and J were found to be 5.9, 6.4, 6.5, 7.9, 6.3, 6.7, 6.7, 6.2, 6.5 and 6.4 respectively as shown in Fig.4.3. The mean score was found to be highest for sample D (7.9).

On the basis of superiority at 5% level of significance following conclusion can be drawn:

[D] > [BCEFGHIJ] > [A]

In the statistical analysis at 5% level of significance sample D was significantly different with other samples and found to be superior on the basis of texture and tenderness of buff meatball.

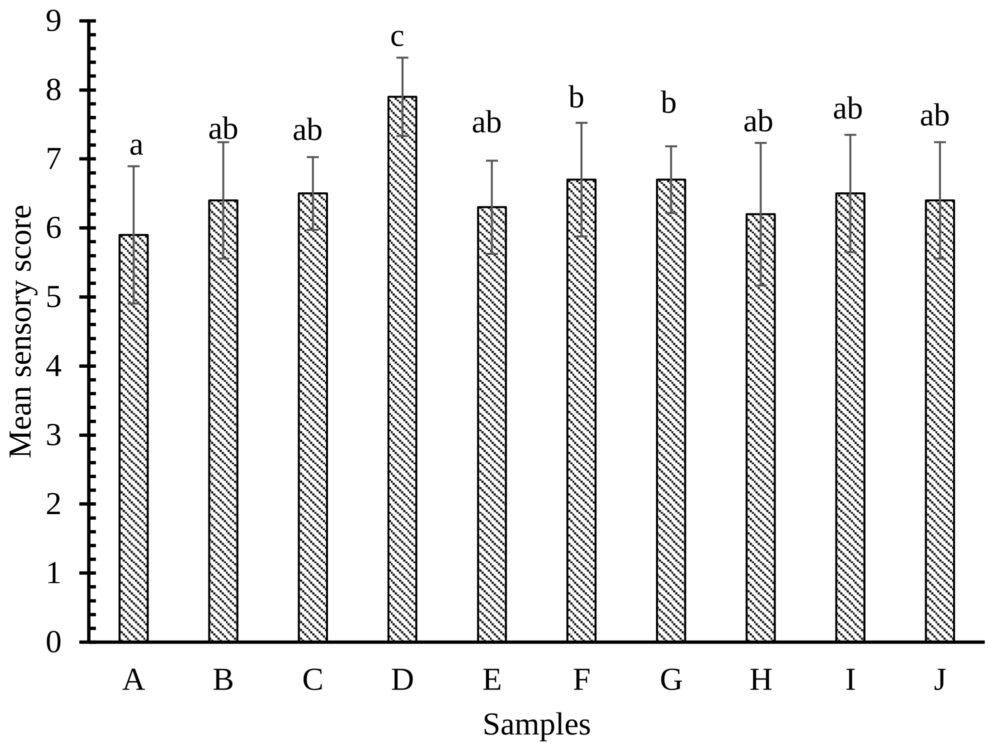

Fig. 4.3 Mean sensory scores for texture and tenderness of buff meatball

Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelists.

In meatball increased in water content with the same fat level decreased fracturability and hardness, while increased in fat with added water increased cohesiveness in all treatments except for few cases (Claus *et al.*, 1989).

4.2.4 Effect of formulations on taste

The mean sensory score for the taste of the samples A, B, C, D, E, F, G, H, I and J were found to be 5.8, 6.6, 6.6, 7.4, 6.6, 6.9, 6.7, 6.2, 6.6 and 7.1 respectively as shown in Fig.4.4. The mean score was found to be highest for sample B (6.6), C (6.6), D (7.4), E (6.6), F (6.9), G (6.7), I (6.6) and J (7.1).

On the basis of superiority at 5% level of significance following conclusion can be drawn:

[BCDEFGIJ] > [H] > [A]

In the statistical analysis at 5% level of significance samples B, C, D, E, F, G, I and J weren't significantly different with each other and found to be superior on the basis of taste of buff meatball.

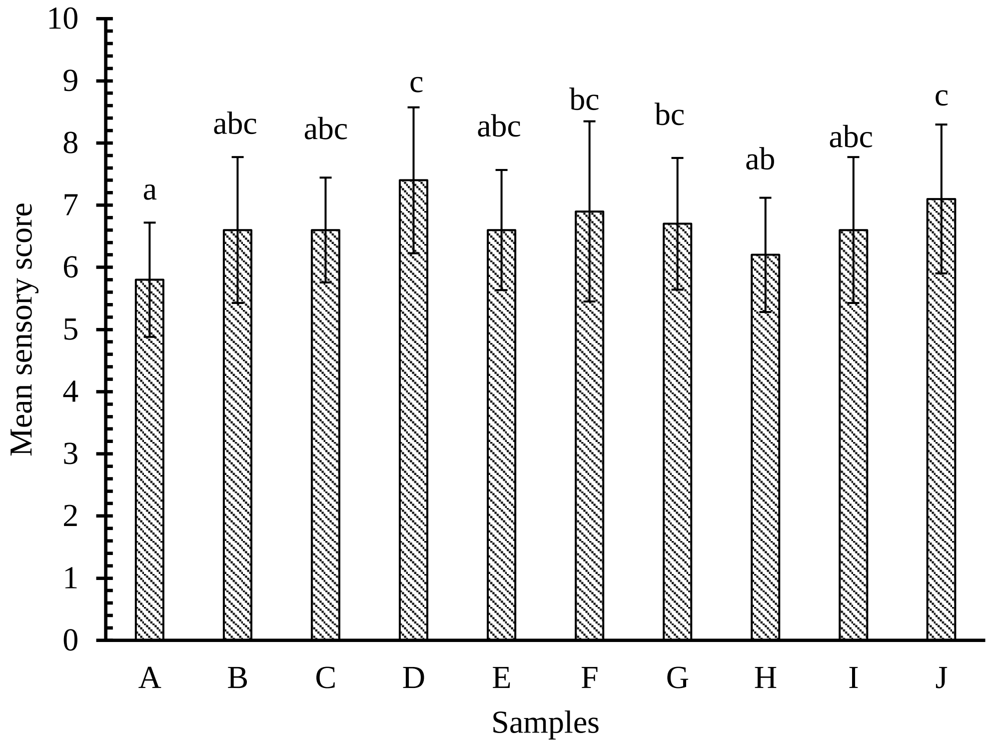

Fig. 4.4 Mean sensory scores for taste of buff meatball

Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelists.

The percentage of fat on meat, water content and the proportion of soy flour and corn starch have effect on the taste of meatballs (Ulu, 2004).

4.2.5 Effect of formulations on juiciness

The mean sensory score for the juiciness of the samples A, B, C, D, E, F, G, H, I and J were found to be 5.8, 6.6, 6.7, 7.4, 6.3, 6.9, 6.5, 6.7 and 7, 7 respectively as shown in Fig.4.5. The mean score was found to be highest for sample C (6.7), D (7.4), F (6.9), H (6.7), I (7) and J (7).

On the basis of superiority at 5% level of significance following conclusion can be drawn:

[CDFHIJ] > [BEG] > [A]

In the statistical analysis at 5% level of significance samples C, D, F, H, I and J weren't significantly different with each other and found to be superior on the basis of juiciness of buff meatball.

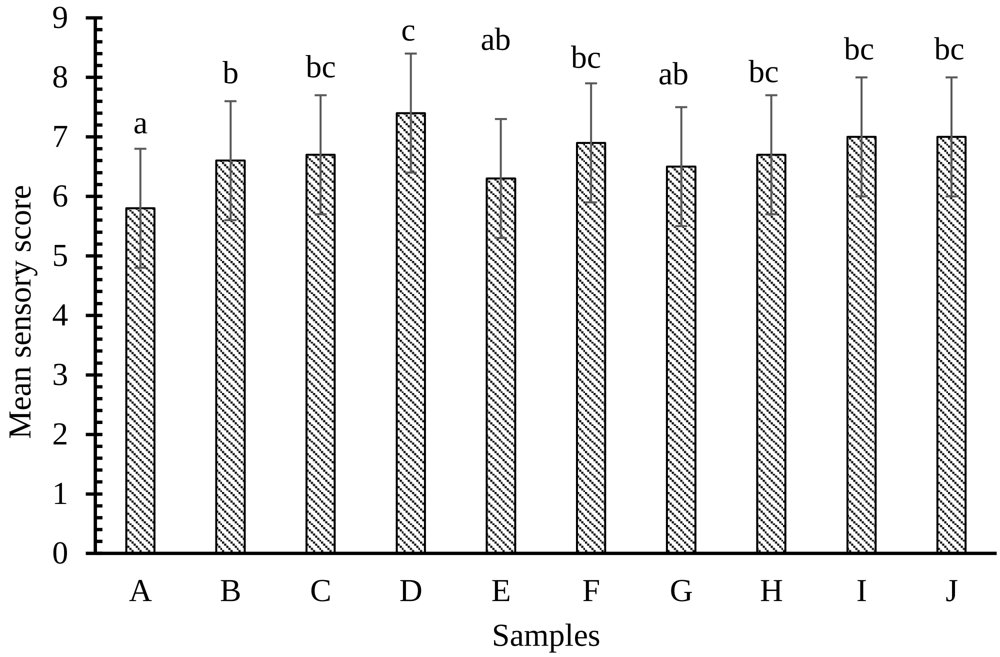

Fig. 4.5 Mean sensory scores for juiciness of buff meatball

Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by panelists.

Juiciness in meat products is largely determined by the combined effect of fat, moisture and salt (Karmer and Twigg, 1973). According to Weir (1960) it is due to initial fluid release and sustained juiciness from the effect of fat on salivary flow. Similar result was obtained by Decker *et al.* (1986) who reported that as the fat content of comminuted meats was reduced from a high (30 and 24 %) to a low level (7.4 %), sensory juiciness become significantly lower.

4.2.6 Effect of formulations on overall palatability

The mean sensory score for the overall acceptability of the samples A, B, C, D, E, F, G, H, I and J were found to be 6.1, 6.4, 6.9, 7.8, 6.7, 6.9, 6.6, 6.2, 6.6 and 6.6 respectively as shown in Fig.4.6. The mean score was found to be highest for sample D (7.9).

On the basis of superiority at 5% level of significance following conclusion can be drawn:

[D] > [BCEFGIJ] > [H] > [A]

In the statistical analysis at 5% level of significance sample D was significantly different with other samples and found to be superior on the basis of overall palatability of buff meatball.

