RECIPE OPTIMIZATION OF *SARGAYANGMA* AND ITS QUALITY EVALUATION

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2017

Recipe Optimization of *Sargayangma* and its Quality Evaluation

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

by

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Approval Letter

This *dissertation* entitled *Recipe Optimization of Sargayangma and its Quality Evaluation* presented by Miss. Pragatee Adhikari has been accepted as the partial fulfillment of the requirements for the B.Tech. degree in Food Technology.

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May 7, 2017

Acknowledgements

It is my great privilege to work for the present investigation under the guidance and kind supervision of Teaching Asst. Mr. Devraj Acharya, Central Campus of Technology, Dharan. I express him my profound sense of gratitude for his continual supervision, encouragement and tireless effort throughout the endeavor. His personal efforts, ideas and valuable time are the backbone of this dissertation work.

I am also deeply grateful to Prof. Dr. Dhan Bahadur Karki, Campus Chief, Central Campus of Technology, Hattisar, Dharan, for providing adequate facilities to make every work convenient and fruitful. My sincere thanks goes to Assoc. Prof. Basanta Kumar Rai, Head of Department, Department of Food Technology, CCT, Hattisar, Dharan for his kind suggestions in every possible way throughout the work.

I am highly indebted to Teaching Asst. Mr. Yadav K.C., Central Campus of Technology, Dharan for his valuable cooperation and time.

A sincere thanks goes to all staffs of Central Campus of Technology for their generous support. I am also thankful to my seniors Prem Kumar Limbu and Nirat Katuwal for their valuable advices. My heartly thanks goes to my classmates without whom this work would not have been this far. My every juniors are also thanked for their helping hands.

At last but not the least, I owe my dissertation to my family whose constant support and blessing made the whole work a inspiring journey.

Thanks to all direct and indirect helping hands whom I am unable to acknowledge individually.

Date of submission: May 7, 2017

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Abstract

The basic raw materials for *sargayangma* preparation were brought from local market of Dharan. The recipe for ingredients was extracted from previous study and Response Surface Methodology (RSM) was employed to optimize the level of addition of fat, blood and *yangben* in the range of 10-25%, 10-30% and 5-20% respectively. The effect of these process variables was studied on responses, processing yield and water holding capacity of *sargayangma* while other parameters were kept constant. Twenty formulations produced by experimental design at three levels +1, 0 and −1 with six centre points were prepared in lab which were also subjected to sensory evaluation to obtain optimum product in terms of aroma, color, blood taste, *yangben* taste, texture, juiciness and overall acceptability.

The processing yield of *sargayangma* was not significantly affected by variation in fat, blood and *yangben* amount. The water holding capacity was significantly affected by blood and *yangben* amount variation whereas variation in fat amount did not show significant effect. Similarly, all the sensory parameters were also significantly affected by ingredients variation. From sensory analysis, formulation with 17.5% fat, 30% blood and 12.5% *yangben* was found to be best while from response analysis, formulation with 10% fat, 10% blood and 20% *yangben* was found to be optimum.

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List of Abbreviations

Abbreviations	Full form
ANOVA	Analysis of variance
CCT	Central Campus of Technology
RSM	Response Surface Methodology
WHC	Water Holding Capacity
PY	Processing Yield
FAO	Food and Agriculture Organization

PART I

Introduction

1.1 General introduction

There exists a diverse food habit in Nepalese society each reflecting the culture and tradition of a particular community. These all peculiar food practices adopted by different ethnic groups can be summed up as indigenous technologies. The preparation methods of such foods is learnt by "doing and learning" system , which is handed from ancestral generations to younger generations (Shrestha, 2012). Nepalese indigenous food are famous for their unique sensory characteristics and health benefits. However, there still remains a broad aspects yet to be revealed and understood (Limbu, 2014).

Sargayangma or *sargemba* is a type of blood sausage famous in Limbu communities. Blood sausage is a traditional food of few communities residing in mountains of Nepal. Sherpa in upper regions make blood sausage from yak or sheep blood while Limbu and Rai from lower regions make from pig blood (Khanal, 2015). Blood sausages are one of the popular traditional meat products in many parts of the world such as Europe, Latin America and Asia. In Europe blood sausages are normally called morcilla and morcella in Spain and Portugal, black pudding in Great Britain, blutwurst in Germany. Most of the blood sausages present dark red to black color with a firm texture due to formation of a gel structure from the interaction of collagen, starch, blood proteins, etc. Blood sausage are basically made with blood, fat and variety of vegetable origin food along with fillers. Moreover the use of meat and offals is also common (Ramos *et al.*, 2013). The making process and the composition differs as a result of type, region and manufacturer. *Sargayangma* is one of the popular type under the diverse group of blood sausages with its own peculiarities and cultural background of Nepal. The use of edible lichen called *yangben* is the principal difference between the *sargayangma* and the regular blood sausages found in market (Kharel *et al*., 2009).

The origin of why people started making *sargayangma* is not clear but it is found to be prepared whenever pig is slaughtered for food and religious purpose. Pigs and pork are at the heart of Limbu culture, cuisine and family customs (Nembang, 2016). It is a good example of by-product utilization as it is prepared from pork belly, blood, liver, heart, killing fat and main ingredients influencing the proximate composition are *yangben* (60-

70%), blood (10-20%), fat, heart, liver and meat used. A typical *sargayangma* contains 12% crude protein, 41% moisture, 16% crude fat, 30% carbohydrate (from *yangben*) and 1% minerals on average. The sausage is rich in iron because of presence of blood (Kharel *et al.*, 2009).

Sargayangma has a unique flavor, aroma, taste and mouth feel produced by *yangben* and is gaining popularity day by day. The principal ingredient *yangben* or edible lichen is mostly found in hills of Eastern region of Nepal like Dhankuta, Taplejung and Terathum (Limbu, 2014). According to research carried out by (Yaqso, 2003), the commonly found species in Nepal are *Parmelia, Ramalina* and *Usnea*. It is a type of edible mossy epiphyte which grows on tree branches as parasite plant. It is also called *Jhyau, Tarey* or *Jhulo* while Rai, Limbu and Sherpa call it *yangben* (Devkota, 2011). This unique ingredient is consumed as food in these ethnic communities only. It does not have its own flavor but with pork meat and blood, it absorbs fat and gives deep earthy flavor to the dish. It gives a dish so unique taste that most of the people outside the ethnic groups have not experienced (Khanal, 2016). Till date, it is praised for its sensory attributes only. The lichens of Nepal have been studied only botanically but many other functional aspects are still unknown. The more research regarding its functional property can promote such valuable product still in shade (Dhungana, 1985). Another important concern for *sargayangma* is due to the incorporation of blood which is an inevitable part of meat production food chain. The physicochemical characteristics and utilization of animal blood and industrial applications been explored to some extent. Many blood derived products with various functionalities have been developed and some blood derived food products are also famous in some parts of world (Bah *et al.*, 2013). *Sargayangma* is a perfect example of such blood derived food consumed in Nepal only. Incorporation of such protein rich component makes *sargayangma* nutritionally rich product with appetizing flavor and appearance.

1.2 Objectives

1.2.1 General objectives

- To optimize the recipe of *sargayangma* and conduct its quality evaluation.

1.2.2 Specific objectives

- To prepare *sargayangma* of different formulations given by experiment design.
- To measure the processing yield and water holding capacity of the samples.
- To conduct sensory analysis of prepared samples.
- To optimize the recipe from RSM (Response Surface Methodology) and sensory analysis.
- To perform different physicochemical analysis of optimized product.

1.3 Statement of the problem

Sargayangma is prepared using locally available materials and techniques. The ingredients used so far are valued for their flavor characteristics only but their technological significance in the product are still not understood. The preparation method differs from place to place and so the product characteristics. The lack of knowledge and scientific research has hindered the improvement in the existing traditional practices. Only countable small scale food market like Noyoz Restaurant in Kathmandu can be found commercializing *sargayangma* and it is still a brand new food item for countless people. Pork meat is being popular day by day among the Nepalese community but the consumption habit in the form of delicious meat dishes is almost limited. So the broadened research is a utmost need for making the manufacturing practices more scientific and bringing the product out of the Limbu kitchen.

The principal ingredient *yangben* being rich in carbohydrate serve to bring about many textural and physicochemical changes in the final product. Such fibrous and carbohydrate rich substance fall under functional non-meat ingredients for improving processing technology and sensory properties (Heinz and Hautzinger, 2007). But the functional property of particular *yangben* has not been assessed yet. Besides, the protein rich ingredient blood also has its own water holding and fat binding properties (Zayas, 1997). It is a rich source of iron and proteins of high nutritional and functional quality. Religious constraints as well as negative consumer perception of blood for direct consumption has made limited its use in food applications which is a great loss from nutritional as well as economic aspect (Bah *et al.*, 2013). A proper research of blood incorporated product highlighting its nutritional safety and significance as well as its effect on various on various parameters of product is thus lacking till date.

1.4 Significance of the study

Sargayangma is a delicious indigenous food of Limbu community which is yet to be commercialized. It is more unique as it contains rarely known food lichen whose nutritious value is comparable with wheat, corn, rice, etc. (Dhungana, 1985). Though assessment of nutritive value of *yangben* is done by Subba (1997) and Yaqso (2003), further study about its other significance has not been assessed yet. Once it is understood various recipe formulation can be done conveniently and its further use in other type of product can also be promoted. The technological significance of *yangben* in *sargayangma* can be thus extensively utilized in other meat products where such high binding property is required. Not only the individual importance but the interaction effect among the ingredients is a critical factor for any product formulation which is the major attempt of this study.

As *sargayangma* is still under the shade of western sausage, to unleash the beauty of this appetizing food, detailed scientific study of its existing manufacturing practices is an obsolete need. It is also a perfect example of bi-product utilization which is vital in terms of economic sustainability as well as environmental sanitation (Irshad *et al.*, 2015). According to Bah *et al.* (2013), with the right collection procedures, blood can be used efficiently as a raw material for products for human consumption as well as for technical products. So, a loss of valuable protein source and serious environmental pollution problems can be minimized by promotion of such products. Also, pig farming is one of the emerging business in Nepal. Commercialization of such pork products accompanied with technical and scientific manufacturing procedures can be a major step for the agricultural development too.

1.5 Limitations of the work

- Consistent quality meat could not be used for all samples preparation.
- Shelf life of *sargayangma* was not studied.
- Parameters other than water holding capacity and yield could not be studied.

PART II

Literature review

2.1 Background of sausage

Sausage is a popular meat product prepared from minced and seasoned meat and formed into cylindrical shape by natural or synthetic casing. The category includes comminuted meats ranging from coarsely ground to fine emulsions. History predates that the ancient Romans gave a vernacular word 'salsus' for the sausage, the prefix sal being the word salt (Young and White, 2008). It literally refers to chopped or minced meat preserved by salting. It is one of the oldest form of processed food, its origin being lost in antiquity. In ancient time, sausage were encased in intestine with more or less cylindrical shape which is now a characteristics of sausage. Thus, sausages are salted and seasoned chopped meat product, that are generally, but not always, cylindrical in shape (Pearson and Tauber, 1984).

The domestication of pigs is a primary source of most sausage which is said to have begun in China and Egypt about 5000 B.C. Sausage production then flourished as pig raising spread all over Europe and Asia. Though a documented proof is lacking, it has been reported that sausages were used by Babylonians and the Chinese about 1500 B.C. They developed a sausage version by wrapping pig intestine around meat to roast on an open flame. Later it became an efficient means of feeding large armies during the expansion of Roman Empire into Western Europe (Young and White, 2008).

Sausages basically contain meat and fat (solid phase) dispersed into ice/water (liquid phase) forming a stable mass that is submitted to a moderate heat treatment (Piotrowicz and Mellado, 2015). They are usually produced by chopping or grinding of meat, followed by mixing with curing and seasoning agents. The mixture is then stuffed into natural or artificial casing whose size depends on origin and culture. The further processing like drying and smoking depends on the end product desired. Some are dried to desired moisture content in short time some are slowly dried and fermented (Hui *et al.*, 2001). As stated by FAO (Food and Agriculture Organization) during sausage processing meat undergo through various modification processes to acquire desirable organoleptic and keeping properties. Sausage are economical as they can be prepared from cheaper cuts of meat and meat by products. In fact early sausage development was also an attempt to utilize the less desirable parts like trimmings, head and shoulders meat, and edible by products from slaughtering. This agrees with the fact that convenience, variety, economy and nutritional value are the main reasons for its wide acceptance by consumers in modern society (Hui *et al.*, 2001).

Sausages made in various countries have much larger variation such that detail catchall coverage of world sausages in a single classification is a tedious job. However, fresh sausages, cooked sausages, fermented sausages and emulsion sausages are the most important four classes of sausages as given by Vignolo *et al.* (2010). Blood sausages are famous cooked type of sausages fabricated as a mixtures of raw un-coagulated blood and other food ingredients which may be meat or non- meat ingredients. It is a useful term generally used to cover all the blood based solid foods around the world. Pig or cattle blood is most commonly used while sheep and goat blood is used to a lesser extent and normally ranges between 5-30%. Generally, they are prepared by cooking blood with filler until it is thick enough to congeal when cooled. Typically fillers include meat, fat, suet, bread, sweet potato, barley, and oatmeal etc. (Toldra, 2010; Heinz and Hautzinger, 2007). Blood and fat being the basic ingredients the addition of meat, pork skin and offals are also common. The use of vegetable origin foods is also diverse including cereals, sugars, fruits, nuts, etc. Despite of diverse manufacturing practices, mixing of raw materials, stuffing, cooking and cooling procedures apply to all of them (Ramos *et al.*, 2013).

2.2 *Sargayangma*

Among thousands of blood sausages made throughout the world, *sargayangma* is a unique one indigenous to Nepal. It is one of the oldest forms of the processed food and there is no exact evidence of its origin in Nepalese society. It is a popular dish in Limbu community of Eastern region of Nepal. According to Limbu language "sa" means meat and "yangma" means vessel (Limbu, 2014). Pork is a celebratory dish in Limbu household and they take pork dish very seriously. It is normal to have excess blood and meat when a pig is slaughtered during pujas and festivals. So not to let any part go waste blood, offals and excess meat are collected and formulated for *sargayangma*. The dish is not specific to any occasion and can be consumed any time of the year. Primarily they are prepared on Dashain, Tihar, *Sisekpa Tongnam* (Saune sakranti), *Kokphewa Tongnam* (Maghe sakranti), *Chasok Tongnam* (Mangsir Purnima) and *Yokwa Tongnam* (Baisakh Purnima) (Nembang, 2016).

The use of special ingredient *yangben* in *sargayangma* makes it different from that of regular blood sausages found in market (Kharel *et al.*, 2009). The basic procedure followed for *sargayangma* preparation in different places is almost similar i.e. mixing minced pork meat including internal organs (heart, liver, etc.), fats, wild edible moss *yangben*, salt and spices together with fresh pig blood, stuffing the mixture in pork intestine and cooking in boiling water until it is fully cooked. Most of the people use *yangben*, blood, liver, heart and killing fats but in few cases people also use rice grain, vegetables, head meat, etc. However, the ingredients used depend on the availability and the by-products are the mostly used one (Limbu, 2014). The boiled sausage is a ready to eat dish though it is chopped, fried and then served with pickle. The sausage can also be smoked and stored over the firewood for several weeks (Khanal, 2015).