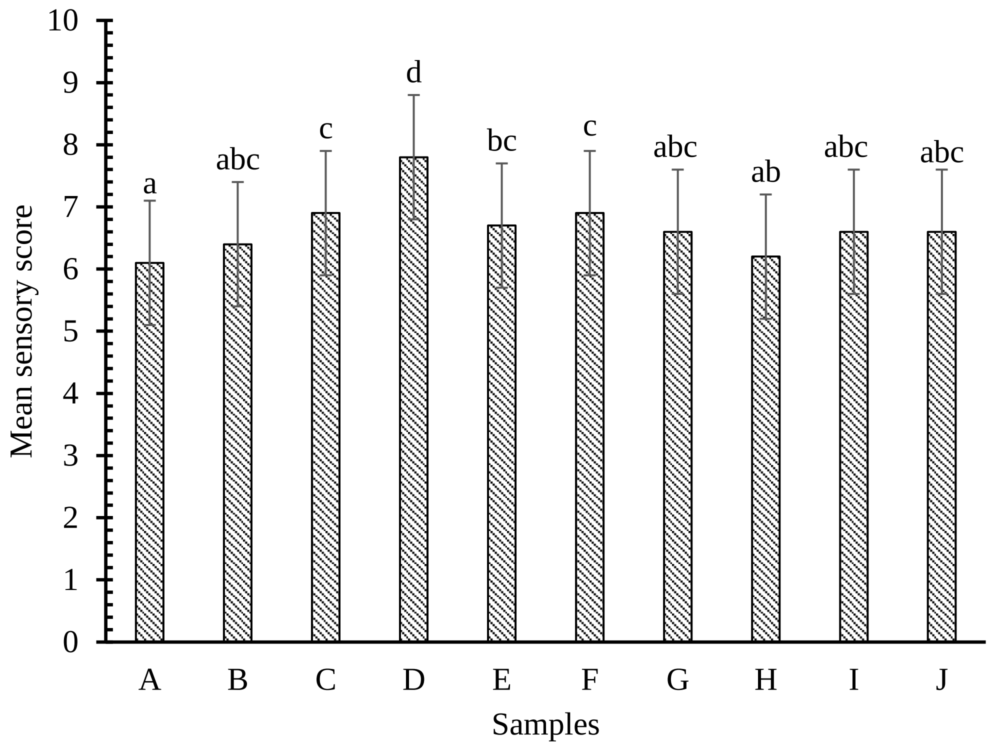

Fig. 4.6 Mean sensory scores for overall palatability of buff meatball

Values on top of the bars bearing similar superscript were not significantly different at 5% level of significance. Vertical error bars represent standard deviation of scores given by panelists.

The sensory evaluation showed that flavor, juiciness and tenderness of the sample D was much liked. Also, statistical analysis showed that higher degree of palatability for sample D.

4.3 Physico-chemical analysis of optimized meatball

From sensory evaluation sample with 80% meat (200 g), 10% soy flour (25 g) and 10% corn starch (25 g) while keeping all other ingredients constant was found to be best. Proximate analysis of optimized sample according to sensory evaluation is shown in Table 4.4.

Table 4.4 Proximate analysis of fried meatball (optimized sample)

S.N.	Parameters	Composition (% dry basis)
1.	Moisture content	55.41 (0.808)
2.	Protein	20.84 (0.229)
3.	Crude fat	3.23 (0.251)
4.	Ash content	2.87 (0.152)
5.	Crude fiber	0.2 (0.1)
6.	Carbohydrate	18.40 (0.561)

From the proximate analysis of best meatball sample, moisture content, protein content, crude fat content, ash content, crude fiber content and carbohydrate content were found to be 55.41%, 20.84%, 3.23%, 2.87%, 0.2%, and 18.40% respectively. All the parameters except fat content was found slightly different then the result obtained by Purnomo and Rahardiyan (2008) Serdaroglu and Abrodimov (2005). According to Ulu (2004) Aukkanita *et al.* (2015) fat content was found different because of incorporation of de-fatted soy flour, corn starch with low percentage of fat and due to use of only lean red meat during the preparation of meatballs. Crude fiber content was also found in the meat ball due to crude fiber content present in the incorporated soy flour and corn starch (Ulu, 2004).

4.4 Effect of different formulation on water holding capacity of buff meatball

The water holding capacity of buff meatballs varied from 50.36% to 67.51%. Table C.1, C.2 and C.3 of appendix show the coefficients of the model and other statistical attributes of water holding capacity. Regression model fitted to experimental results of water holding capacity showed that the model F-value of 15.57 was significant ($p<0.05$). The lack of fit test was not significant ($p>0.05$). The fit of model was also expressed by the coefficient of determination R^2, which was found to be 0.7569, indicating that 75.69 % of the variability of the response could be explained by the model. The predicted R^2 value of 0.5762 was in reasonable agreement with the Adjusted R^2 value. The Adjusted R^2 value 0.7083 and Adequate Precision value 11.638 showed an adequate signal. A ratio greater than 4 is desirable and hence this model may be used to investigate the design space (Myers *et al.*, 2009)

Considering all the above criteria the model (Eq. 4.1) was selected to represent the variation of water holding capacity with the independent variables and further analysis. The linear model fitted for water holding capacity obtained from regression analysis in terms of coded values of the variables is represented by Eq. 4.1.

$$WHC = + 51.92A + 64.89B + 66.17C \qquad \ldots\ldots 4.1$$

Where A, B and C are the coded values of meat (%), soy flour (%) and corn starch (%) respectively.

The positive coefficient of A, B and C indicated that the increase in meat, soy flour and corn starch content of the meatball results increased water holding capacity of the product. Effect of variation of soy flour and corn starch was significant but effect of meat was not significant compared to soy flour and corn starch. Here according to the Eq. 4.1, the positive coefficient of meat showed positive correlation between meat and water holding capacity but according to correlation Fig. 4.8 it was found that there was no any significant effect of meat compared to soy flour and corn starch which was in accordance with (Zayas, 1997). Cheng and Sun (2008), also state that carbohydrate rich plant substances have extensive water binding capacity and gelling capacity as result of which produce final product with high water holding capacity. D-optimal plot for WHC as a function of soy flour, corn starch and meat is shown in Fig. 4.7.

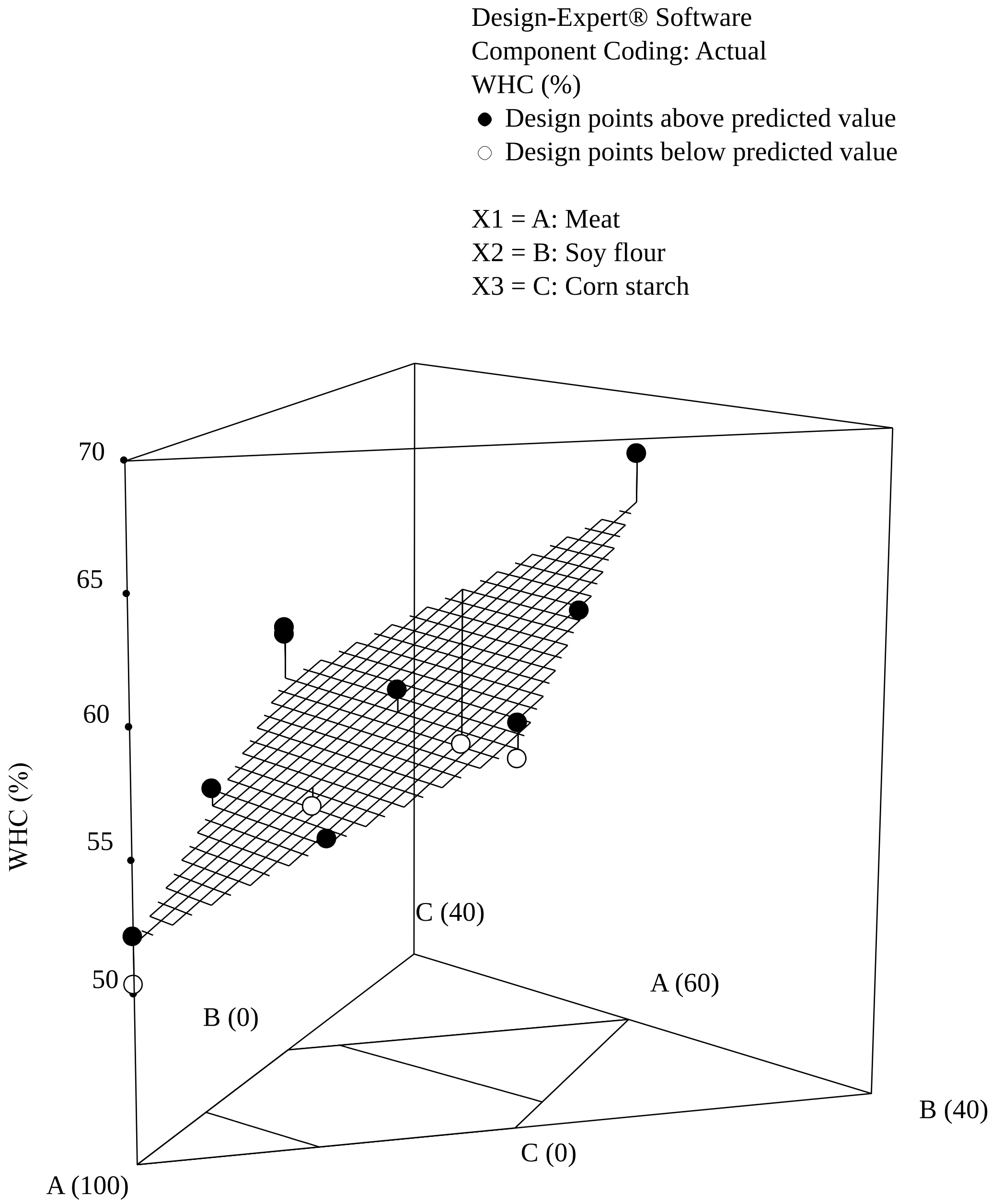

Fig. 4.7 D-optimal plot for WHC as a function of soy flour, corn starch and meat

In the research conducted by Comer and Allan-Wojtas (1988), it has been stated that there exists a competition for moisture between proteins, either meat and non -meat proteins and carbohydrates which directly influence the stability and textural properties of the comminuted meat products.

4.4.1 Correlation of meat on WHC in the formulation

Correlation of meat on WHC in the formulation is shown in Fig. 4.8.