2.3 Ingredients used in *sargayangma*

2.3.1 Pork meat

The principal ingredient of sausage is always the skeletal muscle meat derived from the deboning of carcass (Toldra, 2010). In *sargayangma*, only pork belly is used apart from offals. Meat is defined by the Codex Alimentarius as "All parts of an animal intended for, or have been judged as safe and suitable for, human consumption". In the broadest sense, meat is the edible post mortem component originating from live animals (Hui *et al.*, 2001). Meat is composed of water, protein and amino acids, minerals, fats and fatty acids, vitamins and other bioactive components and small quantities of carbohydrates. Though meat can be derived from different sources, pork *(Sus Domesticus*) is the most widely eaten meat in the world accounting for over 36% of the world meat intake (Bender, 1992). But the pork consumption is forbidden in certain religions, such as Islam and Judaism. It is often eaten unprocessed, but cured (preserved) pork products are also very common (Arnarson, 2016).

2.3.1.1 Pork meat composition

The chemical composition of pork meat per 100 g as given by FAO is presented in Table 2.1.

Table 2.1 Chemical composition of pork meat

Parameters	Pork (lean)	Pork carcass
Water	75.1	41.1
Protein	22.8	11.2
Fat	1.2	47
Ash	1.0	0.6

Source: Heinz and Hautzinger (2007)

2.3.1.1.1 Moisture

Moisture in meat is the greatest contributor to muscle weight and is easily lost from the meat. The water held within meat structure determines the chemical and physical structure of meat. The water of skeletal muscle exists in three forms, referred as bound water, immobilized water and free water (Wenther, 2011).

Bound water is the water that is directly bound to the muscle proteins through severe mechanical or other physical force. It represents 4-5% of the total water found in muscle. Immobilized water is indirectly held by electrically charged reactive groups of meat proteins. The greater the amount of immobilized water, greater is the ability of the product to retain moisture and thus have increased yields. The amount of immobilized water can vary between 35-75%. Free water is the water that is held by muscle membranes and capillary action. Free water is easily lost during different processing steps like communition as the membranes are damaged. This loss of free water from the retail cuts of meat is termed as drip loss or purge loss. Thus, the objective of meat processing is oriented towards converting this free water to immobilized water (Wenther, 2011).

2.3.1.1.2 Protein components

Like all meat pork is rich in high quality protein and contains all the essential amino acids necessary for the growth and maintenance of our body (Arnarson, 2016). A typical mammalian muscle after rigor mortis but before post- mortem degradative changes contains about 19% protein. Among them 11.5% structural protein actin and myosin (myofibrillar), 5.5 % soluble sarcoplasmic protein in the muscle juice, 2% connective tissue (collagen and elastin) encasing the structural protein (Bender, 1992).

Texture, moisture retention and tenderness of processed muscle foods are influenced by the functionality of myofibrillar protein (Xiong, 1994). They are high molecular weight, salt extractable fibrous proteins largely responsible for functional responses within the comminuted meat mass. During the comminution process, the membranes and sarcolemma are disrupted thus releasing myofibrils and myofilaments. Extraction in presence of salt there is shift in the isoelectric point of the myofibrillar protein to a lower pH value, in effet creating a larger negative charge at the existing pH from the ionizable carboxyl groups of the protein. Repulsion between these negatively charged groups causes the protein to open up its spatial arrangement and thus increase the water retention property (Acton *et al.*, 1983).

2.3.1.1.3 Fat components

Pork contains varying amount of fat which depends on the level of trimming and various other factors. It is found throughout the carcass or cuts of meat (Wenther, 2011).

Fat is found at three sites in the body.

a. The largest amount of fat is in the storage deposits under the skin and around the organs. This constitutes the visible fat and can be as much as 40-50% of the total weight in fatty meat. This is composed largely of triglycerides contained in proteinaceous cells with little amount of water.
b. Small amount of fat is visible between the bundles of muscle fibres or in the lean part of the meat termed as intermuscular fat. This fat is also known as marbling fat and can amount to 4-8% of the weight of lean meat.
c. Intramuscular or structural fat is another type of fat present within the muscle structure. This can range from 1-3% of wet weight of muscle (Bender, 1992).

Like other types of red meat, pork is mainly composed of saturated and unsaturated fats, present in approximately equal amounts. The fatty acid composition of pork is slightly different from the meat of ruminant animals. It is low in conjugated linoleic acid and is slightly richer in unsaturated fats (Arnarson, 2016).

2.3.1.1.4 Vitamins and minerals

Pork is rich source of many vitamins and minerals. It is very rich in thiamine as compared with all other animals, nine times as much, but other vitamins do not have much variation. Meat also contain wide variety of minerals. The content of iron, zinc and copper vary considerably in different species and liver being by far the richest source of these minerals. Phosphorous, iron, zinc, potassium are the most abundant among all (Bender, 1992).

2.3.2 *Yangben* or edible lichen

Lichens are the symbiotic organisms, usually composed of a fungal partner, the mycobiont and one or more photosynthetic partners, the photobiont, which is most often either a green alga or cyanobacterium. The nature of the lichen symbiosis is widely debated. However, the most researchers refer lichen as a classical case of mutualism where all the partners gain benefit from the association. Alternatively, they are also regarded as an example of controlled parasitism because the fungus seems to obtain most of the benefit and the photobiont may grow more slowly in lichenized state than when free living (Nash III, 2008). Usually in the association both partners have benefit. The mycobiont has two principle roles: to protect the photobiont from exposure to extreme sunlight and dessication and to absorb mineral nutrients from the underlying surface or from atmosphere. The photobiont also performs two major roles: to synthesize organic nutrients from carbon dioxide and in the case of cyanobacteria, to produce ammonium from N_2 gas by nitrogen fixation (Rankovic, 2015).

Lichens are distributed worldwide and are the pioneer groups of organism that initiate vegetation in the bare area. They have both algal and fungal properties and produce n-alkane, unusual betaine ether glycerol-lipids, and saturated, unsaturated, branched and halogenated fatty acids. Many different bioactive secondary metabolite have also been isolated from different lichen species which have been used in different field of sciences (Hanus *et al.*, 2007). About 13,500 to 17,000 species of lichens extend from the tropics to the polar region. In Nepal, 471 species of lichens have been reported, of which 48 species are endemic. The components of lichen flora are governed by altitudinal variation and growth forms also vary accordingly. In Nepal very little research have been done in lichen till now and most of the known ones were recorded by foreigner (Devkota, 199?) .

Some *Ramalina* species of lichen are used as food in some Central and South Eastern Asian countries. Though the lichen flora of Nepal is complex one, those collected from Dhankuta, Taplejung and Terathum district were identified as *Parmelia nepalensis, Usnea thomsonii, Ramalina farinacea, Ramalina subfarinacea, Ramalina conduplicans* etc. In Nepal, Rai and Limbu communities use them as traditional food usually mixed with other dishes. It is also called *Jhyau, Tarey* or *Jhulo* while Rai, Limbu and Sherpa call it *yangben* (Devkota, 2011). Some species of lichen after being chemically analyzed for food value were found to be rich in carbohydrate, fat, crude fiber and minerals with comparable content of carbohydrate and protein with that of rice. If mixed with food they provide sufficient amount of minerals and other nutrients. The high Fe, Ca and riboflavin content of species *Usnea, Parmelia, Ramalina* and *Peltigera* make them potentially valuable food supplements (Hanus *et al.*, 2007).

2.3.2.1 Chemistry of lichen

Since the lichen thallus is a composite structure, it is not always possible to decide the origin of the chemicals synthesized. Lichen compounds can be classified into two main groups: primary metabolites (intracellular) and secondary metabolites (extracellular). Intracellular products include proteins, amino acids, polyols, carotenoids, polysaccharides and vitamins. They are bound in the cell walls and protoplasts and are often water soluble such that they can be extracted with boiling water. Most of these intracellular products are nonspecific, and also occur in free-living fungi, algae and in higher green plants (Nash III, 2008).

However the majority of the compounds found in lichen are secondary metabolites which may range between 0.1 and 10% of the dry weight, but sometimes up to 30%. More than 800 secondary metabolites are known to produce by lichens most of which are unique to these organisms only. All of the secondary metabolites are of fungal origin and are extremely stable. These compounds are in the form of crystals deposited on the surface of the hyphae. They are poorly soluble in water and can be isolated by organic solvents (Rankovic, 2015).

2.3.2.2 Uses of lichen

Lichens are the important part of nature and are useful to human beings in many ways. Among hundreds of them, the most important ones can be mentioned as:

1. As food

Lichens have been widely used as food for centuries. However some species are mildly toxic, a few are poisonous and most are indigestible in their raw form. The complex carbohydrates difficult to break down in human digestive tract make them poorly digestible. So, they are prepared in the way that improves their digestibility as well as delicacy (Crampton, 2017). The best known lichen polysaccharides are lichenan, isolichenan and galactomannan which are important when it comes to functionality in meat products (Nash III). The fibrous plant substances rich in non-cellulosic polysaccharides were found to have good water holding capacity. The content of soluble non-cellulosic polysaccharides of such fibre rich plant stuffs was found to be positively correlated to water holding capacity by Ngoc *et al*, (2007). This broadens the application of *yangben* in food industries where such functionality is desired.

Reindeer moss or *Cladonia rangiferina* is a staple food of reindeer and caribou. *Umbilicaria esculenta* that grows on rocks is used in Asian cuisine after being fried. Some lichens are boiled and mixed with fruit and flavouring agents such as onions before being served. However, lichen play a major role as food substitute during famine (Crampton, 2017). In Nepal, lichens are taken as meat analog. The commonly found species *Parmelia, Ramalina* and *Usnea* are used as traditional food by Rai and Limbu community. The lichen, commonly called *yangben,* after being collected are sorted and cleaned to remove foreign particles. As stated by Yaqso (2003), lichens have bitter taste due to presence of fumarprotocetraric acid. Subba (1997) suggests that the bitter principle can be removed by boiling lichens in 10% ash solution for 15 minutes. Then, debittered *yangben* is washed to remove ash and sun dried which is then used as food in different ways usually mixed with other items. Blood curry, Pork curry or *Yangben Faksa,* Egg curry, Sausage or *Sargayangma* etc. are the popular dishes in Nepal made out of *yangben* (Dhungana, 1985).

- Blood curry: Rehydrated *yangben* is mixed with blood (especially pork) and then it is cooked

- Pork curry: Rehydrated *yangben* is cooked with pork meat and delicious dish is prepared which resembles the character of meat
- Egg curry: It is prepared by frying the rehydrated *yangben* with egg.
- Vegetable curry: *Yangben* is also consumed by cooking it alone in *karahi* without mixing with any food and taken as vegetable curry with meal (Limbu, 2014).

2. The most important environmental role of lichens is their use as bio indicators. The sensitivity of particular lichen species to a broad spectrum of environmental conditions, both natural and unnatural make them an important biological indicator for pollution. (Nash III, 2008)
3. The genus *Usnea* is most commonly used as medicine due to presence of usnic acid which is used as antibiotic and anti-inflammatory substance (Crampton, 2017). Besides, some species of *Letharia, Ramalina, Cetraria, Cladonia* etc. are also used as traditional medicine in many parts of the world (Rankovic, 2015).
4. Many species of lichen are used for making wool and fabric dyes. Many colors are possible and it depends on the species of lichen and the extraction process used. The most common example is a litmus, which is a mixture of dyes extracted from specific lichens, especially *Rosella tinctoria* (Crampton, 2017).
5. They are used as a ingredients in perfumes and deodorants. *Evernia prunastri* and *Pseudevernia furfuracea* are used for such purpose (Crampton, 2017).
6. The most of the lichen secondary metabolites have wide biological action like antioxidant, antiviral, antipyretic, analgesic and antitumor action which make them suitable candidate for various pharmaceutical purposes (Molnar and Farkas, 2010).

2.3.3 Blood

Blood is the first edible by-product obtained from the animal body after slaughter which used to be discarded for many years. According to the Meat Inspection Act of the United States, blood obtained from healthy animal that has been inspected and passed for use in meat food products is approved for food use. It is the significant part of the animal's body ranging 2.4-8% of the animal's live weight. In average, 3-4 % of blood can be recovered from pig during slaughtering (Jayathilakan; *et al.*, 2012). Blood is made up of two fractions, the cellular fraction containing the red blood cells, white blood cells and platelets, and the plasma fraction in which cellular fraction is suspended. Plasma comprises

65-70% of the total volume of blood and remaining is the cellular fraction. Animal blood contains about 18% protein with reasonably good balance of amino acids. The typical nutrient composition of bovine blood consists of 80.9% water, 17.3% protein, 0.23% lipid, 0.07% carbohydrate and 0.62% minerals. Plasma contains about 7.9% protein, composed mainly of 4.2% immunoglobulins, 3.3% albumins and 0.4% fibrinogen (Nollet and Toldra, 2011). The chemical composition of blood from different species of animals is given in Table 2.2.

Blood is a rich source of iron and proteins having high nutritional and functional quality. Due to high protein content it is sometimes referred as liquid protein. Many recent advances in blood collection and processing techniques have led myriad of blood derived ingredients suitable for use in foods and dietary supplement (Ofori and Hsieh, 2012). In Europe, blood is widely used to make blood sausages, biscuits, bread and blood pudding. In Asia it is widely used to prepare products such as blood curd, blood cake and blood pudding (Nollet and Toldra, 2011). Blood in food is used as an emulsifier, a stabilizer, a clarifier, a color additive and a nutritional component. Owing to the rich nutritional property, it functions as a protein supplement, a milk substitute, a lysine supplement or a vitamin stabilizer and an excellent source of many trace minerals (Silva and Silvestre, 2003).

Table 2.2 Chemical composition of blood

Components	Species(g/1000g of blood)				
	Pigs	Cattle	Sheep	Goats	Horses
Water	790.56	808.9	821.67	803.89	749.02
Dry solids	209.44	191.1	178.33	196.11	250.98
Hemoglobin	142.20	103.1	92.9	112.58	166.9
Other proteins	42.61	69.8	70.8	69.72	69.7
Sugar	0.686	0.7	0.7325	0.829	0.526
Cholesterol	0.444	1.935	1.339	1.299	0.346
Lecithin	2.309	2.349	2.22	2.46	2.913
Fat	1.095	0.567	0.937	0.525	0.611

Contd..

Fatty acids	0.475		0.488	0.395	
Phosphoric acid(as nucleic acid)	0.0578	0.0267	0.285	0.395	0.06
Sodium	2.406	3.636	3.638	3.579	2.691
Potassium	2.309	0.407	0.405	0.396	2.758
Ferric oxide	0.696	0.544	0.492	0.577	0.828
Calcium	0.068	0.069	0.07	0.06	0.051
Magnesium	0.0889	0.0356	0.03	0.04	0.064
Chloride	2.69	3.079	3.08	2.923	2.785
Total phosphorous	1.007	0.4038	0.412	0.307	0.392
Inorganic phosphorous	0.74	0.1711	0.19	0.143	0.174

Source: Pearson and Dutson (1988)

It is the plasma portion that is most widely used in the food industry because of its neutral taste and devoid of dark color arising from red blood cells. The heme component of cellular portion of blood imparts a characteristic dark brown color, odor and metallic taste to the final product which is undesirable for many people. So, the use of whole blood is suitable for only those products like sausages where such black color is expected as well as acceptable (Nollet and Toldra, 2011). When it comes to the meat industry, the bulk of blood protein is used as binder, natural color enhancer, emulsifier and fat replacer (Ofori and Hsieh, 2012).

2.3.3.1 Binder

The use of binder in meat industry is popular to bring about significant improvement in organoleptic properties of product. Binders have a macromolecular structure that have the capacity to form matrices to retain aroma and nutrients along with entrapment of large amount of water released during thermal processing to prevent exudation. Especially in ground meat products like sausages they are used to bind water and fat to stabilize meat emulsion. The plasma portion in fact is the best water and fat binder in the blood portion due to its ability to form gel upon heating. The binding property of plasma protein is comparable to that of egg albumin which is one of the most used binder in food industry.

So, plasma can be a alternative to egg albumin in many cases. However, the expected result in product is found to be affected by type of muscle used and proportion of binder used in the product. Extensive research regarding the optimum amount of plasma protein to get the desired result in a particular product is not found yet (Ofori and Hsieh, 2012). In research carried out by Chen and Lin (2002), water holding capacity of plasma protein was found to increase only at concentrations lower than 8% during optimization of plasma protein to develop a edible gel. The water holding capacity of plasma protein was also found to be significantly affected by pH and fibrinogen concentration also.

2.3.3.2 Color enhancer

As it is said that Man eats with his eyes, color is the most important quality attribute of any food product. Moreover in meat products it is the vital one. The red color of the blood imparted by heme protein of blood represents a good source of natural red colorant. But this red color is not so stable and depends on the oxidation state of heme iron. The heme iron in ferrous form i.e. oxyhemoglobin is bright red in color whereas in its oxidized form (Fe^{3+}) known as methemoglobin imparts undesirable brown color. Thus the stabilized hemoglobin using different techniques is preferred in food industry (Ofori and Hsieh, 2012).

2.3.3.3 Emulsifier

In meat industry, emulsifiers are widely used in emulsified products such as frankfurters, luncheon meat, mortadella etc. where formation of stable emulsion is needed by binding fat, water and proteins together. An emulsion is a heterogeneous system of two immiscible liquids intimately dispersed in another in the form of droplets. Such system possess a minimal stability and thus emulsifier plays a major role in stabilizing them (Adheeb Usaid *et al.*, 2014). It joins two immiscible forms together and also redistribute the fat finely throughout the product. Proteins are the most common emulsifying agents used in food industry owing to their non toxic nature and availability. The emulsifying property of blood protein is found to be comparable with that of casein which is the most popular emulsifier used today. Therefore in many cases blood proteins can adequately replace the expensive casein as the emulsifying agent in meat industries (Ofori and Hsieh, 2012).

2.3.3.4 Fat replacer

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

Many studies have shown that blood proteins have potential to replace fat in meat products. The study carried out by (Viana *et al.*, 2005) and others point out that the use of fat replacer in meat products result in increase in protein and moisture content without significantly affecting the aroma, taste and consistency of the product. According to Confrades *et al.* (2000), plasma protein have greater influence on binding and textural properties of bologna than soy fiber. This supports the fact that blood proteins are comparable fat replacing potential as that of commercial ones. It has been also reported that blood proteins produce superior physical properties in the product than non-blood fat replacers. Blood protein thus is a cheaper alternative as fat replacers in producing many low fat meat products (Ofori and Hsieh, 2012). Whatever be the purpose of incorporation of blood protein in food, it serves as a iron and protein supplement in all cases which is the most desirable nutritional significance (Nollet and Toldra, 2011).

2.3.4 Pork Fat

Fat is another valuable ingredient for *sargayangma* preparation. All type of fat can be incorporated in sausage making. Though carcass fat is widely preferred, internal or body fat can also be used. These fatty tissues are easily separated from other tissues and used as separate ingredient for different product formulation (Savic, 2011).

The effect of fat on food products is complex and depends on the nature of product. The interaction of fat with other component of the food also influences the effect on final product. In general, fat contributes to tenderness, juiciness and flavor along with the significant effect on the physical attributes of the product and all decrease proportionally as fat content decrease. This effect is most important in processed and fabricated product than the whole meat cuts (Paul, 1993). In case of sausage, role of fat depends on the type of sausage varieties. In emulsion type sausage, the added fat is a part of the complex system or emulsion and participates in forming the product's characteristics structure. Whereas in raw sausages, specific taste, aroma and consistency of the product is affected (Savic, 2011).

2.3.5 Meat offals

Offals are also called variety meats and refer to the internal organs usually non-muscular part of slaughtered animals. These offals particularly the glands and organs offer a wide variety of flavor and texture along with nutritional value. Among them mostly heart and liver are incorporated in *sargayangma* preparation. However kidneys and other available by-products can also be a choice depending on the availability (Nembang, 2016).

They have poor binding capacity and do not contribute any technological significance in product. However they improve the palatibility and aid nutritional value to the product if used and processed properly (Savic, 2011).

2.3.6 Salt

Salt is one of the oldest and most ubiquitous ingredient ever known to man. The word was sausage derived from Latin word 'sal' itself describes the importance of salt in sausage making. Salt is added to food basically for two reasons: to enhance flavor and to extend shelf life. Though it holds a rich historical records of use in food its role in meat products has essentially remained the same for thousands of years. The important functions of salt in sausage and other meat products can be outlined as:

a. It assists in reducing and preventing microbial growth.

b. It aids in extracting salt-soluble meat proteins for emulsion stability.

c. It increase the binding capacity of meat during cooking and increase water holding capacity of meat products.

d. It enhances basic meat taste and flavor. (Anonymous., 2010)

The amount of salt used depends on the type of sausage and generally ranges from 1.8-2.2 percent of the sausage mix. The acceptable level may increase up to 3 percent in dry products (Savic, 2011).

2.3.7 Spices

Though spices perform multiple of function in food, spices here in sargayangma are used essentially for flavor and nutritional importance. They have antioxidant, antimicrobial, pharmaceutical and nutritional properties (Peter, 2004). In modern food industry, spices are employed to give appealing and appetizing flavor rather than to act as preservatives. Among the thousands of spices, basically garlic and ginger in grounded form are used in *sargayangma* in amount according to taste. However, the recipe for spices can vary according to consumer preference.

2.3.8 Intestinal casing

Casings are soft cylindrical containers used for stuffing sausage mixes so as to give them a proper shape and appearance. They can be natural or artificial. Natural casings are mainly derived from the small and large intestines of sheep, goat and pigs but also from cattle and horses. Pork intestine is commonly used for *sargayangma* preparation. Among the four anatomical layers of intestinal wall, mucous membrane, muscular layer and serous membrane are peeled off and the remaining sub mucous membrane is processed for sausage casing (Heinz and Hautzinger, 2007).

Small intestines are separated from adhering connective and fatty tissue during which outer layers of serous membrane are removed automatically. The intestinal content is removed manually and flushed with water. Then the internal slime layer is removed manually by using a tablespoon or knives. The internal mucous membrane is also stripped off and the loosened tissues inside the casing are removed manually. The rest of the layers and muscular layers get removed during de-sliming and cleaning operation. The remaining strong elastic sub mucous membrane is used for sausage casing. They are dry salted and stored in a cool place at temperatures not exceeding +15^{o}C. Usually they are not peeled off before consumption but eaten together with the sausage. Thus, they are edible in this sense (Heinz and Hautzinger, 2007).

These all ingredients except *yangben* and blood are the common one for other varieties of blood sausage too. However, ingredients like binders, fillers, extenders, phosphates, nitrite and nitrate and ascorbate are being excessively used as non-meat additives in sausage nowadays (Toldra, 2010). Fillers are mostly the plant substances which are low in protein but rich in carbohydrate such as cereals, roots, tubers and some refined products like starches and flours. They serve to add carbohydrate to the meat products which are very low in carbohydrates and fill up the product volume. Apart from this, they are also used because of their extensive water binding ability by means of physical entrapment. Binders are substances of animal or plant origin with high level of protein that serve as fat and water binder like high protein soy isolate, wheat gluten, milk protein, etc. Such high quality protein act as binder due to protein network structuring (Heinz and Hautzinger, 2007).

It is obvious that the quality of the raw materials selected directly affect the final product quality due to which all the hygienic, economic and processing factors should be considered for the formulation of raw materials required.

2.4 Meat by-products and their utilization

Animal slaughtering not only provides meat but valuable by-products to the mankind. Animal by- product may be defined as every part every part of a slaughtered animal except dressed carcass. After slaughtering of the animal only one third is meat while rest being the by-products and waste. Proper processing and utilization of these by-products is a major challenge for every meat processing industry today (Irshad *et al.*, 2015).

Animal by-product can be divided into two classes, edible and inedible. Edible by products are those by-products that can be consumed as a food by human beings and include blood, liver, kidney, heart, brain intestine, tongue, skin or pelt, spleen etc. The term variety meats is used to indicate all such wholesale edible by-products. Inedible by-products include those which cannot be consumed as food like hides and skin, glands, hoof, bone, etc. (Irshad *et al.*, 2015).

Literature indicates that by-products of cattle and pig represents 66 and 52 percent of live weight respectively. Less than half of the obtained by-products are suitable for normal consumption while remaining are not suitable due to unusual physical and chemical characteristics. So, the improved utilization of such a high amount of by-products can give a good profit to meat processors as well as ensure health and environmental safety (Jayathilakan *et al.*, 2012). Tradition, culture and religion are often important when a meat by-product is being utilized for food. When it comes to Nepal *Sargayangma, Womyuk* etc. are the traditional foods that present meat by products in the form of attractive dishes (Kharel *et al.*, 2009).

2.4.1 Utilization of meat by-products as food

2.4.1.1 Uses of blood

Animal blood is rich source of good quality protein and heme iron and is an important edible by-product. In Europe, animal blood is used to make blood sausages, blood puddings, breads and biscuits. It is used in blood curd, blood cake and blood pudding in Asia (Jayathilakan *et al.*, 2012). Blood is used in food as an emulsifier, a clarifier, a stabilizer, a color additive and as a nutritional component. It is used as a protein supplement, a milk substitute, a lysine supplement and is an excellent source of most of the trace minerals (Silva and Silvestre, 2003).

Blood when separated into different fractions also have therapeutic purposes. It is used as a nutrient for tissue culture media, as a necessary ingredient in blood agar, as peptones for microbial use, as a stabilizer in vaccines, etc. (Jayathilakan *et al.*, 2012).

2.4.1.2 Uses of liver

Liver is the most widely used edible organ as it contains high amount of nutrients, mostly vitamins and minerals (Irshad *et al.*, 2015). It is used in many processed meats such as liver sausage and liver paste (Devatkal *et al.*, 2004). Livers are generally braised or boiled and used directly for human consumption and as sausage ingredient (Irshad *et al.*, 2015).

2.4.1.3 Uses of heart

Heart is generally used as a table meat. Whole heart can be roasted or braised while sliced heart meat is grilled or braised (Jayathilakan *et al.*, 2012). It can also be diced and added to stews and to other meat for additional flavor. It is used as an ingredient for sausage and other processed meat products (Limbu, 2014).

2.4.1.4 Uses of killing fat

Animal fats are the important by-product of the meat industry. The edible fat obtained from clean fatty tissues of pig is called lard. It is obtained by dry or wet rendering in industrial level. Rendered lard can be used as an edible fat without any further processing (Jayathilakan; *et al.*, 2012). Traditionally, lard were used for deep frying. It is also used for margarine and shortenings. Some edible lard is also used in sausages or emulsified products (Shahidi, 2005).

2.5 Basic operations in *sargayangma* preparation

2.5.1 Selection of ingredients

Various ingredients such as pork belly, liver, heart, blood, *yangben*, spices and salt are taken for *sargayangma* preparation. All the ingredients are required to be of hygienic quality. The meat ingredients are trimmed of bones and skin and cut to small pieces. Fat and blood are kept chilled until used. *Yangben* is first soaked in a water for about five minutes and then used. Spices are also washed and used in the form of paste (Limbu, 2014).

2.5.2 Mincing and chopping

The meat chunks of various shapes and sizes are ground to form uniform cylinders of lean meat and fat is also minced separately in a meat mincer. Meat is put into a funnel on the top from where material goes on a horizontal screw conveyer. At the end of the screw conveyer there is a blade installed in front of a fixed hole grinder plate. The rotating blade cuts the compressed meat and minced meat comes out of the machine (Anonymous.). The size of the holes in the grinder plate determines the diameter and the thickness of the plate determine the length of the cylindrical particles (Pearson and Tauber, 1984). Mincing completely destroys the meat structure. Percent extractable protein significantly increased with each step of muscle destruction during grinding and closure contact between meats surfaces aid in reducing cooking loses and increasing binding strength (Thapa, 2016).

Besides meat and fat, offals and *yangben* are chopped separately to a fine chopped mass. The spices are also used in the form of paste. The varieties of spices used depend on the taste of manufacturer. Previously ground spices do not need any preliminary operations. After mincing, chopping is one of the key step in sausage processing but in traditional *sargayangma* preparation chopping of ingredients in a meat chopper is not a part of the process.

2. 5.3 Stuffing

The sausage mix obtained from chopper is then transferred to stuffers for extruding into casings. Casing may be natural or artificial in sausage stuffing. For *sargayangma*, natural pork intestines well processed into casing are used for stuffing. This is the principle step that determines the size and shape of the product. Piston type, pump type and one combining the feature of both are three types of stuffers commonly used for sausage stuffing (Pearson and Tauber, 1984).

2. 5.4 Linking and tying

The encased mass after stuffing is tied with thread or fastened with metal clips. Stuffed casing are twisted or drawn together to produce links which is done either by hand or with mechanical devices for commercial sausage production (Pearson and Tauber, 1984). The similar process applies for *sargayangma* too in which encased mass is tied at a regular intervals of about 10 cm in length which are then ready to cook (Limbu, 2014).

2.5.5 Cooking

Various cooking methods are used nowadays for sausage cooking. Oven cooking employing steaming, smoking, drying or a combination of all are common ones. According to Limbu (2014), *sargayangma* in traditional process is cooked in a vessel in hot water at 85°C for about 45 min. Whatever the methods used it should be effective for achieving ambient and product core temperature (Vignolo *et al.*, 2010).

The objective of cooking sausage is to

a. firm them by protein coagulation and partial dehydration
b. fix the color of the cured sausage by denaturation of myoglobin and ultimate formation of pink nitrosyl haemochromogen
c. to extend the shelf life (Limbu, 2014)

Heat treatment strongly affects the texture, protein and other important quality factors such as color, flavor and juiciness of the final product (Abdulhameed *et al.*, 2016).

2.6 Quality attributes of *sargayangma*

The quality attributes are the combination of intrinsic as well as extrinsic characteristics of the product. Intrinsic properties refer to the functional properties whereas extrinsic ones are those that project the image of the product on the basis of which consumer judge the product (Ventura-lucas, 2002). In other way, quality may be nutritional or acceptance quality. Nutritional quality is a objective measure while acceptance quality is highly subjective approach (Rivera, 2006). In case of processed meat products like sausage different technological functional properties like water holding capacity, emulsion capacity, protein solubility, etc. and other sensory parameters are the major ones to be considered for assessment of the quality.

2.6.1 Sensory attributes

2.6.1.1 Appearance

Appearance is one of the important factor that catches consumer's eyes while eating fresh as well as processed meat products. It is a overall judgment of properties such as color, surface structure, shape and any defects present (Thapa, 2016).

Color is the primary criterion that determines the acceptability and rejection of product. It reflects the freshness as well as hygienic quality of product (Pearson and Dutson, 1994). For processed product like *sargayangma*, the ingredients used play a major role. The typical reddish black color contributed by blood and the black color of *yangben* affect the color perception of consumers. Moncel (2016) states that fat also affects the visual appearance of food by imparting glossy and moist texture and due to its ability to refract the light. The other visual factors are affected by particle size, uniformity of mixing and processing methods.