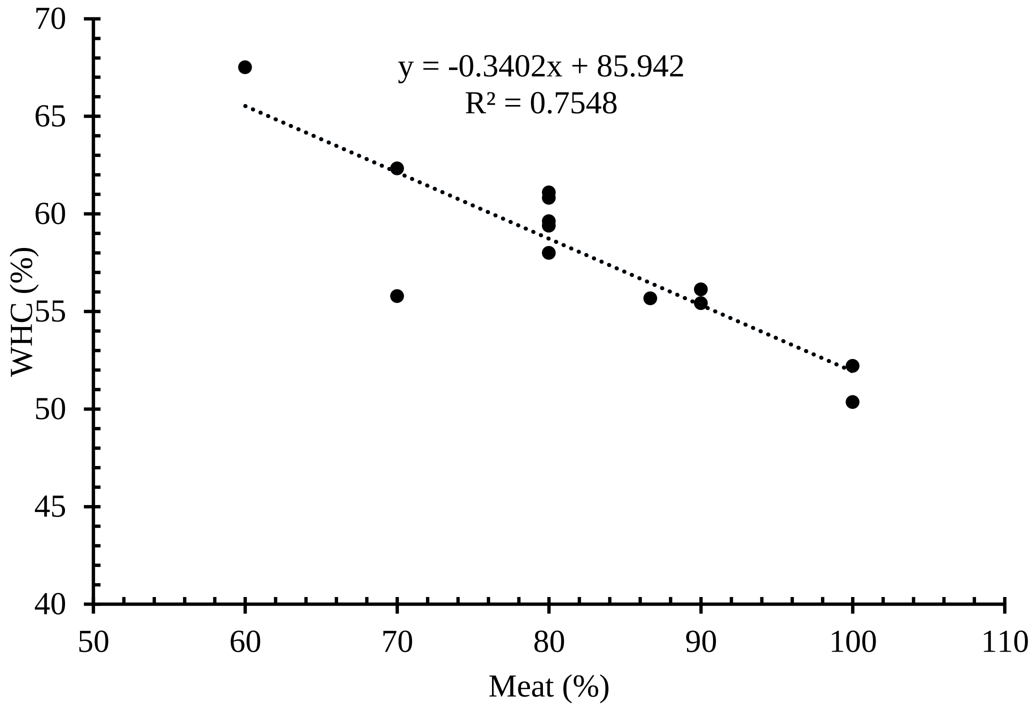

Fig. 4.8 Graph showing correlation of meat on WHC

The Fig. 4.8 shows the correlation of meat on WHC which shows that on increase in meat percentage in the formulation results decrease in WHC %. This was due to less significant effect of meat compared to soy flour and corn starch on the WHC of meatball. The result shows that WHC is inversely proportional to the amount of meat parts in meatball (Odiase *et al.*, 2013). But the result of RSM regression equation shows there is positive correlation between meat and WHC. So we can conclude that in comparison to soy flour and corn starch incorporated meatball, increase in meat proportion relatively decrease the WHC.

4.4.2 Correlation of soy flour on WHC in the formulation

Correlation of soy flour on WHC in the formulation is shown in Fig. 4.9.

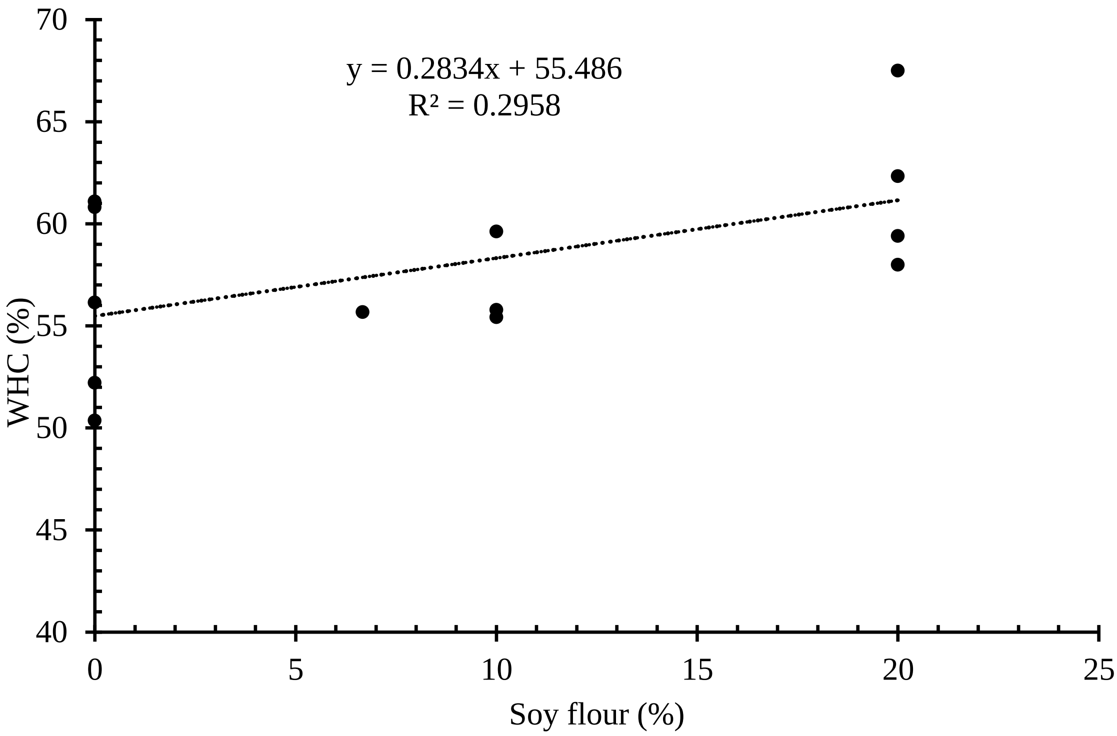

Fig. 4.9 Graph showing correlation of soy flour on WHC

ANOVA table (Table C.2, Appendix C) shows that WHC of different samples are significantly different ($p<0.05$). The increase in WHC of soy flour, corn starch incorporated buff meatball may be due to the decrease in proportion of meat and increase in proportion of corn starch. So we can say that, with increase in percentage of soy flour and corn starch in the formulation, WHC also increases because of higher water holding capacity of soy flour and corn starch than that of meat (Odiase *et al.*, 2013).

The Fig. 4.9 has R^2 value of 0.2958 which is very low. From this we can conclude that the correlation between WHC and soy flour on meatball was not significant.

4.4.3 Correlation of corn starch on WHC in the formulation

Correlation of corn starch on WHC in the formulation is shown in Fig. 4.10.

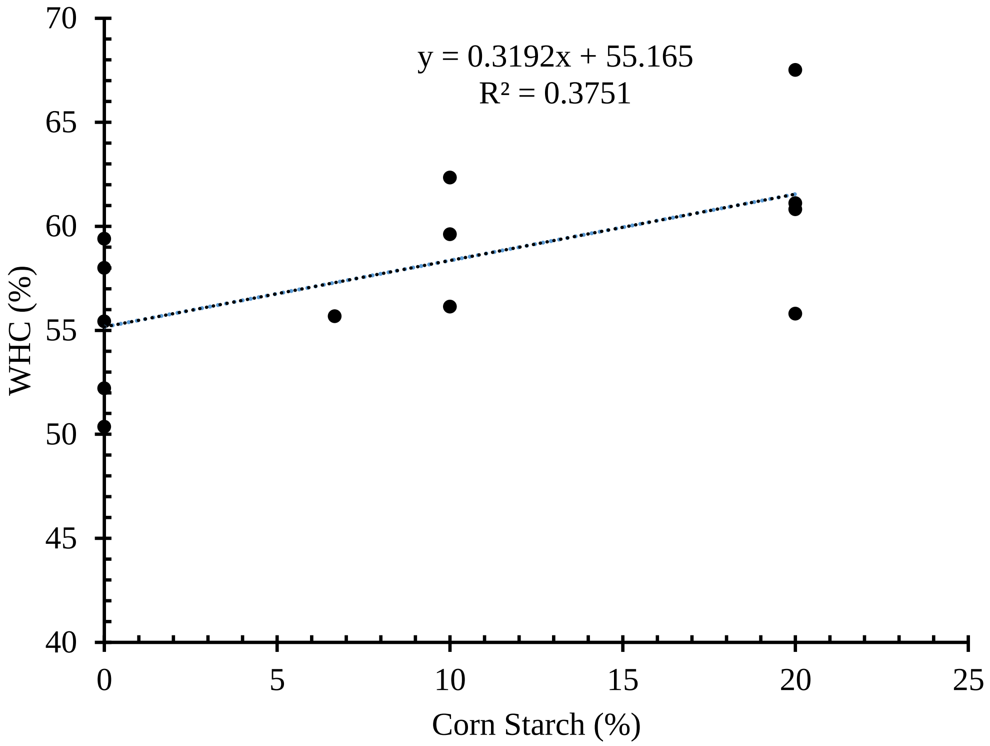

Fig. 4.10 Graph showing correlation of corn starch on WHC

ANOVA table (Table C.2, Appendix C) shows that WHC of different samples are significantly different ($p<0.05$). The increase in WHC of soy flour, corn starch incorporated buff meatball may be due to the decrease in proportion of meat and increase in proportion of corn starch. We can say that, with increase in percentage of soy flour and corn starch in the formulation, WHC also increases because of higher water holding capacity of soy flour and corn starch than that of meat (Aaslynga *et al.*, 2002).

The Fig. 4.10 has R^2 value of 0.3751 which is very low. From this we can conclude that the correlation between WHC and cornstarch on meatball was not significant.

4.5 Effect of process variables on processing yield

The measured yield of the products varied from 101 % to 119 %. Table C.4, C.5 and C.6 of appendix show the coefficients of the model and other statistical attributes of processing yield. Regression model fitted to experimental results of processing yield showed that the model F-value of 144.60 was significant ($p<0.05$). The lack of fit test was not significant (p>0.05). The fit of model was also expressed by the coefficient of determination R^2, which was found to be 0.993, indicating that 99.30 % of the variability of the response could be explained by the model. The predicted R^2 value of 0.871 was in reasonable agreement with the Adjusted R^2 value. The Adjusted R^2 value 0.986 and Adequate Precision value 44.3 showed an adequate signal.

Considering all the above criteria the model (Eq. 4.2) was selected to represent the variation of processing yield with the independent variables and further analysis. The special cubic model fitted for processing yield obtained from regression analysis in terms of coded values of the variables is represented by Eq. 4.2.

Processing yield = 101 A + 100 B + 108C + 12.7AB + 0.102AC + 58.5BC - 121 ABC………………………4.2

Where A, B and C are the coded values of meat (%), soy flour (%) and corn starch (%) respectively.