2.6.1.2 Flavor

Flavor is a complexed sensation comprising mainly of odor and taste. It is sensed collectively by the oral and olfactory senses. It results from the combination of the basic tastes sweet, salt, bitter, sour and umami. Flavor and odor active volatiles include alcohols, aldehydes, aromatic compounds, esters, ethers, furans, hydrocarbons, lactones, etc. (Toldra, 2010). Flavor has profound effect on overall acceptability of the product. The raw meat is characterized by salty, metallic and bloody taste with sweet aroma. During cooking process typical taste and aroma is developed as a result of complex interaction of precursors derived from lean and fat compositions of meat. like maillard reaction, lipid interaction (Pearson and Dutson, 1994). Lipids generally have the greatest influence on the production and release of aroma and flavor components (Tran and Thu, 2006).

The flavor in *sargayangma* is a summed up effect of typical ingredients used. Besides the typical meat taste and aroma, the metallic blood taste from blood and deep earthy flavor from *yangben* are the major palatable flavors found (Khanal, 2016; Ofori and Hsieh, 2012).

2.6.1.3 Texture and tenderness

Texture is the attribute of a substance resulting from a combination of physical properties and perceived by the sense of touch, sight and hearing (Thapa, 2016). It is a complex sensory attributes of chewing and mouthfeel with multiple descriptors like fiber cohesiveness, softness, friability, adhesion, chew count, rubberiness and hardness. Though texture can be assessed by objective method too, tenderness as perceived by subjective approach is one of the sensory attribute used to assess the overall texture of meat (Juárez *et al.*, 2012).

Meat tenderness depend on several factors such as size of meat fibers, activity of the muscle, distribution of connective tissue, aging, cooking methods, fat marbling and other mechanical treatments applied to increase the tenderness of muscle fibers. In mouth, tenderness also depend on other characteristics of meat including water holding capacity and fat content (Juárez *et al.*, 2012).

2.6.1.4 Juiciness

Juiciness is another important attribute that reflects the texture of meat and meat products. It occupies third importance behind tenderness and flavor (Pearson and Dutson, 1994). According to Weir (1960), juiciness is a combined effects of initial fluid release and the sustained juiciness during continuous chewing resulting from stimulating effect of fat on salivary flow. Tenderness and juiciness can be closely related as the more tender the meat, the more juice is released on chewing and the more juicy the meat appears. One of the most important factor affecting the juiciness of meat is the cooking procedure. Method which causes greatest retention of meat fluid results the product with enhanced juiciness. As a result moisture content, salt, water holding capacity of meat and marbling or intramuscular fat directly influence the juiciness of product (Weir, 1960). According to Pearson and Dutson (1994), incorporation of meat by-products like heart, tripe, liver etc. also have been found to have significant effect on juiciness of the product.

2.6.2 Physicochemical attributes

2.6.2.1 Water holding capacity

Water holding capacity is the ability of meat to hold onto both inherent water or added water when force is applied. Sometimes two different terms, water holding capacity and water binding capacity, are used to refer the ability to hold natural water and added water respectively. Apart from natural moisture processed meat product contain added water as part of recipe. To retain both of the water to a consistent optimum extent is a primary factor to be attended during meat manufacturing process so as to improve quality as well as yield (Ranken, 2000).

2.6.2.2 Processing yield

Processing yield or cooking loss is one of the important part of interest. This parameter of meat can be correlated with many other attributes of meat like juiciness, water holding capacity etc. A high cooking loss gives an expectation of less optimal eating quality (Aaslynga *et al.*, 2002). The total loss that occur during cooking of meat include the dripping loss and volatile loss. The volatile loss is from evaporation of water and include volatile substances from decomposition of fat and volatile aromatic substances. The dripping loss include fat, water, salts and both nitrogenous and non-nitrogenous extractives (Lowe, 1932).

2.6.3 Mechanism of water holding in meat

Water is the major component of the muscle tissue. Most of the water is held in the spaces between the thick and thin filaments of the muscle cell, and accumulated between fiber bundles and between fibers, while a small proportion is also held by electrostatic attraction between proteins. The amount varies depending on different pre and post-mortem factors (Cheng and Sun, 2008). The forces with which the water is held in the meat are not fully understood yet. Though it is estimated that 5 percent may be chemically bonded to proteins, 24 percent is held by capillary forces which can be squeezed out under pressure and 45 percent is held firmly whose mechanism is not known (Ranken, 2000). The overall two effect, static effect and net charge effect can be best used to describe the mechanism of water holding in meat.

2.6.3.1 Static effect

The thousands of spaces present within myofibrils, between myofibrils, between myofibrils and cell membranes, between individual cells and bundle of cells and the connective tissue layers of skeletal muscle are the critical parts where water is held. Any processing steps that alter these spaces, denature the protein or reduce the total available spaces decrease the water holding capacity of meat (Anonymous., 2012).

2.6.3.2 Net charge effect

It is the protein water interaction and greatly influenced by processing conditions and added ingredients. Meat components are in a charged state. The opposite electrical charges on meat component attract and may be believed to hold water. Conversely, similar charges repel and as a result the charged components spread apart. Thus the compartments created between sarcomeric myofilaments provide space to hold water. This enlargement is possible only when the proteins are not denatured by any processing condition. The meat components usually are in the negatively charged state. Water molecules being dipolar are thus attracted towards muscle proteins by ionizable acidic and basic groups (Anonymous, 2012).

2.6.4 Factors affecting water holding capacity

The water holding capacity of fresh meat as well as the processed product is influenced by numerous factors starting from live animal handling to final processing steps. All of these factors directly and indirectly affect the rate and extent of pH decline and thus the final water holding capacity (Huff-Lonergan, 2010).

2.6.4.1 Post mortem pH

The pH of meat affects WHC tremendously. Immediately after slaughter of animal, the pH of muscle changes from neutral to about 5.5-5.7 which is the ultimate pH of the post rigor meat. The electronic charge on the protein changes as the pH changes. The higher the net negative charge on the proteins, higher is the repulsive charge and thus the space between the filaments. This leads to higher water holding capacity of meat. If pH of meat falls to the isoelectric pH ($pH_{I)}$ of meat proteins i.e 5.1 at which the net electronic charge on the protein is zero, WHC of meat is the lowest. This is due to the minimal attraction between the proteins and water. Thus, to improve the water holding capacity of processed meat products focus should be on maintaining the pH of meat above or below pH_I (Puolanne and Halonen, 2010). Rapid pH decline below ultimate pH while the muscle is still warm causes the denaturation of many proteins resulting severe purge or drip loss. This condition is commonly called PSE (Pale, Soft and Exudative) and is found in product from pigs. On the other hand meat with very high ultimate pH is dark in color and the surface appears dry with very firm texture. Such meat is referred as DFD (Dark, Firm and Dry) meat and has high water holding capacity. This type of condition arise when the animal experience pre-harvest stress such that glycogen is depleted and less lactate is formed for pH decline (Huff-Lonergan, 2010). The stress condition and pre-slaughter handling procedures thus greatly influence the final pH of meat. Thus, it is obvious to follow optimized pre handling procedures and feeding strategies to obtain the product with better water holding capacity (Cheng and Sun, 2008).

2.6.4.2 Storage condition

Chilling of meat after slaughtering is widely preferred for safety as well as quality purpose. According to Savell *et al.* (2005), the chilling process after slaughter greatly influences post-mortem biochemical and structural changes in the muscle. The internal muscle temperature reaching 10^oC after 12 hour and $2\text{-}4^oC$ after 24 hour of slaughter is recommended to avoid PSE meat. The rate of chilling is also found to affect the post-mortem pH decline i.e slower chilling results more rapid drop in pH and vice-versa, which in result affect the final water holding capacity. But it is also important to maintain the possible low temperature without freezing as the physical disruption caused by ice crystals formed in the meat increase the drip loss on thawing (Huff-Lonergan, 2010).

2.6.4.3 Processing ingredients

The number of ingredients are used in different meat product formulation. Salt is one of the basic ingredient among all which act as yield enhancer and binder (Anonymous, 2012). The water holding capacity of meat is increased up to a concentration of about 5% in lean meat and then decrease. The water holding capacity at 11% concentration is same as that of unsalted lean meat (Heinz and Hautzinger, 2007). Salt in meat provide positive Na ions and negative Cl ions which can interact with the protein and water and aid in holding water in meat. According to Cheng and Sun (2008), chloride ion bind to the meat protein filaments and increase the electrostatic repulsive force between them. Due to repulsive force the protein structure matrix unfolds and swelling occurs. The swelling provides a number of protein side chains to bind water which in turn increases the water holding capacity. The increase in water holding capacity is observed above pH_I and the same decreases below $pH_{I.}$ (Pomeranz, 1985). Similarly, phosphate also act in the same way as salt and improve the water holding capacity of meat by increasing pH and ionic strength (Cheng and Sun, 2008). The fat content of meat can also be correlated with water holding capacity of final product. Muscles with high level of intramuscular fat tend to have higher water holding capacity (Lawrie, 1991; Wood, 1993). Lawrie (1991) suggests that intramuscular fat loosens up the microstructure of meat causing more incorporation of water. Also the reduction of fat level in sausage also reduces the effective salt concentration and thus water holding capacity (Zayas, 1997). Hence, fat content also affects the water holding capacity in various ways.

Besides salt and phosphate, many other non-meat ingredient are also used increasingly to improve the water holding capacity of meat and meat products. Modified food starch, functional animal and plant protein are commonly used ones and are considered much safer than salt and phosphate regarding health concerns. Such ingredients are usually the gelating adjuncts and are used as binders and extenders for increasing the ability of water retention of meat products (Cheng and Sun, 2008). Functional plant proteins such as soy protein isolate, common bean flour, milk powder, casein and corn germ protein flour have been found to have ability to increase protein functionality by binding fat and water. They also have special gelling property which is essential for binding meat chunks and fat together (Ranken, 2000). However these functional effects are described based on the individual effects of such binders and fillers used but the correlation between the functional properties of meat and such non-meat filler ingredients and functional effects in comminuted meat products have not been investigated much. Comer and Allan-Wojtas (1988) have reported that the stability and the textural properties of comminuted meat products with fillers and binders is also influenced by the competition for moisture between proteins and carbohydrates and by protein interactions with water, fat and other proteins. The different ingredients used as binders do not have absolute bind values but the relative functional performance of ingredients is affected by the composition of the total system or interactions between them. But the papers regarding such microstructural effects of fillers in meat products are limited and requires extensive research.

2.6.4.4 Processing factors

Various physical processes are involved during processing of different meat products like mincing, chopping, mixing, tumbling, massaging, etc. These processes disrupt the cellular structural integrity and open the channels from where water may be lost (Puolanne and Halonen, 2010). On contrary, it is also found that mincing of meat in the production of sausages destroys the sarcolemma and considerably intensifies the swelling and water holding capacity of the myofibrillar system (Hamm, 1975). Cooking is another important processing step adopted for most of the meat product. The primary purpose of cooking is to make meat more palatable through flavor, aroma and color development and reduce the microbial load. Besides these several changes occur in the structural and functional property of meat proteins. Depending on the temperature of cooking sarcoplasmic, myofibrillar and connective tissue protein are heat shrinked which impair the water holding capacity of proteins. The temperature for protein shrinkage and denaturation ranges 60-70°C for sarcoplasmic proteins, 65-70°C for myofibrillar protein and 55-60°C for connective tissue protein (Ranken, 2000).

2.6.5 Factors affecting cooking loss

2.6.5.1 Time and temperature of cooking

The extent of fluid loss depend greatly on cooking temperature and to some extent on cooking time. There is large cooking loss in the temperature range of 50-60°C. It is found that 80-100% of the total loss occurs by the time the temperature reaches 80°C. In the case of larger meat piece overall loss depend on the temperatures reached at different depths. Besides, the rate of cooking also affects the loss as the rate of protein denaturation differs with the rate of cooking.. Under same centre temperature for a particular product, the faster the cooking, the greater the loss and slower the cooking, smaller is the loss (Ranken, 2000). In general, cooking loss is found to vary proportionately with cooking temperature (Lowe, 1932). Increasing the cooking temperature causes the denaturation of myofibrillar proteins and consequently results in shrinkage which leads to higher cooking loss (Murphy and Marks, 2000).

2.6.5.2 Composition of meat

The cooking loss is related with fat content of meat such that the greater the fat content of meat greater is the cooking loss. Meat with high percentage of fat cooked under standardized condition has a greater loss than the lean meat cooked under same condition. The drip loss is also higher for the fatty meat than similar lean cuts for the same kind of animal (Lowe, 1932). In research carried out by Roseland et al, (200?), no significant difference in cooking yield of ground pork was noticed in three levels of fat content while cooking yield was inversely related to fat content in ground beef products. Thus, it can be concluded that cooking yield is not affected by fat alone but also the species and type of meat. Similarly, the use of binders and fillers in the meat products also affect the cooking yield. According to Heinz and Hautzinger (2007) and Ranathunga *et al.* (2015), binders and fillers are added during meat processing to improve the texture, cooking yield and as an aid to improve quality attributes like taste and juiciness by binding the water in the system and allowing the meat proteins to form a firm gel structure. The cooking loss in pork meat was also related to the water holding capacity and pH of the meat by Aaslynga *et al.* (2002) who reported that low water holding capacity and low pH result in higher cooking loss while medium and high WHC and pH result no difference in cooking loss. The connection between the water holding capacity and cooking loss was not linear as increase in WHC or pH beyond a certain level did not decrease the cooking loss. Thus, the overall cooking loss can be attributed to the raw meat quality as well as interaction of ingredients affecting the composition of meat.

2.6.5.3 Surface area

Cooking loss is also directly affected by surface area of meat pieces. There is greater cooking loss for meat chunks with large exposed surface area as the possibility of loss of water soluble components increase with increase in surface area. Also the compact meat pieces with small surface areas result lower cooking loss than irregular shaped pieces with larger surface areas (Lowe, 1932).

PART III

Materials and methods

3.1 Raw materials

3.1.2 Pork meat

Pork belly, blood, liver and heart was bought from VRC meat shop of Dharan market after 3-4 hours of slaughter. They were washed, trimmed, deboned and deskinned with the help of sharp knife on a chopping board. The fat was also trimmed and separated from meat. All of the ingredients were kept in refrigeration until processing.

3.1.2 *Yangben*

Yangben was also bought from the local market of Dharan. The ash processed *yangben* was bought. So, the sorted and processed *yangben* was used directly after soaking.

3.1.3 Pork intestine

Pork large intestine was brought from VRC meat shop in Dharan. The adhered fat was removed and then intestine was manually cleaned, flushed with water and processed for intestinal casing. It was reversed and then inflated with water to check pinholes. It was kept soaked in water and used for stuffing.

3.1.4 Salt and spices

Salt, garlic, ginger and chili powder were bought from the market. Garlic and ginger were finely ground in a electric grinder. All of the ingredients were weighed out and used as per required.

3.2 Chemicals, Glassware and equipments

Following chemicals, glasswares and equipments of lab of Central Campus of Technology (CCT) were used for the work.

3.2.1 Glasswares

Beakers, petridish, funnel, measuring cylinder, volumetric flask, conical flask, crucible, burette, pipette, glass rods.

3.2.2 Equipments

Meat mincer, Stuffer, Hot air oven, Soxhlet apparatus, Muffle furnace, Kjeldahl digestion and distillation set, Dessicator, Electronic balance, Thermometer, Whatman no.1 filter papers.

3.2.3 Chemicals

Catalytic mixture (K_2SO_4+$CuSO_4.5H_2O$+SeO_2), Mixed indicator solution, Boric acid, Phenolphthalein, Conc. H_2SO_4, NaOH, Diethyl ether/ Petroleum ether, Conc. HCl

3.3 Methodology

3.3.1 Experimental design

Response surface methodology (RSM) was adopted in the experimental design as it emphasizes the modelling and analysis of the problem in which response of interest is influenced by several variables. The main advantage of RSM is reduced number of experimental runs needed to provide sufficient information for statistically acceptable results. A three-level, three-factor central composite face-centered design was selected for which Design Expert (7.1.5 version, State-Ease Minneapolis, MN, USA) was used. The independent variables selected for the experiments were fat, blood and *yangben* concentration. Response variables were water holding capacity, processing yield and jelly and fat separation.

The three level of the process variables were coded as -1, 0 and 1 and the values of variables at three levels are given in Table 3.1.

Table 3.1 Values of independent variables at three levels of the design

Independent Variables	**Uncoded**	**Levels in coded from**		
		-1	**0**	**+1**
Fat content (%)	A	10	17.5	25
Blood content (%)	B	10	20	30
Yangben content (%)	C	5	12.5	20

The upper and lower limit of the ingredients were set as above on the basis of the survey carried out by Limbu (2014) on *sargayangma* and the effect was studied within the range. The scheme of recipe given by Limbu (2014) is presented in Table 3.2.

Table 3.2 Recipe formulation for *sargayangma*

Composition	Amounts (g/100g)
Blood	20
Belly	50
Killing fat	5
Liver	15
Heart	10

Yangben was added 5% of above formulation. Here, in this study the percentage concentration of fat, blood and *yangben* were taken in the range 10-25%, 10-30% and 5-20% respectively of the belly and offals (liver and heart) taken. The range were so taken such that the recipe given by Limbu (2014) falls within the limits and thus the effect could be studied within the range. The experimental designs in coded form and actual levels for response surface analysis are given in Table 3.3 and Table 3.4 respectively.

Table 3.3 Experimental design in coded form for response surface analysis

Coded variables			Combinations	Replication	No. of Expt.
A	B	C			
±1	±1	±1	8	1	8
±1	0	0	2	1	2
0	±1	0	2	1	2
0	0	±1	2	1	2
0	0	0	1	6	6

Code '0' is for centre point of the parameter range investigated '±1' for factorial points.

A- Fat content (%), B- Blood content (%), C-*Yangben* content (%)

Table 3.4 Experimental combinations at actual levels for response surface analysis

S.N.	Uncoded variables		
	A: Fat (%)	B: Blood (%)	C: *Yangben* (%)
1	17.5	20	12.5
2	25	20	12.5
3	17.5	10	12.5
4	25	10	20
5	17.5	20	20
6	10	30	5
7	25	30	20
8	25	30	5
9	25	10	5
10	10	20	12.5
11	17.5	20	12.5
12	17.5	20	12.5
13	10	10	20
14	17.5	20	12.5
15	17.5	20	12.5
16	17.5	20	5
17	10	10	5
18	17.5	20	12.5
19	17.5	30	12.5
20	17.5	20	12.5

3.3.2 Preparation of raw materials

Pork belly, liver, heart and blood were brought from the market. Belly and offals were trimmed, deskinned and adhering fat were removed. The separated fat was chilled until processing. Meat, heart and liver were cut to small pieces before mincing. *Yangben* was soaked in warm water for 5 minutes before cutting. Similarly, blood was also bought, mixed well and kept under refrigeration. Intestines were washed, processed and kept soaked in salt solution for casing. Spices were ground in electric grinder and weighed amount of salt and spices for different formulation were prepared. The recipe formulation for fat, *yangben* and blood was done according to the combination shown in Table 3.3. The salt concentration was taken as 1.5% of total ingredients and 7% spices was used for all samples. The flow chart for preparation of *sargayangma* is given in Fig. 3.1.

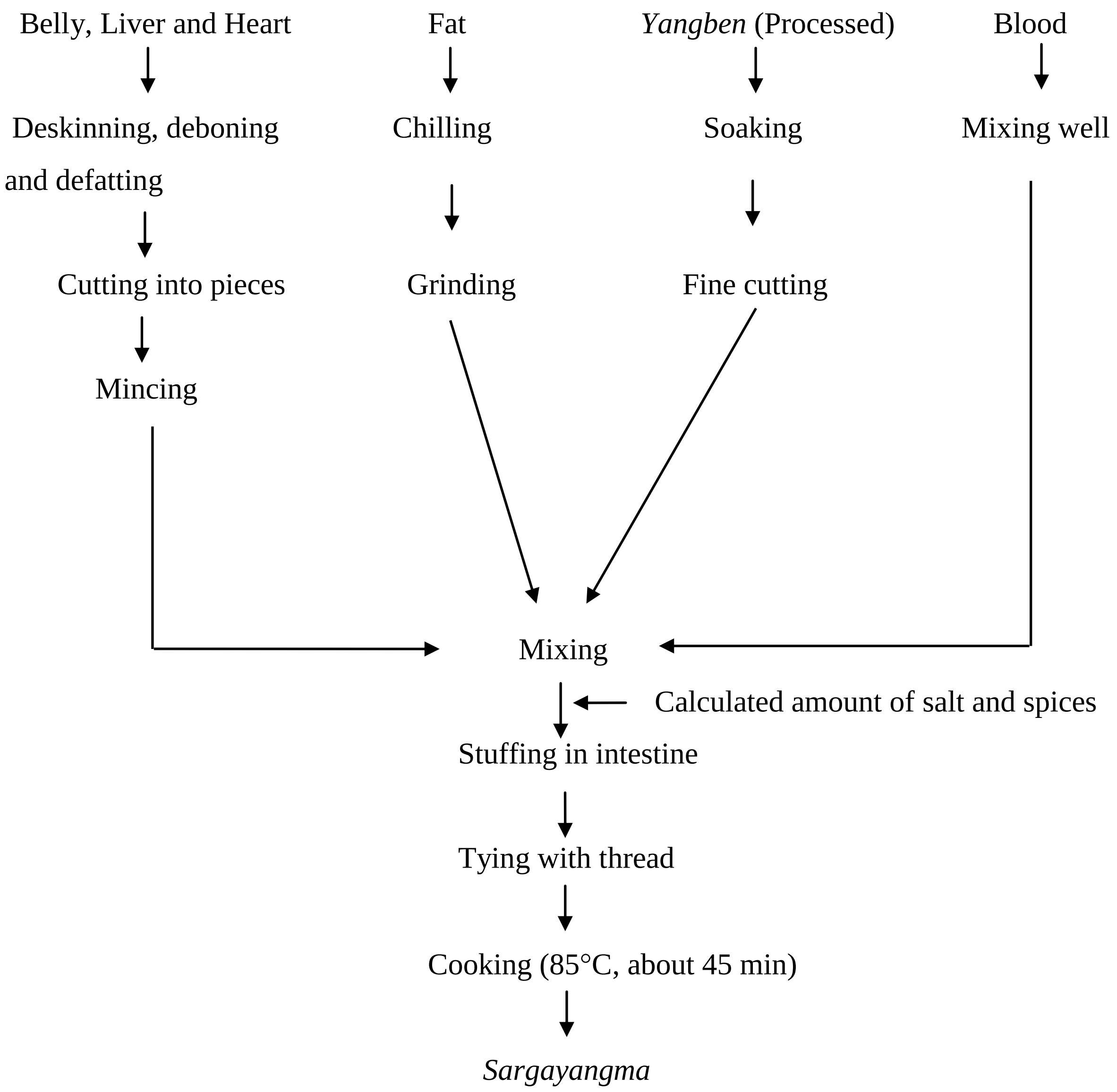

Fig. 3.1 Flow chart for preparation of *sargayangma*

Source: Limbu (2014)

3.3.3 Mincing and chopping

Belly meat was minced in a meat mincer to obtain a fine mass. Fat was also minced in the similar way. Liver and heart were chopped in a chop board with a knife to a finely chopped mass. *Yangben* was also cut in a similar way to obtain fine fibrous mass.

3.3.4 Mixing

All the ingredients were weighed out according to the formulations. The chopped meat ingredients were mixed with addition of chopped *yangben*. The salt and spices were also added. The mixing was done manually till a homogenous mass was obtained. Later, blood was also added and mixed well.

3.3.5 Stuffing

The well mixed sausage batter was stuffed in casing with the help of stuffer. The stuffed casing were linked and tied at a regular intervals of about 10cm in length. The sausages were then ready to cook.

3.3.6 Cooking

The *sargayangma* were cooked in a vessel in hot water at 85°C for about 45 minutes.

3.3.7 Cooling and storage

The cooked *sargayangma* were allowed to cool and then packed in packaging plastics and kept under refrigeration for further use.

3.5 Chemical analysis

3.5.1 Moisture content

The moisture content was determined by using hot air oven method as given by Subba (2010).

3.5.2 Crude fat

The crude fat was determined by extracting fat by Soxhlet extraction apparatus using petroleum ether according to the method cited in Subba (2010).

3.5.3 Crude protein

The crude protein was determined by estimating nitrogen content in the sample by Kjeldahl factor 6.25 according to Subba (2010).

3.5.4 Crude fiber

The crude fiber content of the product was determined by recovering the ash free residue after sequential treatment of ground 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 and crude fiber was determined according to Subba (2010).

3.5.5 Ash

Ash content of the meat was determined by gravimetric method according to Subba (2010).

3.5.6 Carbohydrate

Carbohydrate content was determined by difference.

Carbohydrate (%): 100 - (% moisture + crude protein + crude fat + crude fiber + ash)

3.5.7 Water holding capacity

Water holding capacity was determined according to Kowale *et al.* (2008). For determining the WHC, 500 mg weighed sausage sample was placed between the centers of two weighed Whatman no.1 filter papers. The filter papers were kept over a flat surface and covered by polyethene sheet above and below it and pressed by 2.81 kg for 5 min. The sample was weighed after pressing. The filter papers were dried and weighed. Then WHC (%) was calculated as:

$$\text{WHC (\%)} = \frac{\text{Actual weight of sausage sample}}{\text{Sample weight}} \times 100\%$$

Where, actual weight of sausage sample = weight of sample after pressing + subtraction of weight of filter paper before and after pressing.

3.5.8 Processing yield

For processing yield (%) of *sargayangma*, weight of sausage before and after cooking was noted. The processing yield was calculated as percentage weight of cooked sausage to weight of raw sausage (Kowale *et al.*, 2008).

$$\text{Processing yield}(\%) = \frac{\text{Weight of sausage before cooking}}{\text{Weight of sausage after cooking}} \times 100\%$$

3.5.9 Fat and jelly separation

For fat and jelly separation the procedure given by Kowale *et al.* (2008) was followed.

3.6 Sensory evaluation

3.6.1 Sample preparation

The *sargayangma* samples were warmed, cut into thin slices of about 1mm and were descriptively evaluated for their different sensory attributes through hedonic taste. The sensory evaluation of *sargayangma* was performed by 10 member panelists. The panelists were research students and teachers from CCT. The panelists were introduced about the product and its sensory characteristics to make them familier with the product and thus considered to be semi-trained panelists. Sensory evaluation was performed by 9 point hedonic rating test as described by Ranganna (2015). The parameters for sensory evaluation were taken to be color, aroma, blood taste, *yangben* taste, texture, juiciness and overall acceptability. Sensory evaluation was carried out in individual booth with adequate light and free from obnoxious odors. Each panelist was provided with samples coded with random numbers and evaluation card. They were provided with water for rinsing between the samples.

3.6.2 Statistical analysis

The responses water holding capacity and processing yield for different experimental combinations were related to the coded variables (xi, i=1and 2,) by a second degree polynomial equation as given below:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon$$

The coefficients of the polynomial were represented by β_0 (constant), β_1, β_2 (linear effects); β_{11}, β_{22}, (quadratic effects); β_{12} (interaction effects); and ε (random error).

A complete second order quadratic model was employed to correlate the independent process variables. The second order polynomial coefficient for each term of the equation was determined through multiple regression analysis using design expert. Data were fitted to the selected models and the statistical significance of the terms was examined by analysis of variance for each response. The adequacy of the model was tested considering R^2 (the coefficient of multiple determination of the amount of variation around the mean explained by the model), adjusted R^2 (a measure of the amount of variation around the mean explained by the model adjusted for the number of terms in the model), predicted R^2 (a measure of how good the model predicts a response value) and Fischer's F-test (Myers *et al.*, 2009). Coefficient of determination R^2 is defined as the ratio of the explained variation to the total variation and is a measure of degree of fit. When R^2 approaches to unity, the better the empirical model fits the actual data. The smaller the value of R^2, the less relevance the dependent variables in the model have in explaining the behavior variation. The models were then used to interpret the effect of various predictors on the response. The analysis of variance (ANOVA) tables were generated and the significances of all terms in the polynomial equation were judged statistically by computing the F- value at 5% level of significance by the help of Design Expert.

The analyses were carried out in triplicate. Statistical calculations were performed in Microsoft office Excel 2010. All the data obtained from the sensory analysis was interpreted using statisticsl software Genstat Release 12.1 (Discovery Edition 12 developed by VSN International Limited). From this mean ANOVA at 5% level of significance and LSD was obtained and thus the significant difference between the samples was evaluated.

PART IV

Results and discussion

Sargayangma is a type of blood sausage with its own peculiarity and cultural background of Limbu communities of Nepal. The basic procedure followed for *sargayangma* preparation is almost similar and ingredients used are also consistent. Yangben, blood, liver, heart and killing fats are the common raw materials used and other additives depend on the availability. For the study, pork meat, blood, liver, heart, yangben and spices were brought from the local market of Dharan. The meat and yangben were analysed for their proximate composition and *sargayangma* of various formulations were prepared in lab which were then subjected to further sensory and other physicochemical analysis.

4.1 Proximate composition of raw materials

4.1.1 Proximate composition of meat

Pork belly was taken as lean meat for the preparation of *sargayangma*. The chemical composition of pork belly (fat trimmed) is presented in Table 4.1.

Table 4.1 Proximate composition of pork belly

Parameters	Value (% db)
Moisture	317.71 ± 0.54
Protein	83.87 ± 2.31
Fat	11.21 ± 1.08
Ash	4.29 ± 0.03
Carbohydrate	0.63 ± 0.04

* The values in the table are the mean of the triplicates ± standard deviation. The values obtained above are in dry basis.

The values obtained above were slightly different than the values given by Heinz and Hautzinger (2007) which may be due to difference in species, sex, breed, plane of nutrition, physiological maturity, climatic conditions, etc. (Lawrie, 1966). According to Okrouhla *et al.* (2008), the values for moisture, protein, fat and ash in lean pork meat were found to be 263.63%, 84.29%, 5.67% and 5.09% on dry basis respectively. The fat content was found to be little high as the meat was not completely trimmed. These values also support the data provided by Matvaretabellen (2016) for fatty pork belly.

4.1.2 Proximate composition of *yangben*

The ash processed *yangben* was used for the preparation of *sargayangma* whose proximate composition is given in Table 4.2.

Table 4.2 Proximate composition of *yangben*

Parameters	Value (% db)
Moisture	12.70 ± 0.33
Protein	9.93 ± 0.58
Fat	2.47 ± 0.28
Carbohydrate	71.32 ± 0.68
Crude fibre	11.93 ± 0.15
Ash	4.36 ± 0.27

* The values in the table are the mean of the triplicates ± standard deviation. The values obtained are in dry basis.