The positive coefficient of A, B and C indicated that the increase in meat, soy flour and corn starch content of the buff meatball results increased processing yield of the product. The result in processing yield was the overall effect of meat, soy flour and corn starch in the formulation. The negative coefficient of ABC indicates the combined effect of meat, soy flour and corn starch on processing yield of meatball is inversely proportional. While the combined effect of meat and soy flour, meat and corn starch and soy flour and corn starch are directly proportional to processing yield of meatball as shown in Eq. 4.2. No significant effect of meat variation in chicken meatball in the cooking yield was also reported by Roseland *et al.* (2009) and similar result was found in buff meatball. On this contrary, Young et al. (1991) reported positive correlation between meat percentage in meatball and cooking loss. The reasons for both of these findings are not known. According to Ranathunga *et al.* (2015), yield of comminuted meat product can be increased by use of fillers and binders. D-optimal plot for processing yield as a function of soy flour, corn starch and meat is shown in Fig. 4.11.

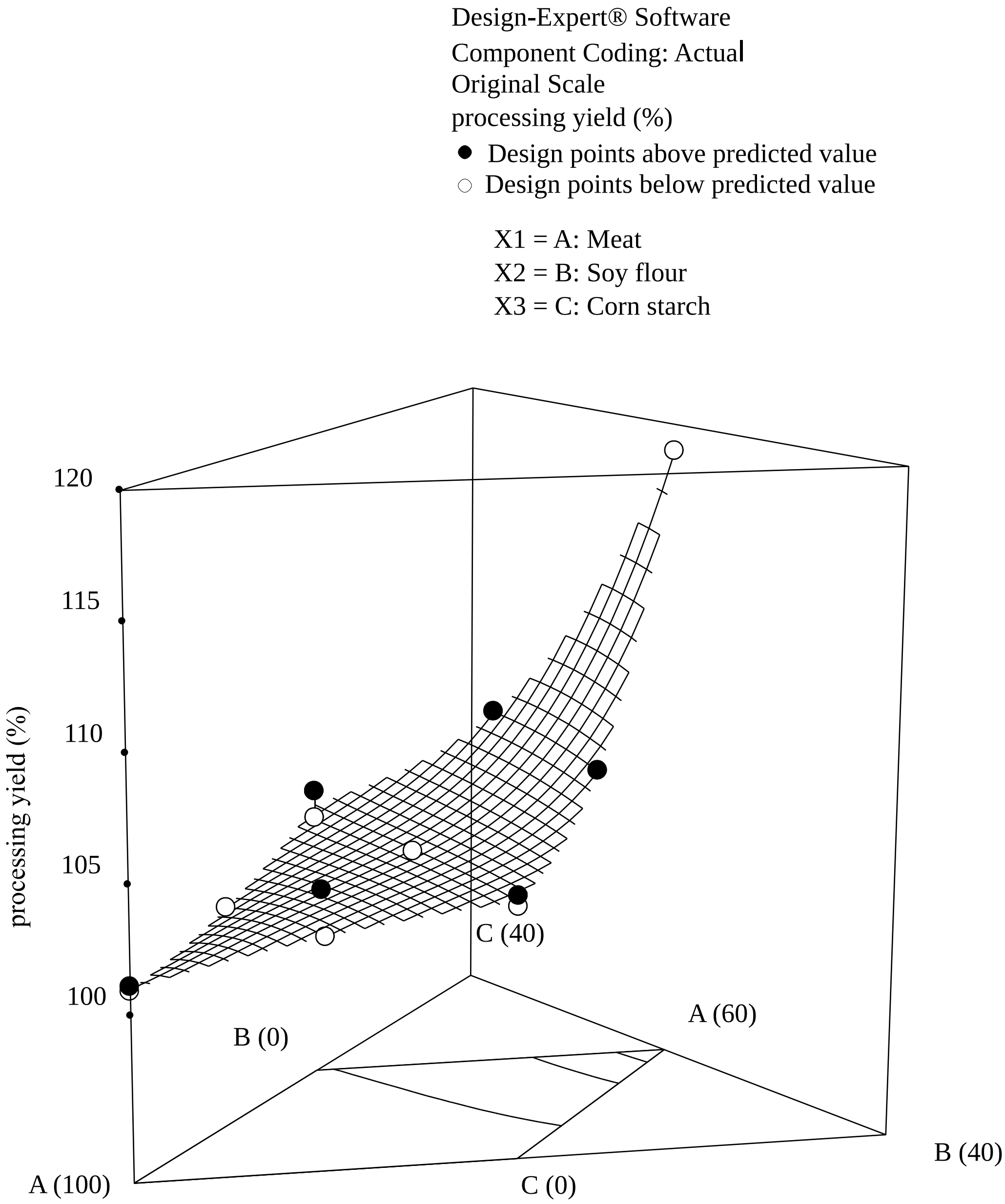

Fig. 4.11 D-optimal plot for processing yield as a function of soy flour, corn starch and meat

But Aaslynga *et al.* (2002) reported no significant effect of ingredients in cooking loss in a meat system with medium or high water holding capacity. So, the improved water holding capacity of the buff meatball by soy flour and corn starch might be the reason for no significant effect of meat as well as overall effect in cooking loss of meatball. Fig. 4.11 shows the response surface plot for the effect of process variables on the processing yield of buff meatball.

4.5.1 Correlation of meat on processing yield in the formulation

Correlation of meat on processing yield in the formulation is shown in Fig. 4.12.

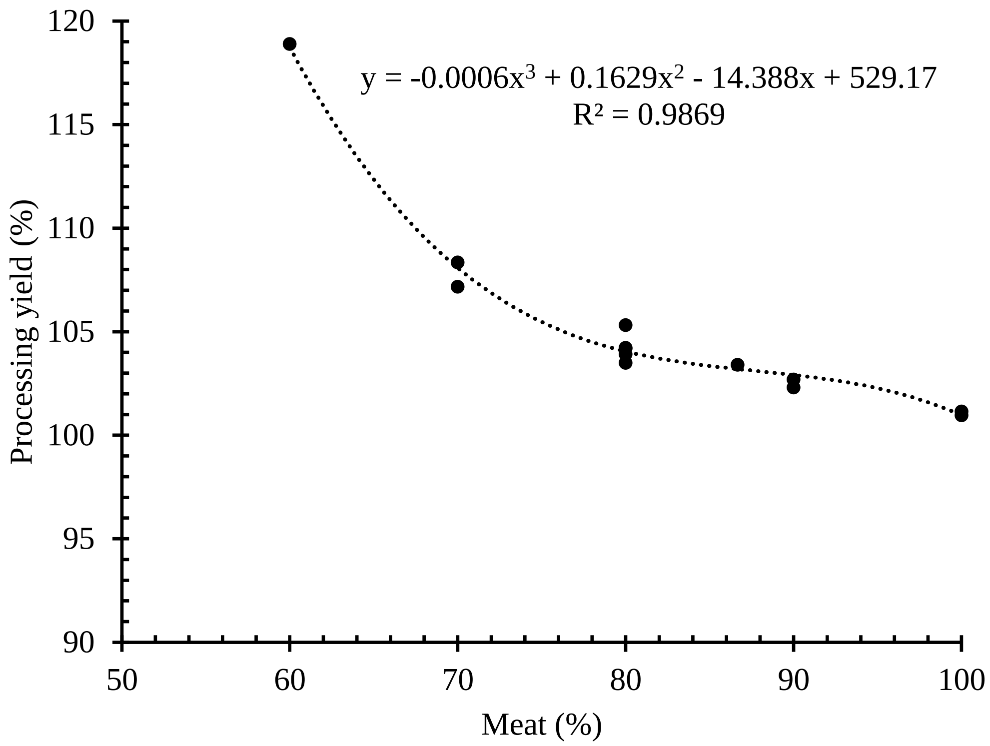

Fig. 4.12 Graph showing correlation of meat on processing yield

The Fig. 4.12 shows the correlation of meat on processing yield which shows that on increase in meat percentage in the formulation results decrease in processing yield. This was due to less significant effect of meat compared to soy flour and corn starch on the processing yield of meatball. The result shows that processing yield is inversely proportional to the amount of meat parts in meatball (Odiase *et al.*, 2013). But the result of RSM regression equation shows there is positive correlation between meat and processing yield. So we can conclude that in comparison to soy flour and corn starch incorporated meatball, increase in meat proportion relatively decrease the processing yield.

4.5.2 Correlation of soy flour on processing yield in the formulation

Correlation of soy flour on processing yield in the formulation is shown in Fig. 4.13.

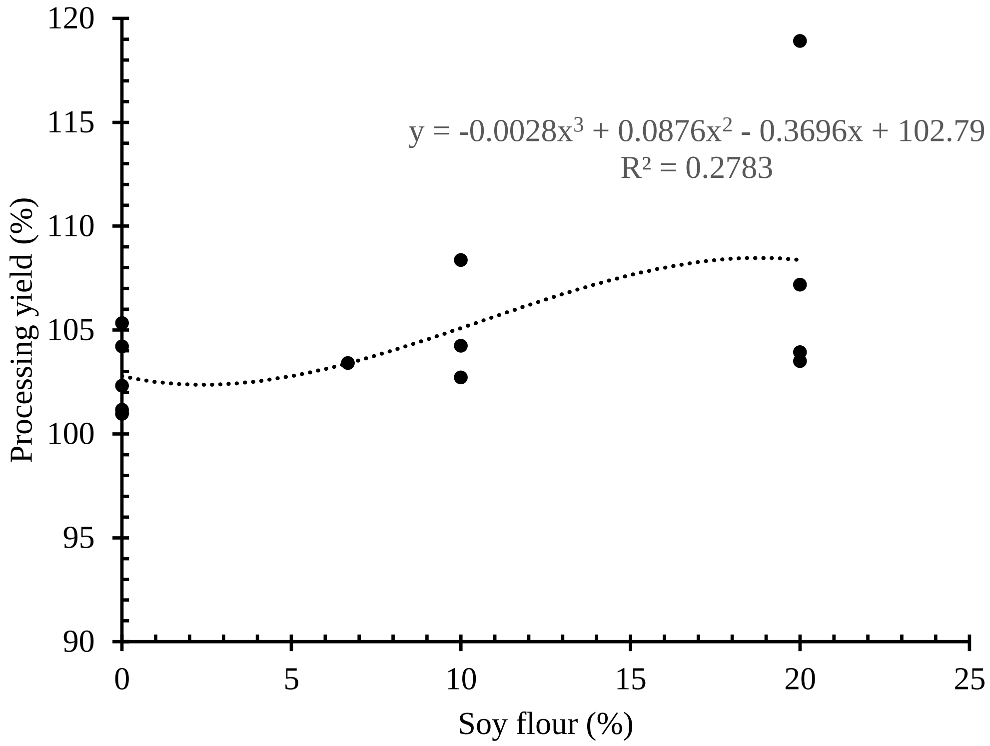

Fig. 4.13 Graph showing correlation of soy flour on processing yield

ANOVA table (Table C.5, Appendix C) shows that processing yield of different samples are significantly different ($p<0.05$). The increase in processing yield of soy flour, corn starch incorporated buff meatball may be due to the decrease in proportion of meat and increase in proportion of corn starch. The result shows that processing yield is directly proportional to the amount of soy flour parts in meatball (Odiase *et al.*, 2013).