According to Dhungana (1985), the respective values for species *Usnea* were 18.18% moisture, 6.37% protein, 2.63% fat, 14.28% crude fibre, 4.3% ash and 75.02% carbohydrate on dry basis. According to Peter *et. al.* (2005), moisture, carbohydrate and ash content of lichen range 65-67%, 2-10% and 3-10% respectively on wet basis. The slight difference in proximate composition was due to the species as the *yangben* contain mixture of species mainly *Parmelia, Ramalina* and *Usnea* (Yaqso, 2003). Thus, the proximate composition vary from species to species.

4.2 Effect of process variables on the properties of *sargayangma*

4.2.1 Effect of process variables on sensory attributes of *sargayangma*

All the prepared samples of *sargayangma* were subjected to sensory analysis for aroma, color, blood taste, yangben taste, texture, juiciness and overall acceptability. Among twenty experiments, only fifteen samples of *sargayangma* were subjected to sensory evaluation as six experiments were centre point with same formulations. The coding of different formulations for sensory analysis was done as shown in Table 4.3.

Table 4.3 Coding of different formulations for sensory analysis

Samples	Fat	Blood	Yangben
A	10	10	5
B	25	10	5
C	10	30	5
D	25	30	5
E	10	10	20
F	25	10	20
G	10	30	20
H	25	30	12.5
I	10	20	12.5
J	25	20	12.5
K	17.5	10	12.5
L	17.5	30	12.5
M	17.5	20	5
N	17.5	20	20
O	17.5	20	12.5

Sensory scores obtained from 10 semi trained panelists using 9-point hedonic rating scale (9 =like extremely, 1 = dislike extremely) for different formulations of *sargayangma* was statistically analyzed. From the statistical analysis ($p<0.05$), products were found to be significantly different in terms of all sensory parameters.

4.2.1.1 Effect of formulation on aroma

The mean sensory scores for aroma of fifteen samples with their standard deviation are given in Appendix C. The statistical analysis showed that there was significant effect ($p<0.05$) of fat, blood and *yangben* variation on aroma at 5% level of significance.

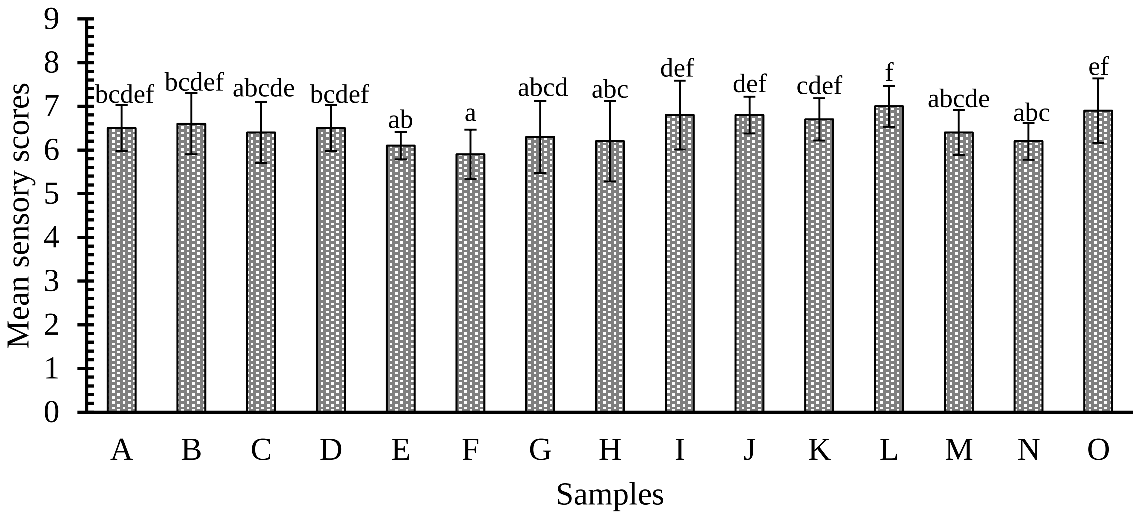

Fig. 4.1 Mean sensory scores for aroma of *sargayangma*

The obtained mean values of samples are represented in Fig 4.1. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

The mean sensory score for sample L (7.0 ± 0.47) was highest which was significantly different from samples C, E, F, G, H, M and N but not significantly different from rest of the samples. Sample F (5.9 ± 0.57) had the lowest score which was significantly different from samples A, B, D, I, J, K, L and O but not different from rest of the samples. LSD showed no significant differences between the samples A, B, C, D, I, J, K, M and O. Most of the samples showed no significant difference in terms of aroma and also the mean scores for the samples did not show much variation. However, samples with 12.5 % *yangben* were more preferred than with highest and lowest amount. According to Pearson (1966) and Rabe *et al.* (2003), aroma compounds are more lipophilic than hydrophilic. Thus, release of volatiles responsible for typical aroma in meat products is affected by fat content. But such effect was not seen in s which might be due to the dominating effect of *yangben* and blood aroma in the product. On the basis of superiority at 5% level of significance, following conclusions for aroma of *sargayangma* were drawn.

Sample L > Sample O > Sample J = Sample I > Sample K > Sample B > Sample A = Sample D > Sample M = Sample C > Sample G > Sample N = Sample H > Sample E > Sample F

4.2.1.2 Effect of formulation on color

The mean sensory scores for color of fifteen samples with their standard deviation are given in Appendix D. The statistical analysis showed that there was significant effect of variation in fat, blood and *yangben* concentration on the color at 5% level of significance.

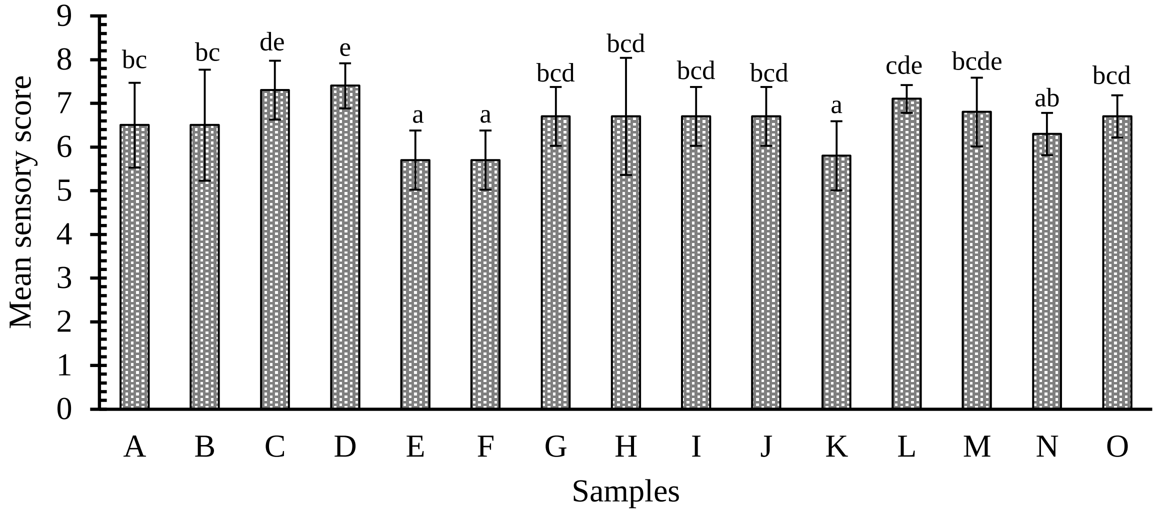

Fig. 4.2 Mean sensory scores for color of *sargayangma*

The obtained mean values of samples are represented in Fig 4.2. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

LSD showed that samples A and B were significantly different from the samples C, D, E, F and K. Similarily, samples C&E, C&F, C&K and C&N were significantly different to each other. The mean sensory score was highest for the sample D (7.4 ± 0.52) which was not signficantly different from samples C and M but significantly different from rest of the samples. Similarily samples E and F with mimimum scores (5.7 ± 0.67) were not significantly different from samples K and N but signifcantly different from rest of the samples.

The highest score for the sample D was found to be slightly intense (7.4) which showed that preference was highest for the sample with highest amount of blood and lowest amount of *yangben*. Samples with relatively high amount of *yangben* but low amount of blood were poorly rated which may be due to the masking effect of blood color by black color of *yangben*. Samples G, H, I and J had the same scores and were similar to each other in terms of color which might be due to same ratios of blood and *yangben* amount despite of their different concentration. Thus the color factor was positively related with blood amount but negatively related with *yangben*. The fat content was also found to have some influence on color of product as the samples with higher amount of fat but similar blood and *yangben* amount had higher scores than with lower amount of fat. This supports the functions of fat in food as described by Moncel (2016). Hence, the color perception of panelists were effected by proportion all of the three ingredients in *sargayangma*. On the basis of superiority at 5% level of significance, following conclusions for color of *sargayangma* were drawn.

Sample D > Sample C > Sample L > Sample M > Sample H = Sample G = Sample I = Sample O = Sample J > Sample A = Sample B > Sample N > Sample K > Sample F = Sample E

4.2.1.3 Effect of formulation on blood taste

The mean sensory scores for blood taste of fifteen samples with their standard deviation are given in Appendix E. The statistical analysis showed that there was significant difference on the blood taste of *sargayangma* due to fat, blood and *yangben* variation at 5% level of significance.

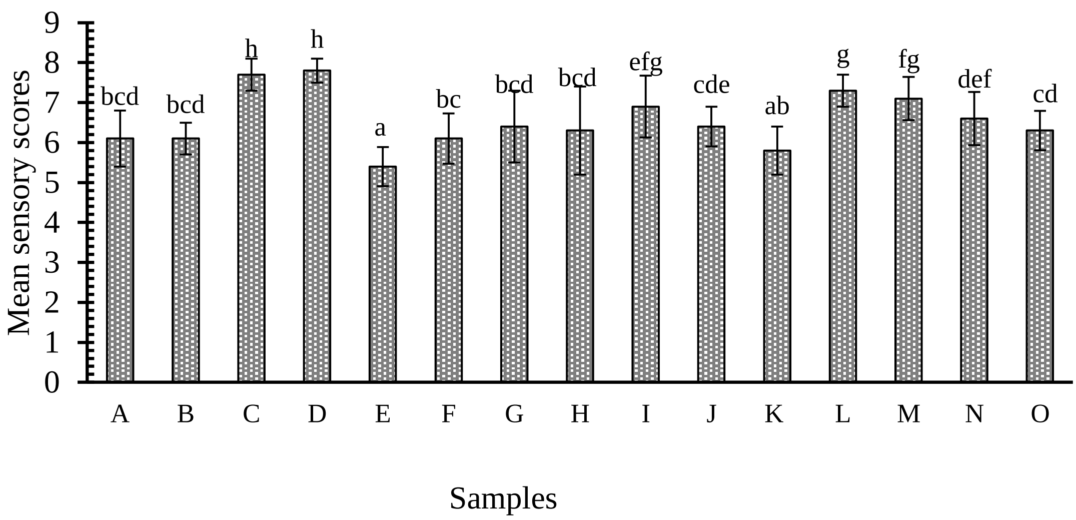

Fig. 4.3 Mean sensory scores for blood taste of *sargayangma*

The obtained mean values of samples are represented in Fig 4.3. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

LSD showed that the samples A, B, G and H were significantly different from samples C, D, E, I, L and M. Samples C and D with high scores were significantly different from all the other samples while the sample E with minimum score was not significantly different from sample K only but significantly different from rest of the samples.

The blood taste is readily detectable part of the *sargayangma* and it was found quite intense in sample D with highest score (7.9 ± 0.3). The intense blood taste was preferred by panelists in the samples which contained relatively low amount of *yangben*. The result was similar to that for color where blood taste was positively correlated with blood content but negatively correlated with *yangben* content. This might be due to the masking effet of *yangben* on the blood taste of samples. Though the metallic taste of blood is not preferred by consumer for most of the product it was highly expected and acceptable in sausage product like *sargayangma* as described by Nollet and Toldra (2011). Similarily, the fat content of sausage also affected the order of preference by panelists as samples with high amount of fat but similar amount of blood were high scored than those with low fat content. This may be due to the influence of fat in releasing the flavor components of blood and make it more profound (Tran and Thu, 2006). On the basis of superiority at 5% level of significance, following conclusions for blood taste of *sargayangma* were drawn.

Sample D > Sample C > Sample L > Sample M > Sample I > Sample N > Sample J > Sample O > Sample H = Sample G > Sample B > Sample A > Sample F > Sample K > Sample E

4.2.1.4 Effect of formulation on *yangben* taste

The mean sensory scores for *yangben* taste of fifteen samples with their standard deviation are given in Appendix F. The statistical analysis showed that there was significant difference in *yangben* taste between the samples at 5% level of significance.

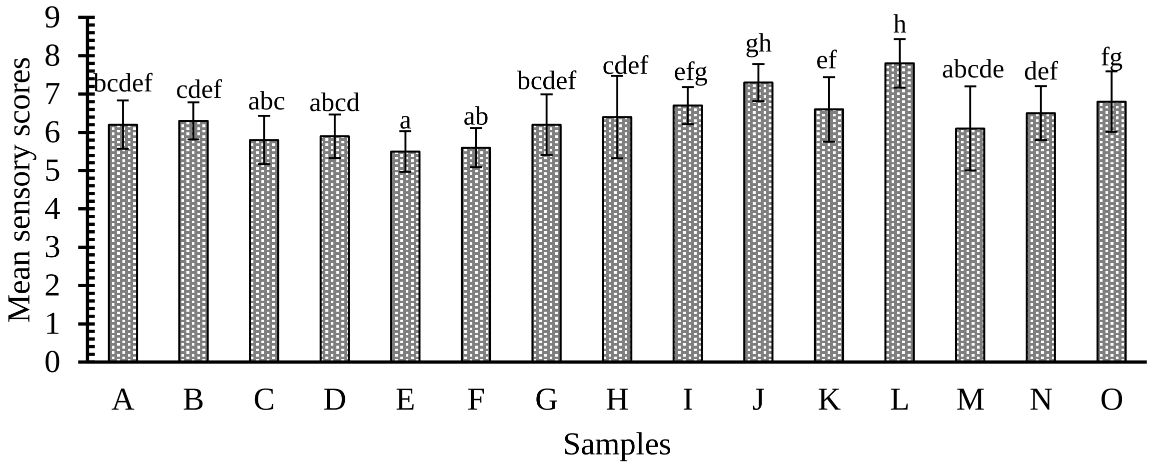

Fig. 4.4 Mean sensory scores for *yangben* taste of *sargayangma*

The obtained mean values of samples is represented in Fig 4.4. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

Sample L (7.8 ± 0.69) had the highest score which was not significantly different from sample J (7.3 ± 0.74) but significantly different from rest of the samples. Sample E (5.5 ± 0.53) had the lowest score which was significantly different from samples A, B, G, H, I, J, K, L, N and O but showed no significant difference from rest of the samples. The majority of the panelist liked the samples with 12.5% *yangben* concentration while the samples with 20% *yangben* concentration were poorly rated. Samples L and J with same amount of *yangben* but different fat and blood content showed no significant difference which might be due to the interaction of fat and blood to give same distinct *yangben* flavor. The highest *yangben* concentration was liked only for the samples with high blood and fat amount such that unaccetably high *yangben* taste could be masked. The preference for samples with lowest amount of *yangben* was high for samples with low amount of fat and blood which meant that unique and distinct *yangben* taste of *sargayangma* was liked by majority of panelists. Thus, the demand for the samples with moderate fat and blood amount giving distinct *yangben* taste was high. On the basis of superiority at 5% level of significance, following conclusions for *yangben* taste of *sargayangma* were drawn.