The Fig. 4.13 graph has R^2 value of 0.2783 which is very low. From this we can conclude that the correlation between processing yield and soy flour on meatball was not significant.

4.5.3 Correlation of corn starch on processing yield in the formulation

Correlation of corn starch on processing yield in the formulation is shown in Fig. 4.14.

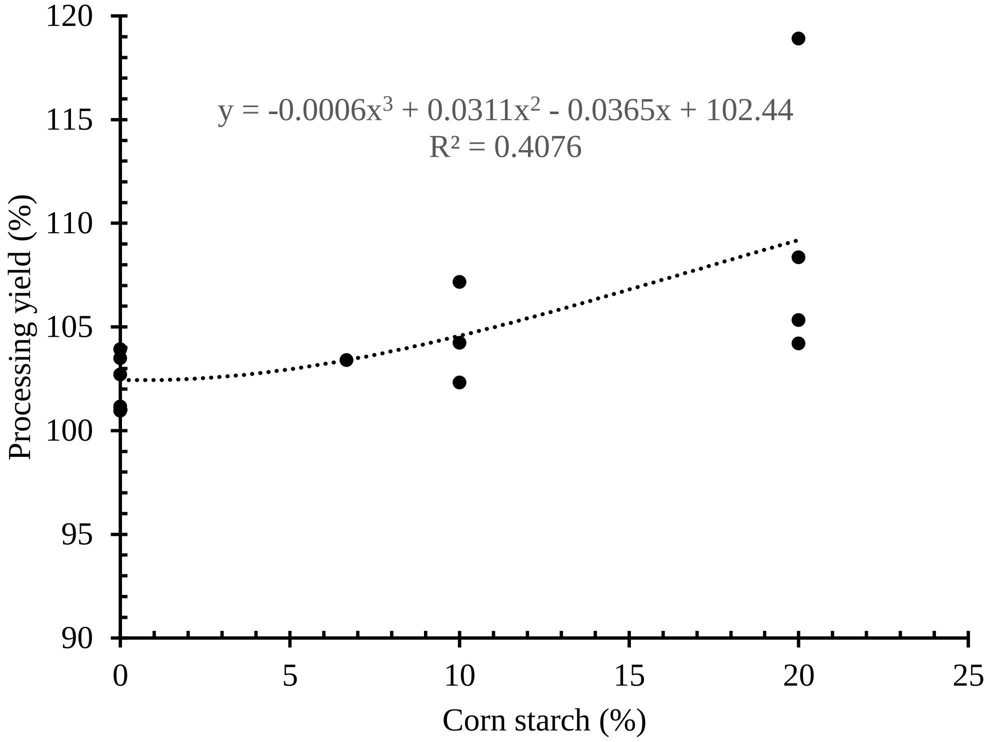

Fig. 4.14 Graph showing correlation of corn starch on processing yield

ANOVA table (Table C.5, Appendix C) shows that processing yield of different samples are significantly different ($p<0.05$). The increase in processing yield of soy flour, corn starch incorporated buff meatball may be due to the decrease in proportion of meat and increase in proportion of corn starch. The result shows that processing yield is directly proportional to the amount of corn starch parts in meatball (Aaslynga *et al.*, 2002).

The Fig. 4.14 has R^2 value of 0.4076 which is very low. From this we can conclude that the correlation between processing yield and corn starch on meatball was not significant**.**

4.5.4 Optimization of meatball from RSM with respect to process variables

On the basis of formulation of recipe total starting point was found to be 110. Among 110 recipe only 2 solutions were found to be desirable on the basis of maximized WHC and processing yield. The maximum value of WHC for solution 1 is 65.529 and for processing yield is 118.904 with desirability of 0.904 for the recipe (60% meat 20% soy flour and 20% corn starch). Similarly for solution 2 the WHC and processing yield were found to be 61.207

and 106.680 respectively with desirability of 0.449 for recipe (73.33% meat, 6.67% soy flour and 20% corn starch). The solution table as presented in appendix C.7.

4.6 Effect of process variables on fat and jelly separation

Though fat and jelly separation was selected as a response, during the study no detectable amount of fat and jelly could be separated from most of the samples. Thus, no further analysis of the response was done.

4.7 Cost evaluation

The cost for control was NRs 524.28 per kg of buff meatball while NRs 457.08 was needed for the manufacture of same amount of soy flour and corn starch incorporated meatball. The cost calculation for meatball is shown in Appendix D.

Part V

Conclusions and recommendations

5.1 Conclusions

Based on the physico-chemical and sensory analysis of the lab prepared meatball samples, the following conclusions can be drawn.

- The processing yield of all the formulated samples were found to be increases with increase in percentage of soy flour and corn starch.
- The water holding capacity (WHC) of all the formulated samples were found to be increases with increase in percentage of soy flour and corn starch.
- The variation in formulation had significant effect on all the sensory attributes of buff meatball.
- The variation in meat content did not affect the water holding capacity and processing yield of meatball significantly.
- Meatball with 80% (200 g) buff meat incorporated with 10% (25 g) Soy flour and 10% (25 g) corn starch keeping all the other ingredients constant in all the formulations was best from sensory evaluation
- The processing yield and water holding capacity of sensory optimized sample was found to be 104.23% and 59.62% respectively.
- Meatball with 60% buff meat, 20% soy flour and 20% corn starch for solution 1 and meatball with 73.33% buff meat, 6.67% soy flour and 20% corn starch for solution 2 was found to be optimized from RSM.
- The processing yield and water holding capacity of RSM optimized sample was found to be 118.904% and 65.529% respectively with desirability of 0.904 for the recipe (60% meat 20% soy flour and 20% corn starch) and again the processing yield and water holding capacity of RSM optimized sample was found to be 106.680 and 61.207 respectively with desirability of 0.449 for recipe (73.33% meat, 6.67% soy flour and 20% corn starch).

5.2 Recommendations

From the research work, the following suggestions are recommended for future work:

- Shelf life and microbiological quality of the buff meatball can be studied.
- Binding properties of binding agent in different samples variation can be studied.
- Effect of frying temperature and time on the sensory quality of meatball can be studied.

Part VI

Summary

Meatballs are processed comminuted meat and contain valuable nutrients like protein, minerals (Ca, P, Fe, etc.), fat, etc. Generally meatballs are produced by emulsifying fine ground meat with starch of some sort, mixing salt and certain herbs specific to the ethnic cuisine and finally shaping into balls. It is then cooked in boiling water, steam or deep fried depending on the cuisine.

The raw buffalo meat was purchased from local meat shop in Dharan. Soy flour, corn starch and other ingredients were also bought from local shop in Dharan. Raw buffalo meat was washed, trimmed and cut into small pieces. Raw buff meat was minced through a mincer. Meatball batter was prepared by mixing buff meat, soyflour, corn starch with calculated amount of water and kneading. For each different formulation calculated and weighed amount of buff meat, soy flour and corn starch was mixed and chopped in a bowl chopper. Necessary species were also weighed and mixed in the chopper with the batter.

Chemical composition of raw buff meat, soy flour and corn starch, which was used for preparation of meatball was carried. Sensory evaluation of Thirteen different samples having different formulations, which was prepared according to Design Expert was performed to find the best product. All the results of sensory evaluation were obtained by ANOVA in Genstat program taking two-way ANOVA no blocking method. Processing yield and WHC of all the samples were studied using central composite face centered design of response surface mothodology (RSM). The effect of meat, soy flour and corn starch variation on the responses processing yield and water holding capacity was investigated. The data were anayzed using Design Expert®10.

From the sensory evaluation sample prepared with 80% meat, 10% soy flour and 10% corn starch was found to be significantly best as compared to other samples. The moisture content, crude fat, crude protein, ash, crude fiber and carbohydrate for optimized product on dry basis were found to be 55%, 3.2%, 20.58%, 2.7%, 0.2% and 18.32% respectively. Processing yield and water holding capacity (WHC) of all the thirteen samples were observed and calculated. The values of processing yield and WHC of thirteen samples shows that, increase in percentage of soy flour and corn starch in the batter formulation with meat, there was increased in WHC as well as in processing yield.

From the sensory evaluation, meatball with 80% meat, 10% soy flour and 10% corn starch was found to have optimized recipe where as from response surface methodology meatball with 60% meat, 20% soy flour and 20% corn starch from solution 1 and meatball with 73.33% meat, 6.67% soy flour and 20% corn starch from solution 2 was found to be as optimized recipe. Processing yield and WHC of optimized sample acording to sensory evaluation was found to be 104.23% and 59.62% respectively. Likewise processing yield and WHC of optimized sample according to RSM was found to be 118.904% and 65.529% for solution 1 and 106.680% and 61.207% for solution 2 respectively.

References

Aaslynga, M., Bejerholma, C., Ertbjergb, P., Bertram, H. C. and Andersen, H. J. (2002). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. *Food Quality and Preferenc.* **14**, 277-288.

Anjaneyulu, A. S. R., Lakshmanan, V., Sharma, N. and Kondaiah, N. (1990). Buffalo meat production and meat quality: A review. *Indian Food Packer*. **44** (4), 21-31.

Anjaneyulu, A. S. R., Sengar, S. S., Lakshmanan, V. and Joshi, B. C. (1985). Meat quality of male buffalo calves maintained in different levels of protein. *Buffalo Bulletin*. **4**, 44-47.

Anjaneyulu, A. S. R., Sharma, N. and Kondaiah, N. (1989). Evaluation of salt, polyphosphates and their blends at different levels on physicochemical properties of buffalo meat and patties. *Meat Sci.* **25** (4), 293-306.

Anjaneyulu, A. S. R., Sharma, N. and Kondaiah, N. (1994). Effect of salt and polyphosphate blends on the quality of buffalo meat patties under hot, chilled and frozen conditions. *J. Food sci. Technol.* **31**, 404-408.

Anjaneyulu, A. S. R., Thomas, R. and Kondaiah, N. (2007). Technologies for value added buffalo meat products-a review. *American J. Food Technol.* **2**, 104-114.

Annonymous. (2016). History of the Meatball. Retrieved from https://www.sites.google.com/site/meatballsrus/home/history-of-the-meatball. (Last update March 23, 2016). [Accessed 3 Jan, 2018].

Anonymous. (1828). Definition of cornstarch. Merriam-Webster. USA.

Anonymous. (1998). Soy Flour. Indiana Soybean Board. Retrieved from http://www40.pair.com/rstevens/soyfoodsdescriptions/soyflour.html. [Accessed 9 January, 2018].

Anonymous. (2011/12). LIvestock farming. South Asia Subregional Economic Corporation (SASEC. Retrieved from http://www.sasecrtn.edu.np/. [Accessed 10 January, 2018].

Anonymous. (2017). Soy Flour. Soyfoods Association of North America. Retrieved from http://www.soyfoods.org/soy-products/soy-fact-sheets/soy-flour-fact-sheet. [Accessed 9 January, 2018].

AOAC. (2005). "Official Methods of Analysis" (18th ed.). Association of Official Analytical Chemists. Washington D.C.

Aukkanita, N., Kemngoena, T. and Ponharna, N. (2015). Utilization of corn silk in low fat meatballs and its characteristics. *Procedia - Social and Behavioral Sciences*. **197**, 1403-1410.

Bhutta, Z. (1999). Protein: digestibility and availability. *In:* "Encyclopedia of Human Nutrition". (M. Sadler, J. Strain and B. Caballero, Eds.). pp. 1646-1656. San Diego: Academic Press.

Bosselmann, A., Moeller, C., Steinhart, H., Kirchgessner, M. and Schwarz, F. J. (1995). Pyridinoline cross-links in bovine muscle collagen. *J. Food Sci.* **60** (5), 953-958.

Charles, D. D. (1982). Meat tenderness and palatibilityof swamp buffalo and four breeds of cattle. *animal production*. **34**, 79-85.

Cheng, Q. and Sun, D. (2008). Factors affecting the water holding capacity of red meat products: A review of recent research advances. *Food Sci. and Nutr.* **48**, 137-159.

Cilantro. (2015). Meatballs! History and Recipes. cilantrocooks.com. Retrieved from https://www.cilantrocooks.com/cilantro-blog/meatballs-history-and-recipes/. (Last update July 16, 2015). [Accessed 3 Jan, 2018].

Claus, J. R., Hunt, M. C. and Kastner, C. L. (1989). Effect of substituting added water for fat on the textural, sensory and processing characteristics of bologna. *J. Muscle Food*. 1-21.

Comer, F. W. and Allan-Wojtas, P. (1988). Functional and microstructural effects of fillers in comminuted meat products *Food structure*. **7** (1), 25-46.

CRA. (2006). "Corn Starch" (11th ed.). Corn Refiners Association. 1701 Pennsylvania Avenue, N.W. Washington.

De Mendoza, G. M., De Moreno, L. A., Huerta-Leidenz, N., Uzcátegui-Bracho, S., Beriain, M. J., and and Smith, G. C. (2005a). Occurrence of conjugated linoleic acid in longissimus dorsi muscle of water buffalo (Bubalus bubalis) and zebu-type cattle raised under savannah conditions. *Meat Sci.* **69**, 93-100.

De Mendoza, G. M., De Moreno, L. A., Huerta-Leidenz, N., Uzcátegui-Bracho, S., Beriain, M. J. and Smith, G. C. (2005b). Occurrence of conjugated linoleic acid in longissimus dorsi muscle of water buffalo *(Bubalus bubalis)* and zebu-type cattle raised under savannah conditions. *Meat Sci.* **69**, 93-100.

Decker, C. D., Conley, C. C. and Richert, C. H. (1986). Use of isolated soy protein in the development of frankfurters with reduced levels of fat, calories and cholesterol. Presented at European meeting of Research workers. p. 333.

Dimov, K., Kalev, R., Tzankova, M. and and Penchev, P. (2012). Fatty-acid composition of the lipids in m. longisimus dorsi of bovine and buffalo calves and buffalo cows. *Bulgar. J. Agric. Sci.* **18**, 778-783.

Dosi, R., Di Maro, A., Chambery, A., Colonna, G., Costantini, S., Geraci, G. and and Parente, A. (2006). Characterization and kinetics studies of water buffalo *(Bubalus bubalis)* myoglobin. *Comp. Biochem. Physiol*. **145**, 230-238.

El-Magoli, S., Laroia, S. and Hansen, P. M. T. (1996). Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. *Meat Sci.* **42** (2), 179-193.

Esposito, S. (2013). Is Spaghetti and Meatballs Italian? smithsonianmag.com. Retrieved from https://www.smithsonianmag.com/arts-culture/is-spaghetti-and-meatballs-italian-94819690/?no-ist. (Last update june 6, 2013). [Accessed 19 december, 2017].

Faustman, C., Yin, S., Tatiyaborworntham, N. and and Naveena, B. M. (2010). "Oxidation and Protection of Red Meat.". Vol. 2. Woodhead Publishers,. Cambridge, UK.

Gerrard, F. (1976). "Sausage and Small Goods Production" (6th ed.). North Wood Publication Ltd. London 36.

Gujral, H. S., Kaur, A., Singh, N. and Sodhi, N. S. (2002). Effect of liquid whole egg, fat and textured soya protein on the textural and cooking properties of raw and baked patties from goat meat. *J. Food Engineering*. **53**, 377-385.

Higgs, J. (2000). The changing nature of red meat: 20 years of improving nutritional quality. *Trends Food Sci. Technol.* **11**, 85-95.

Hongsprabhas, P. and Barbut, S. (1999). Effect of pre-heated whey protein level and salt texture development of poultry meat batters. *Food Research International*. **32**, 145-149.

Honikel, K. O. (1983). Water binding and fat emulsification during the processing of Bruhwurst mixtures. *Fleischweirtscharft*. **63** (7).

Hoogenkamp, H. W. (1997). Formulated meat patties. *J. Meat Production and Meat Processing*. **5** (9), 26-30.

Hsu, S. Y. and Chung, H. Y. (1998a). Effects of processing factors on qualities of emulsified meatball. *J. Food Eng*. **33**, 62-66.

Hsu, S. Y. and Chung, H. Y. (1998b). Effects of processing factors on qualities of emulsified meatball. *J. Food Eng*. **36**, 337-347.

Hsu, S. Y. and Yu, S. H. (1999). Effects of phosphate, water, fat and salt on qualities of low-fat emulsified meatball. *J. Food Eng.* **39** (2), 123-130.

Huang, S. C., Shiau, C. Y., Liu, T. E., Chu, C. L. and Hwang, D. F. (2005). Effects of rice bran on sensory and physico-chemical properties of emulsified pork meatballs. *Meat Science*. **70**, 613-619.

Irurueta, M., Cadoppi, A., Langman, L., Grigioni, G. and and Carduza, F. (2008). Effect of aging on meat characteristics from water buffalo grown in delta del Paraná in Argentina. *Meat Sci.* **79**, 529-533.

Jedlicka, J., Mojto, J., Vancisin, J., Kenetova, E. and Palwik, S. (1980). Examination of the effects of stress before slaughter by biochemical criteria and its effect on bull and cow meat quality. *Fleischwirtschaft*. 60 (8) 1532-1534. Deutscher Fachverlag GmbH.

Jha, R. (2010). Quality evaluation and storage stability of chicken-pork patty. B. Tech Thesis. Tribhuvan Univ., Nepal.

Joksimovic, J. and Ognjanovic, A. (1977). Comparison of carcass yield, carcass composition and quality characteristics of buffalo meat and beef. *Meat Science*. **1** (2), 105-110.

K.C., J. B. and Rai, B. K. (2007). "Basic Food Analysis Handbook" (1st ed.). Prompt Printers Pvt. Ltd.

Kandeepan, G., Anjaneyulu, A., Kondaiah, N., Mendiratta, S. K. and Lakshmanan, V. (2009a). Effect of age and gender on the processing characteristics of buffalo meat. *Meat Sci.*. **83** (1), 10-14.

Kandeepan, G., Anjaneyulu, A. S. R., Kondaiah, N., Mendiratta, S. K. and V., L. (2009b). Effect of age and gender on the processing characteristics of buffalo meat. *Meat Sci.* **83**, 10-14.

Kandeepan, G. and Biswas, S. (2007). Effect of domestic refrigeration on keeping quality of buffalo meat. *J. Food Technol.* **5** (1), 29-35.

Kandeepan, G., Biswas, S. and Rajkumar, R. S. (2009c). Buffalo as a potential food animal. *Int. J. Livestock Production*. **1** (1), 1-5.

Kandeepan, G., Mendiratta, S. K. and Shukla, V. (2013a). Processing characteristics of buffalo meat—a review. *J. Meat Sci. Technol.* **1**, 01-11.

Kandeepan, G., Mendiratta, S. K., Shukla, V. and Vishnuraj, M. R. (2013b). Processing characteristics of buffalo meat- a review. *J. Meat Sci. Technol.* **1** (1), 1-11.

Karmer, A. and Twigg, B. A. (1973). Meat and Meat product. *In:* ""Quality Control for the food industry"" (3 ed., Vol. 1).). pp. 34-39. AVI publishing Co. Inc.

Karvir, M. G. (1985). Canned corned beef. *Ind. Food Packer*. **34**, 35-38.

Kesava Rao, V., Anjaneyulu, A. S. R., Lakshmanan, V., Sharma, N. and Kondaiah, N. (1985). Meat and fat characteristics of male buffalo calves. *Indian J. Animal Sci.* **55** (7), 596-598.

Kesava Rao, V. and Kowale, B. N. (1991). Changes in phospholipids of buffalo meat during processing and storage. *Meat Sci.* **30**, 115-129.

Kim, C. J. and Lee, E. S. (2003). Effects of quality grade on the chemical, physical and sensory characteristics of Hanwoo (Korean native cattle) beef. *Meat Sci.* **63**, 397-405.

Kondaiah, N., Anjaneyulu, A. S. R., Kesava Rao, V., Sharma, N. and Joshi, H. (1985a). Effect of salt and phosphate on the quality of buffalo and goat meats. *Meat Sci.* **15**, 183-192.