Sample L > Sample J > Sample O > Sample I > Sample K > Sample N > Sample H > Sample B > Sample A = Sample G > Sample M > Sample D > Sample C > Sample F > Sample E

4.2.1.5 Effect of formulation on texture

The mean sensory scores for texture of fifteen samples with their standard deviation are given in Appendix G. Statistical analysis showed that there was significant difference in texture between the samples due to variation in fat, blood and *yangben* at 5% level of significance.

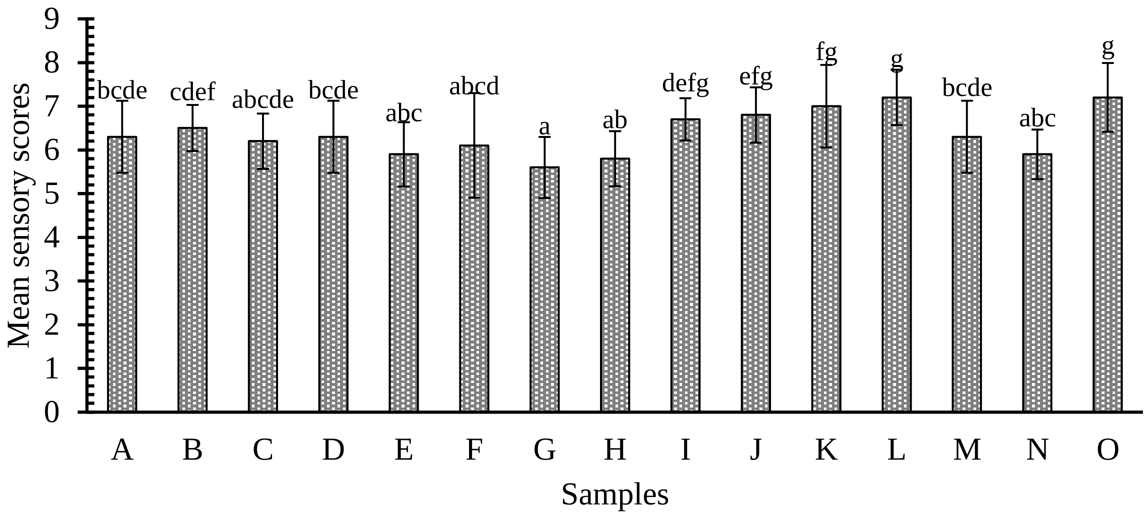

Fig. 4.5 Mean sensory scores for texture of *sargayangma*

The obtained mean values of samples are represented in Fig 4.5. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

Samples L (7.2 ± 0.63) and O (7.2 ± 0.79) had the highest scores and these both samples were not significantly different from samples I, J and K but significantly different from rest of the samples. Sample G (5.6 ± 0.7) had the lowest mean score which was not significantly different from samples C, E, F, H and N while significantly different from rest of the samples. The similarities between the samples L, O, I, J and K showed that the *yangben* and fat had profound effect on the texture of product while blood content had only a little influence. The lowest rating for the samples with highest concentration of *yangben* indicated the poor texture of sausages on increasing the *yangben* concentration. Similarily, the average rating was for the samples with lowest *yangben* concentration among which those with high fat content were more preferred than sausages with low fat content. According to Purslow (1985), the presence of higher amount of fat within or between the muscles improve the texture of meat because of easier disruption of muscle fibers during chewing. But the blood amount did not seem to affect the preference of sausages as most of the samples with same amount of fat and blood but varying blood amount showed no significant differences. On the basis of superiority at 5% level of significance, following conclusions for texture of *sargayangma* were drawn.

Sample O = Sample L > Sample K > Sample J > Sample I > Sample B > Sample M = Sample A = Sample D > Sample C > Sample F > Sample N = Sample E > Sample H > Sample G

4.2.1.6 Effect of formulation on juiciness

The mean sensory scores for juiciness of fifteen samples with their standard deviation are given in Appendix H. Statistical analysis showed that there was significant difference in juiciness between the samples at 5% level of significance.

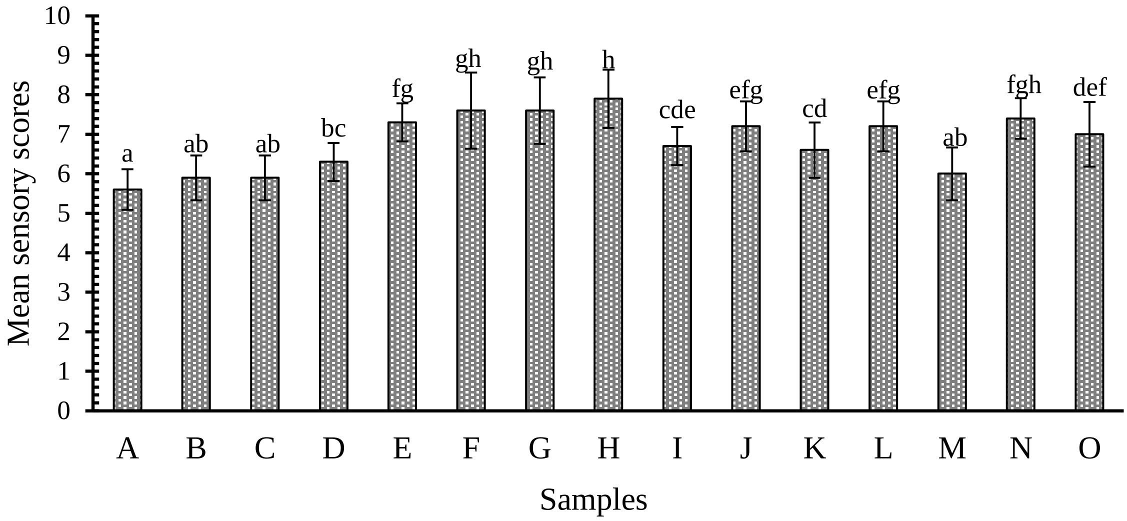

Fig. 4.6 Mean sensory scores for juiciness of *sargayangma*

The obtained mean values of samples are represented in Fig 4.6. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

Sample H (7.9 ± 0.73) had the highest score and was not significantly diffeent from samples F, G and N while significantly different from rest of the samples. These samples had all of the three ingredients in higher amount as compared to other samples. Among them too samples with high fat and blood amount appeared to be more juicy and had higher scores. Sample A (5.6 ± 0.52) had the minimum score and was not significantly different from the samples B, C and M while significantly different from rest of the samples. These all minimally rated samples contained lower amount of *yangben* while most of them had low fat and blood content. This showed that *yangben* had the most profound effect on juiciness followed by fat and blood.

According to Ofori and Hsieh (2012), blood has good fat and water binding capacity in grounded meat product which enhances the juiciness of final product. Ramos *et al.* (2013), also states that fat has direct influence on the peceived juiciness of the product. This may be the reason for liking the product with high fat and blood content. On the basis of superiority at 5% level of significance, following conclusions were drawn for juiciness of *sargayangma* were drawn.

Sample H> Sample F= Sample G > Sample N> Sample E > Sample J = Sample L > Sample O > Sample I > Sample K > Sample D > Sample M > Sample B = Sample C > Sample A

4.2.1.7 Effect of formulation on overall acceptability

The mean sensory scores for overall acceptability of fifteen samples with their standard deviation are given in Appendix I. Statistical analysis showed that there was significant difference in overall acceptability between the samples at 5% level of significance.

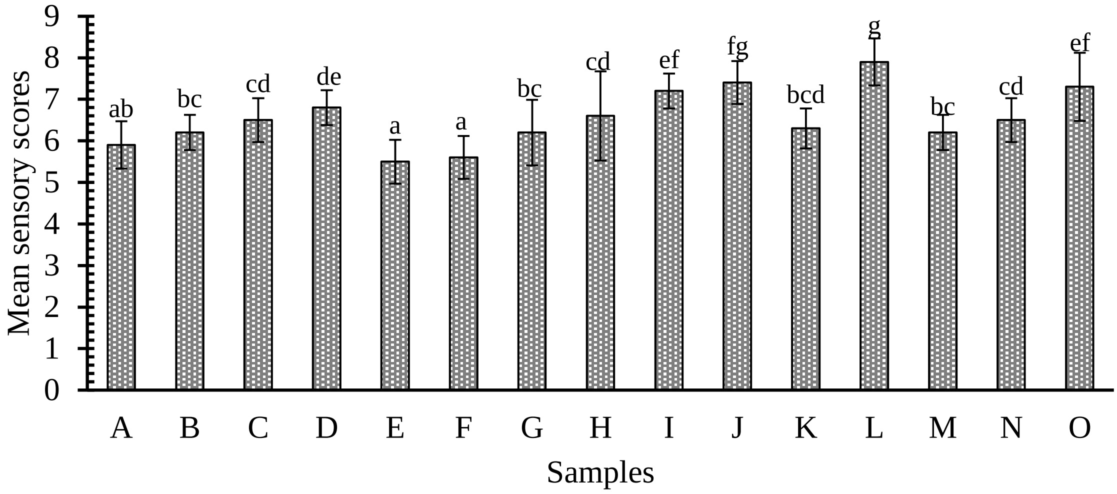

Fig. 4.7 Mean sensory scores for overall acceptability of *sargayangma*

The obtained mean values of samples are represented in Fig 4.7. Values on top of the bars bearing similar superscript are not significantly different at 5% level of significance. Vertical error bars represent ± standard deviation of scores given by 10 panelists.

LSD showed that there was significant differences in overall acceptability of the samples at 5% level of significance. The mean sensory scores for sample L was highest (7.9 ± 0.57) and was significantly different from all the samples except J. According to Ofori and Hsieh (2012), blood proteins are the cheaper alternative for fat replacers and provide protein and moisture to the product without significantly affecting other sensory parameters. This might be the reason for no significant difference between the samples L and J. Sample L was followed by samples I, J and O which were not significantly different from each other while significantly different from rest of the samples. This showed that *yangben* and blood mostly affected the overall acceptability of *sargayangma* as they contained similar amount of yangben and blood. The increasing mean scores for them with increasing fat content also pointed out some influence of fat content on the overall acceptability of *sargayangma*. On the basis of superiority at 5% level of significance, following conclusions for overall acceptability of *sargayangma* were drawn.

Sample L > Sample J > Sample O > Sample I > Sample D > Sample H > Sample N = Sample C > Sample K > Sample B = Sample M = Sample G > Sample A > Sample F > Sample E

By sensory data and obtained data interpretation sample L was found to be best in most of the parameters as well as overall palatibility. However, the responses given by panelists were not consistent and it was difficult to point out the distinct differences between the samples. This may be due to large number of samples with slight variations in formulations and the panelists were also semi trained and only slightly familier with *sargayangma*. Based on average scores, the formulation with 17.5 % fat, 30% blood and 12.5 % *yangben* was the best product. The conclusion thus derived in the present study is based on sensory analysis of limited number of panelists. The result may be different when subjected to other populations. So, the experimental finding needs to be taken with some reservations.

4.2.2 Effect of process variables on processing yield

The measured yield of the products varied from 85.58 to 96.49 %. Table 4.3 and 4.4 show the coefficients of the model and other statistical attributes of processing yield. The ANOVA result of processing yield showed that the model F-value of 0.94 was not significant relative to the noise at $p<0.05$. Table 4.3 showed that there were no any significant model terms which means variation in concentration of fat, blood and *yangben* had no significant effect on the processing yield. Thus, the ANOVA result showed no significant effect of formulation on the processing yield of *sargayangma*.

The result in cooking loss was the overall effect of fat, blood and *yangben* in the formulation. No significant effect of fat variation in ground pork in the cooking yield was also reported by Roseland *et al.* (200?) and similar result was found in *sargayangma*. On this contrary, Young *et al.* (1991) reported positive correlation between fat content and cooking loss. The reasons for both of these findings are not known. According to Heinz and Hautzinger (2007) and Ranathunga *et al.* (2015), yield of comminuted meat product can be increased by use of fillers and binders. 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 *sargayangma* by *yangben* might be the reason for no significant effect of *yangben* as well as overall effect in cooking loss of *sargayangma.* Fig. 4.8, Fig. 4.9 and Fig. 4.10. show the response surface plot for the effect of process variables on the processing yield of *sargayangma*.

Table 4.4 Analysis of variance for effect of process variables on processing yield

Factor	Coefficient of estimate	Sum of squares	Mean square	Df	F-value	p-value
Model	92.43	86.12	9.57	9	0.94	0.5318
A	-0.44	1.95	1.95	1	0.19	0.6705
B	1.18	13.95	13.95	1	1.37	0.2687
C	0.95	9.10	9.10	1	0.89	0.3665
AB	-0.56	2.49	2.49	1	0.24	0.6317
AC	-0.71	4.03	4.03	1	0.40	0.5430
BC	0.80	5.06	5.06	1	0.50	0.4968
A^2	-1.14	3.57	3.57	1	0.35	0.5668
B^2	2.02	11.18	11.18	1	1.10	0.3192
C^2	-3.28	29.57	29.57	1	2.91	0.1190

Table 4.5 Analysis of variance result

Response	Source	Sum of squares	df	Mean squares	F- value	p-value
Processing yield	Regression	86.12	9	9.57	0.94	0.5318
	Lack of fit	79.28	5	15.86	3.54	0.0960
	Pure error	22.42	5	4.48		
	Residual	101.70	10	10.17		
	Total	187.82	19			
	R^2 value	0.4585				
	Adjusted R^2	-0.0288				
	Adequate precision	3.443				

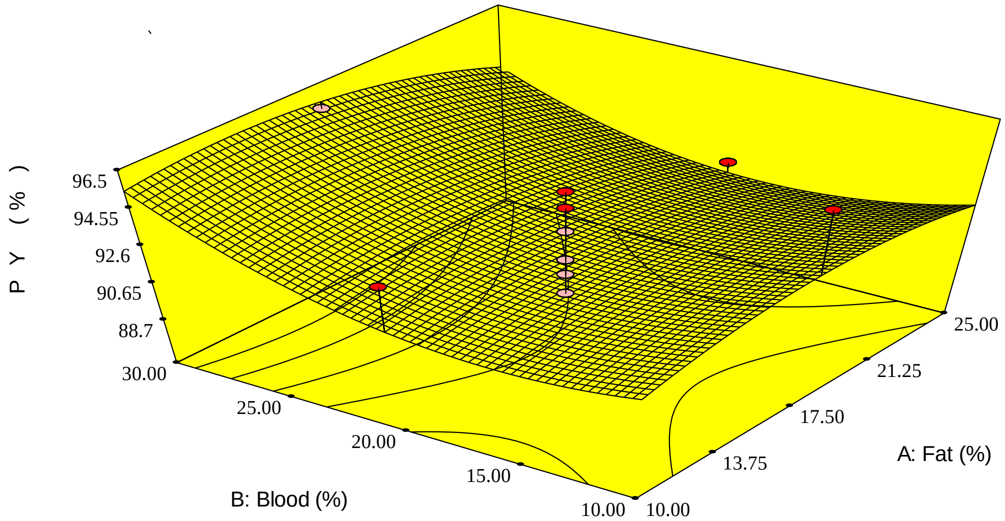

Fig. 4.8 Response surface plot for Processing Yield as a function of fat and blood content in *sargayangma*