Kondaiah, N., Kesava Rao, V., Anjaneyulu, A. S. R., Sharma, N. and Lakshmanan, V. (1986a). Evaluation of variety meats from buffaloes for some quality parameters. *J. Food Sci. and Technol.* **23** (2), 113-115.

Kondaiah, N., Lakshmanan, V., shama, N. and Rao, V. K. (1981). Studies on type and conformation of buffaloes for meat. *J. Animal Sci.* **15**, 107-111.

Kondaiah, N., Rao, V. K., Anjaneyulu, A. S. R., sharma, N. and Joshi, H. B. (1985b). Effect of salt and phosphate on the quality of buffalo and goat meats. *Meat sci.* **15**, 183-192.

Kondaiah, N., Rao, V. K., anjaneyulu, A. S. R., Sharma, N. and Lakshmanan, V. (1986b). Evaluation of variety meats from buffaloes for some quality parameters. *J. Food Sci. Technol.* **23**, 113-115.

Kowale, B. N., Kulkarni, V. V. and Rao, V. K. (2008). "Methods in Meat Science" (1st ed.). Jaypee Brothers Medical Publishers (P) ltd. Delhi.

Kulkarni, V. V., Kowale, B. N., Kesava Rao, V. and Murthy, T. R. K. (1993). Storage stability and sensory quality of washed ground buffalo meat and meat patties during refrigerated storage. *J. Food Sci. Technol.* **30** (3), 169-171.

Kumar, M. and Sharma, B. D. (2004). The storage stability and textural, physico-chemical and sensory quality of low-fat ground pork patties with carrageenan as fat replacer. *Int. J. Food Sci. Technol.* **39**, 31-42.

Landrigan, M. (2016). The true origin of the meatball Retrieved from https://www.theatlantic.com/technology/archive/2016/03/history-of-the-meatball/475083/. (Last update March 24, 2016). [Accessed 3 January, 2018].

Lawrie, R. A. (1985). Sensory attributes of meat and meat products Indian food industry. *Meat Science*. **14** (3), 24.

Lawrie, R. A. (1998). Water holding capacity and juiciness. *In:* "Lawrie's Meat Science." (6th ed.). (R. A. Lawrie, Ed.). Cambridge, England. Woodhead Publishing Limited.

Lawson, H. (1995). "Food oils and fats, Technology, utilization and Nutrition" (1st ed.). Vol. 1. CBS publications and distributors. Australia.

Macedo-Silva, A., Shimokomaki, M., Vaz, A. J., Yamamoto, Y. Y. and Tenuta-Filho, A. (2001). Textured soya protein quantification in commercial hamburger. *J. Food Composition and Analysis*. **14** (5), 469-478.

Macleod, G. and and Seyyedain, A. M. (1981). "Natural and Simulated Meat Flavors". *In:* (Vol. 14).). pp. 309-437. United States. National institute of health.

Maher, S. C., Mullen, A. M., Moloney, A. P., Drennan, M. J., Buckley, D. J. and Kerry, J. P. (2004). Colour, composition and eating quality of beef from the progeny of two charolais sires. *Meat Sci.* **67** (1), 73-80.

Mamino, E. N. and Horn, S. P. (1996). Chemical, processing and sensory characteristics of carabeef ham from philmurrah x phil-murrah crossbred. Presented at Proceedings of the 2nd Asian Buffalo Association Congress. Manila, Philippines. October 9-12. pp. 252-260.

Mancini, R. A. and Hunt, M. C. (2005). Current Research in meat color. *Meat Sci.* **71**, 100-121.

Marinova, P., Renerre, M., Pinkas, A., Polikronov, A. and Lacourt, A. (1985). Comparision of carcass composition and some meat qualities of buffaloes and cattle. *Proceding 31st European Meeting of Meat and Research Work*. **31**, 278-285.

Marmer, W., Maxwell, R. and Williams, J. (1984). Effects of dietary regimen and tissue site on bovine fatty acid profiles. *J. Anim. Sci.* **59**, 109-121.

Mohan, B., Narahari, D. and Prasad, J. (1987). Studies on influence of age and sex on the chemical composition of Japanese quail *(Coturnix coturnix japonica)* meat. *Indian J. Poultry Sci.* **22** (2), 182-184.

Moncel, B. (2017). What Is Corn Starch. The Spruce. Retrieved from https://www.thespruce.com/what-is-corn-starch-1328466. (Last update October 23, 2017). [Accessed 9 January, 2018].

Morgan, J. B., Wheeler, T. L., Koohmaraie, M., Savell, J. W. and Crouse, J. D. (1993). Meat tenderness and the calpain proteolytic system in longissimus muscle of young bulls and steers. *J. Animal Sci.* **71** (6), 1471-1476.

Muchenje, V., Dzama, K., Chimonyo, M., Strydom, P. E., Hugo, A. and and Raats, J. G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chem*. **112**, 279-289.

Myers, R. H., Montgomery, D. C. and Anderson-Cook, C. M. (2009). "Response Surface Methodology" (3rd ed.). John Wiley & Sons, Inc. U.S.A.

Naveena, B. M., Mendiratta, S. K. and Anjaneyulu, A. S. R. (2004a). Tenderization of buffalo meat using plant protease from *Cucumis trigonus ruxb (Kachri)* and *Zingiber officinale roscoe (Ginger rhizome). Meat Sci.* **68**, 363-369.

Naveena, B. M., Sen, A. R., Muthukumar, M., Babji, Y. and and Kondaiah, N. (2011). Effects of salt and ammonium hydroxide on the quality of ground buffalo meat. *Meat Sci.* **87**, 315-320.

Neath, K. E., Del Barrio, A. N., Lapitan, R. M., Herrera, J. R. V., Cruz, L. C., Fujihara, T., Muroya, S., Chikuni, K., Hirabayashi, M. and Kanai, Y. (2007b). Difference in tenderness and pH decline between water buffalo meat and beef during postmortem aging. *Meat Science*. **75** (3), 499-505.

NHMRC. (2006). Nutrient Reference Values for Australia and New Zealand including Recommended Dietary Intakes [Report]. Commonwealth Department of Health and Ageing. Canberra, Australia.

Odiase, O. M., Igene, J. O., Evivie, S. E. and Ebabhamiegbebho, P. A. (2013). Determination and sensory evaluation of soy flour-meat combinations in the production of meatballs. *J. Applied and Natural Sci.* **5** (2), 482-487.

Ognjanovic, A. (1974). Meat and Meat production in : The Husbandry and Health of the domestic buffalo. *In*.). Rome. Fao.

Paleari, M. A., Camisasca, S., Beretta, G., Renon, P., Tessuto, I. and Benedetti, G. (1998). Ostrich meat: Physico-chemical characteristics and comparison with turkey and bovine meat. *Meat Sci.* **48**, 205-210.

Palka, K. (2003). The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine semitendinosus muscle. *Meat Sci.* **64** (2), 191-198.

Payne, R. (2007). "The Development of Statistical Design and Analysis Concepts at Rothamsted". VSN International. Herts. UK.

Purnomo, H. and Rahardiyan, D. (2008). Indonesian Traditional Meatball. *Int. Food Res. J.* **15** (2), 101-108.

Ragab, M. T., Darwish, M. Y. M. and Malek, A. G. A. (1966). Meat production from Egyptian buffaloes. *J. Animal Production*. **6**, 31-32.

Ranathunga, R. A. A., Jayawardena, B. C. and Gunasekara, G. T. N. (2015). Effect of different extenders on physical, chemical and sensory characteristics of sausage production. *Int. J. Infor. Res. Rev.* **2** (11), 1311-1314.

Ranganna, S. (1986). "Handbook of Analysis and Quality Control for Fruit and Vegetable Products". TaTa McGraw Hill Publishing Co. Ltd.

Roseland, J. M., Williams, J. R., Duvall, M., Showell, B., Patterson, K. Y., Douglass, L. W., Daniel, M., Howe, J. C. and Holden, J. M. (2009). Effect of meat type and cooking method on cooking yields. USDA. Retrieved from https://www.ars.usda.gov/ARSUserFiles/80400525/Articles/ICoMST2012_MeatCookingYield2.pdf. [Accessed 15 January, 2018].

Sami, S., Augustini, C. and Schwarz, F. J. (2004). Effects of feeding intensity and time on feed on performance, carcass characteristics and meat quality of Simmental bulls. *Meat Sci.* **67** (2), 195-201.

Schaafsma, G. (2000). The Protein Digestibility-Corrected Amino Acid Score. *J. Nutr.* **130**, 1865-1867.

Seideman, S. C., Koohmaraie, M. and Crouse, J. D. (1987). Factors associated with tenderness in young beef. *Meat Sci.* **20**, 281-291.

Sengar, S. S., Lakshmanan, V., Joshi, B. C. and and Anjaneyulu, A. S. R. (1985). Meat quality of male buffalo calves maintained in different levels of protein. *Buff. Bull*. **4**, 44-47.

Serdaroglu, M. and Abrodimov, K. (2005). Quality of low-fat meatballs containing legumes flours as extenders. *Meat Sci.* **70**, 99-105.

Shiba, N., Matsuzaki, M. and Isuneishi, E. (2004). Effects of preslaughter nutritional condition on intramuscular collagen solubility, pyridinoline cross links and meat tenderness in aged goats. *Animal Sci. J.* **75** (4), 319-324.

Smith, G. C., Gross, H. R., Carpenter, Z. L., Murphy, C. E., Abraham, H. C. and Davis, G. W. (1982). Relationship of USDA maturity groups to palatability of cooked beef. *J. Food Sci.* **47**, 1100-1101.

Subba, D. (2010). "Analysis of meat and meat products" (2 ed.). National college of food science and technology. Kathmandu.

Suman, S. P. and Sharma, B. D. (2003). Effect of grind size and fat levels on the physico-chemical and sensory characteristics of low-fat ground buffalo meat patties. *Meat Sci.* **65** (3), 973-976.

Swan, J. E. and Boles, J. A. (2006). Functionality of cow beef in coarse and fine ground model systems. *Meat Sci.* **72** (1), 25-33.

Syed Ziauddin, K., Mahendrakar, N. S., Rao, D. N., Ramesh, B. S. and Amla, B. L. (1994a). Observations on some chemical and physical characteristics of buffalo meat. *Meat Science*. **37** (1), 103-113.

Syed Ziauddin, K., Mahendrakar, N. S., Rao, D. N., Ramesh, B. S. and Amla, B. L. (1994b). Observations on some chemical and physical characteristics of buffalo meat. *Meat Sci.* **37**, 103-113.

Tateo, A., De Palo, P., Quaglia, N. C. and and Centoducati, P. (2007). Some qualitative and chromatic aspects of thawed buffalo *(Bubalus bubalis)* meat. *Meat Sci.* **76**, 352-358.

Theno, D. M., Siegal, D. G. and Schmidt, G. R. (1978). Meat massaging, effects of salt and phosphates on the ultrastructure of cured muscle. *J. food sci.* **43**, 447-448.

Turgut, H. (1984). Emulsifying capacity and stability of goat, water buffalo, sheep and cattle muscle proteins. *J. Food Sci.* **49**, 168-171.

Ulu, H. (2004). Effect of wheat flour, whey protein concentrate and soya protein isolate on oxidative processes and textural properties of cooked meatballs. *Food Chemistry*. **87** (4), 523-529.

Uriarte, A. M. E., Spaeth, C. W. and and Bolsen, K. K. Nutritional factors affecting sheep meat quality. *Animal Sci. and Industry*. 1-12.

USAID. (2016). Defatted Soy Flour Commodity Fact Sheet. United States Agency for International Development. Retrieved from https://www.usaid.gov/what-we-do/agriculture-and-food-security/food-assistance/resources/defatted-soy-flour-commodity-fact. (Last update November 14, 2016). [Accessed 9 january, 2018].

Valin, C., Pinkas, A., Drahnev, H., Boikovski, S. and Polikronov, D. (1984). Comparative study of buffalo meat and beef. *Meat Sci.* **10**, 69-84.

Warriss, P. D. (2000). "Meat Science: An Introductory Text". Vol. 23. CABI Publishing. Wallingford, UK.

Weir, C. E. (1960). Palatability characteristics of meat. *In:* "The Science of Meat and Meat Products".

Yadava, B. S. and Singh, L. N. (1985). Age Related changes in chemical composition of buffalo skeletal muscles. *Indian J. Animal Sci.* **55** (4), 272-277.

Zayas, J. F. (1997). Water binding in comminuted meat products. *In:* "Funtionality of Proteins in Food" (1st ed.). (J. F. Zayas, Ed.). pp. 89-94. New York. Springer-Verlag Berlin Heidelberg.

Appendices

Appendix A

1. Sensory evaluation card

Sensory evaluation sheet of Buff meatball

Prepared by: Saroj Ghimire

Purpose: Dissertation for the *partial fulfillment of the requirements for the degree* of Bachelor's Degree in Food Technology (B.Tech Food)

Name of panelist……………………….. Date……………………

Name of the product: *Buff Meat Ball*

Dear panelist, you are given 13 sample of *Buff Meat Ball* on each proportion with variation on *Buff Meat, Soya Flour and Corn Starch* Please taste the sample and score how much you prefer the each one. Please give points for your degree of preference for each parameter as shown below using the scale given.

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13
Appearance													
Flavor/Aroma													
Texture and Tenderness													
Taste													
Juiciness													
Overall palatability													

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 (if any)…………………………………………………………………….

Signature………………………….

Appendix B

1. Sensory evaluation of the product

Table B.1.1 Two way ANOVA for Appearance (color)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	12	44.6923	3.7244	4.44	<.001
Panelist	9	42.1615	4.6846	5.59	<.001
Residual	108	90.5385	0.8383		
Total	129	177.3923			

Since F Pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.2 Two way ANOVA for Flavor/Aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	12	22.7077	1.8923	3.51	<.001
Panelist	9	19.0846	2.1205	3.93	<.001
Residual	108	58.2154	0.539		
Total	129	100.0077			

Since F Pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.3 Two way ANOVA for Juiciness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	12	23.2769	1.9397	2.81	0.002
Panelist	9	48.4308	5.3812	7.79	<.001
Residual	108	74.5692	0.6905		
Total	129	146.2769			

Since F Pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.4 Two way ANOVA for Taste

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	12	21.9692	1.8308	1.97	0.034
Panelist	9	45.7615	5.0846	5.47	<.001
Residual	108	100.3385	0.9291		
Total	129	168.0692			

Since F Pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.5 Two way ANOVA for Texture and Tenderness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	12	32.7077	2.7256	4.47	<.001
Panelist	9	13.6923	1.5214	2.49	0.013
Residual	108	65.9077	0.6103		
Total	129	112.3077			

Since F Pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Table B.1.6 Two way ANOVA for Overall palatability

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	12	32.7077	2.7256	4.47	<.001
Panelist	9	13.6923	1.5214	2.49	0.013
Residual	108	65.9077	0.6103		
Total	129	112.3077			

Since F Pr. <0.05, there is significant difference between the samples so LSD testing is necessary.

Appendix C

Outputs from Design-Expert 10.0.1

Response 1: WHC

Table C.1 Fit and model summary statistics for WHC

Source	Std. Dev	Lack of fit p-value	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.43	0.0501	0.757	0.708	0.576	113	Suggested
Quadratic	2.42	0.0415	0.832	0.713	0.0793	224	
Special Cubic	2.05	0.0586	0.897	0.793	-0.487	362	
Cubic	1.71	0.0520	0.952	0.856	_	_	

Table C.2 ANOVA for Response Surface Linear model (for WHC)

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Model	184	2	92.2	15.6	0.000848	Significant
Linear mixture	184	2	92.2	15.6	0.000848	
Residual	59.2	10	5.92	-	-	
Lack of Fit	56.5	7	8.07	8.87	0.0501	Not significant
Pure Error	2.73	3	0.910			
Cor Total	244	12				

Table C.3 ANOVA for best point for components (for WHC)

Component	Coefficient Estimate	Df	Standard Error	95% CI low	95% CI high
A-Meat	51.9	1	1.29	49.1	54.8
B-Soy flour	64.9	1	2.65	59.0	70.8
C-Corn flour	66.2	1	2.65	60.3	72.1

Response 2: Processing yield

Table C.4 Fit and model summary statistics for processing yield

Source	Std. Dev	Lack of fit p-value	R-Squared	Adjusted R-Squared	Predicted R-Squared	PRESS	
Linear	2.57	0.0063	0.747	0.696	0.424	150	
Quadratic	1.34	0.0340	0.951	0.917	0.667	86.5	
<u>Special Cubic</u>	<u>0.546</u>	<u>0.390</u>	<u>0.993</u>	<u>0.986</u>	<u>0.871</u>	<u>33.7</u>	Suggested
Cubic	0.563	0.238	0.995	0.985	-	-	

Table C.5 ANOVA for Response Surface special cubic for processing yield

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	
Model	258.2742	6	43.0457	144.599	< 0.0001	significant
Linear Mixture	239.0253	2	119.5127	401.4666	< 0.0001	
AB	0.958169	1	0.958169	3.21868	0.123	
AC	6.17E-05	1	6.17E-05	0.000207	0.989	
BC	7.430192	1	7.430192	24.95948	0.00246	
ABC	10.86046	1	10.86046	36.48243	0.000931	
Residual	1.786141	6	0.29769			
Lack of Fit	1.048441	3	0.34948	1.421229	0.390	not significant
Pure Error	0.7377	3	0.2459			
Cor Total	260.0603	12				

Table C.6 ANOVA for best point for components (for processing yield)

Component	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High
A-meat	100.9839	1	0.382936	100.0469	101.921
B-Soy flour	99.96325	1	3.584498	91.1923	108.7342
C-Corn starch	108.2473	1	3.584498	99.4763	117.0182
AB	12.66159	1	7.057477	-4.60743	29.93062
AC	0.101594	1	7.057477	-17.1674	17.37062
BC	58.52477	1	11.71445	29.86054	87.18899
ABC	-121.372	1	20.09445	-170.541	-72.2025

Table C.7 Constraints for the optimization of recipe for solution 2

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Meat	is in range	60	100	1	1	3
B:Soy flour	is in range	0	20	1	1	3
C:Corn starch	is in range	0	20	1	1	3
processing yield	maximize	100.96	118.9	1	1	3
WHC	maximize	50.36	67.51	1	1	3

Table C.8 Solution table for maximized WHC and processing yield

Number	Meat	Soy flour	Corn starch	processing yield	WHC	Desirability	
1	60	20	20	118.904	65.52867	0.940463	Selected
2	73.33333	6.666667	20	106.6799	61.20714	0.449066	

Table C.9 Constraints for the optimization of recipe for solution 2

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Meat	is in range	60	100	1	1	3
B:Soy flour	is in range	0	20	1	1	3
C:Corn starch	is in range	0	20	1	1	3
processing yield	maximize	100.96	118.9	1	1	3
WHC	maximize	50.36	67.51	1	1	3

Table C.10 Solution table for maximized WHC and processing yield

Number	Meat	Soy flour	Corn starch	processing yield	WHC	Desirability	
1	60	20	20	118.904	65.52867	0.940463	
2	73.33333	6.666667	20	106.6799	61.20714	0.449066	Selected

Appendix D

D.1 Cost comparison of fried control buff meatball and fried soy flour and corn starch incorporated buff meatball

Table D.1 Price calculation per 250 g of fried controlled buff meatball (100 parts of meat) is shown in table.

Type	Ingredients	Amount	Rate	Cost (Rs)
Control buff meatball	Buff lean meat	250 g	350/kg	87.5
	Salt	5 g	20/kg	0.1
	Onion	50 g	80/kg	4
	Ginger-garlic paste	25 g	300/kg	7.5
	Oil	37.5 ml	70/l	2.63
	MSG	0.75 g	10/5 g	1.5
	Black pepper	1.5 g	10/5 g	3
	Red pepper	1.5 g	10/g	3
				109.23
Overhead cost (20%)				21.84
Total		371.25 g		131.07

Table F.2 Price calculation per 250 g of fried soy flour and corn starch incorporated buff meatball (Containing 80 parts meat, 10 parts of soy flour and 10 parts of corn flour by weight) is shown in table:

Type	Ingredients	Amount	Rate	Cost (Rs)
Soy-buff meatball	Meat	200 g	350/kg	70
	Soy flour	25 g	70/kg	1.75
	Corn starch	25 g	70/kg	1.75
	Salt	5 g	20/kg	0.1
	Onion	50 g	80/kg	4
	Ginger-garlic paste	25 g	300/kg	7.5
	Oil	37.5 ml	70/l	2.63
	MSG	0.75 g	10/5 g	1.5
	Black pepper	1.5 g	10/5 g	3
	Red pepper	1.5 g	10/g	3
				95.23
Overhead cost (20%)				19.04
Total		371.25 g		114.27

Color plates

Cooking meatball

Meatballs after frying

Samples of meatball

Sensory evaluation of meatball