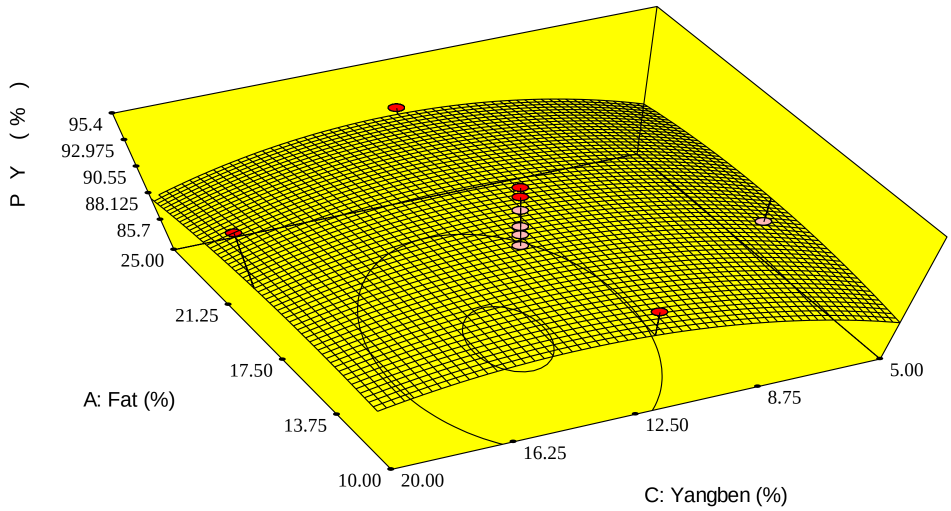

Fig. 4.9 Response surface plot for Processing Yield as a function of fat and *yangben* in *sargayangma*

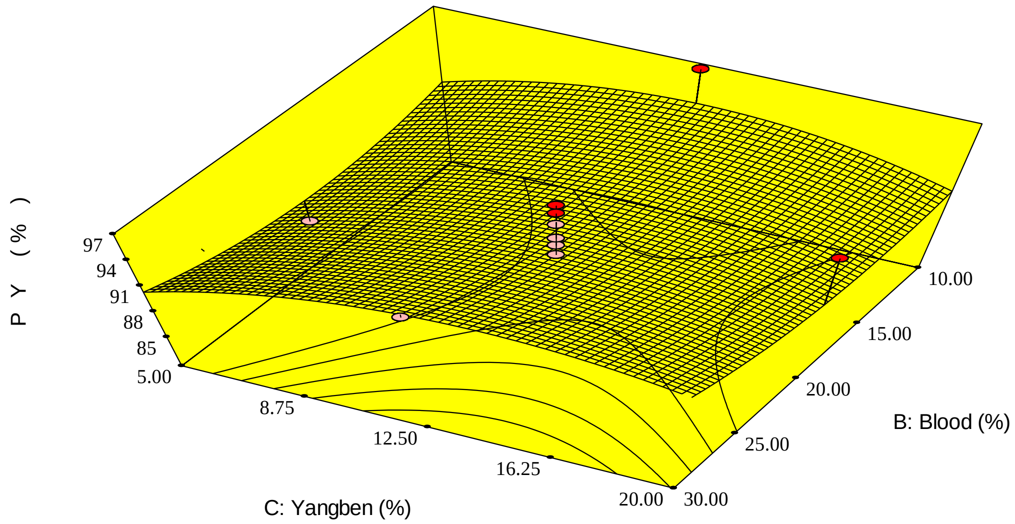

Fig. 4.10 Response surface plot for Processing Yield as a function of blood and *yangben* in *sargayangma*

4.2.3 Effect of process variables on product water holding capacity

The water holding capacity of the *sargayangma* varied from 65.51% to 90.32%. Table 4.5 and 4.6 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 12.92 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 0.9208, indicating that 92.08 % of the variability of the response could be explained by the model. The predicted R^2 value of 0.6548 was in reasonable agreement with the Adjusted R^2 value. The Adjusted R^2 value 0.8496 and Adequate Precision value 13.858 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 (equation 4.1) was selected to represent the variation of water holding capacity with the independent variables and further analysis. The quadratic model fitted for water holding capacity obtained from regression analysis in terms of coded values of the variables is represented by Eq. 4.1.

$$WHC = 72.05 + 1.30A - 2.52B + 5.14C + 2.80A^2 + 0.15B^2 - 0.077\ C^2 + 2.64\ AB + 0.074\ AC - 5.07\ BC \quad \ldots\ldots (4.1)$$

Where A, B and C are the coded values of fat (%), blood (%) and *yangben* concentration (%) respectively.

In this case B, C, AB and BC are significant model terms. Other interactions were not significant ($p<0.05$). The positive coefficient of A and C indicated that the increase in fat and *yangben* content of the *sargayangma* results increased water holding capacity of the product. But the effect of *yangben* variation was significant one whereas that of fat was not significant. The positive coefficient of fat showed positive correlation bewteen fat and water holding capacity which was in accordance with Lawrie (1991), Zayas (1997) and Wood (1993) though there was no signficant effect. According to Ngoc *et al.* (2012), the water holding capacity of fibrous stuffs is positively correlated with amount of soluble non cellulosic polysachharides. The same relation between water holding capacity and *yangben* was found in *sargayangma*. Such water binding property of *yangben* is due to the dominant polysaccharides lichenan, isolichenan and galactomanan found in lichen (Nash III, 2008). Heinz and Hautzinger (2007) and 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. The negative coefficient of B showed that increase in blood content in *sargayangma* significantly increased its water holding capacity. This result can be related with the research carried out by Chen and Lin (2002) where plasma protein exhibited high water holding only at very low concentration. But in the equation positive coefficient for blood was observed for quadratic term B^2. Thus, it could be concluded that plasma protein increased the water holding capacity of sausage at very low and very high concentrations only. But according to Ofori and Hsieh (2012) and Ranken (2000), plasma proteins are good fat and water binder used extensivvely as binder in meat products.

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. Here the interaction term BC showed significant effect on the water holding capacity which means that there was high interaction between the blood and *yangben* amount. The negative coefficient for the term BC indicated that there was decrease in water holding capacity with increase in amount of both blood and *yangben*. Since the effect of *yangben* was more significant, increase in water holding could be observed by increasing the *yangben* concentration and decreasing blood concentration which was also demonstrated by Fig. 4.12. As stated by Comer and Allan-Wojtas (1988), due to very high water binding properties of *yangben*, competition between blood protein and *yangben* could be expected to occur which led to reduced availability of water to bind for plasma proteins. Thus, extensively high concentration of plasma proteim was required to overcome the competitive effect ($+B^2$). It was also reported that the functional effects of ingredients is dependent upon the composition of total system and the ingredients do not have absolute binding values. Thus, this interaction between the blood and *yangben* may be the reason for negative effect of blood on water holding capacity of *sargayangma.*

Fig. 4.11, Fig. 4.12 and Fig. 4.13. show the respose surface plot for the effect of process variables on the water holding capaity of *sargayangma*.

Table 4.6 Analysis of variance for effect of process variables on water holding capacity

Factor	Coefficient of estimate	Sum of squares	Mean square	df	F-value	p-value
Model	72.05	646.53	71.84	9	12.92	0.0002***
A	1.30	16.87	16.87	1	3.04	0.1121
B	-2.52	63.50	63.50	1	11.42	0.0070**
C	5.14	264.09	264.09	1	47.50	<0.0001***
AB	2.64	55.92	55.92	1	10.06	0.0100**
AC	0.074	0.044	0.044	1	7.827E-0.03	0.9312
BC	-5.07	205.54	205.54	1	36.97	0.0001***
A^2	2.80	21.61	21.61	1	3.89	0.0769*
B^2	0.15	0.060	0.060	1	0.011	0.9191
C^2	-0.077	0.016	0.016	1	2.919E-003	0.9580

*Significant effect at p<0.1, ** Significant effect at p<0.05, ***Significant effect at p<0.001

df: degree of freedom

Table 4.7 Analysis of variance results of equation 4.1

Response	Source	Sum of squares	Df	Mean squares	F- value	p-value
Water holding capacity	Regression	646.53	9	71.84	12.92	0.0002
	Lack of fit	46.40	5	9.28	5.05	0.0501
	Pure Error	9.20	5	1.84		
	Residual	55.59	10	5.56		
	Total	702.12	19			
	R^2 value	0.9208				
	Adjusted R^2	0.8496				
	Adequate precision	13.858				

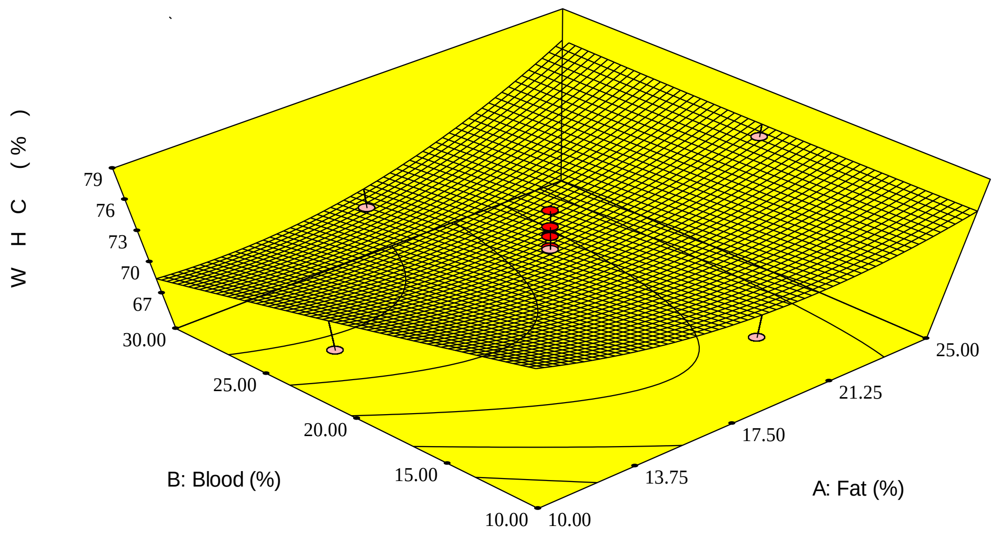

Fig. 4.11 Response surface plot for Water Holding Capacity as a function of fat and blood content in *sargayangma*

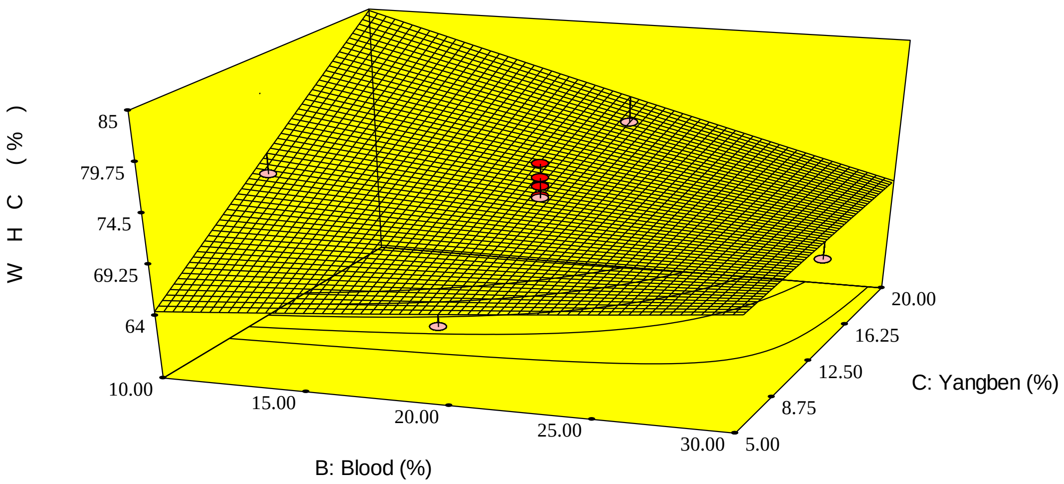

Fig. 4.12 Response surface plot for Water Holding Capacity as a function of blood and yangben content in *sargayangma*

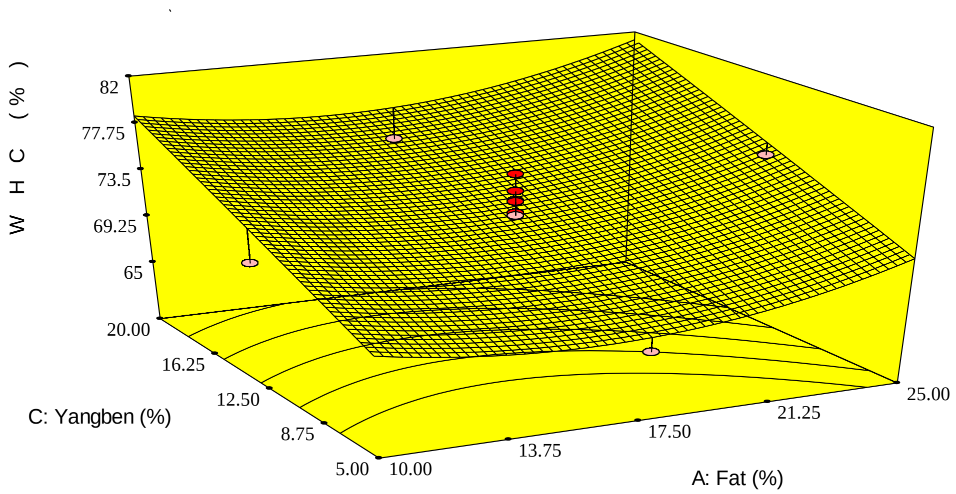

Fig. 4.13 Response surface plot for Water Holding Capacity as a function of *yangben* and fat content in *sargayangma*

4.2.4 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 specially from the samples containing high amount of yangben. Thus, no further analysis of the response was done.

4.3 Optimization study

A numerical multi-response optimization technique was applied to determine the optimum combination of fat, blood and *yangben* composition on *sargayangma*. The assumptions were to develop a product which would have a maximum water holding capacity and processing yield in range. All the other paramaters were kept in range and optimization study was carried out. Under these assumptions by design expert, the uncoded optimum amount of fat, blood and *yangben* for development of *sargayangma* were 10%, 10% and 20% respectively. The responses predicted by the software for these optimum proportions ingredients reported 88.92% water holding capacity and 89.59% processing yield with desirability of 94.4%. But from sensory analysis sample L with 17.5% fat, 30% blood and 12.5% *yangben* was found to be best. Chemical analysis and sensory evaluation are different techniques to optimize the product and they are hard to correlate. On comparison between numerical optimization and sensory analysis, sensory analysis was preferred because consumer preference should be taken into account for the documentation of new product. The values for constraints during optimization are given in Table 4.7.

Table 4.8 Multi response optimization constraints of *sargayangma*

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Fat	is in range	10	25	1	1	3
Blood	is in range	10	20	1	1	3
Yangben	is in range	5	20	1	1	3
WHC	maximize	65.51	90.32	1	1	3
PY	is in range	85.58	96.49	1	1	3

4.4 Proximate analysis of sensory optimized product

The chemical composition of optimized *sargayangma* is given in Table 4.8.

Table 4.9 Proximate composition of sensory optimized product

Parameters	Value (% db)
Moisture	149.5 ± 0.86
Protein	35.3 ± 0.47
Fat	52.27 ± 1.01
Crude fiber	2.22 ± 0.12
Ash	3.89 ± 0.40
Carbohydrate	6.32 ± 0.91

* The values in the table are the mean of the triplicates ± standard deviation. The values obtained above are in dry basis.

The moisture, protein, fat and ash was the contribution of almost all the ingredients used in the preparation whereas crude fiber and carbohydrate are due to yangben and spices used in formulation.

PART V

Conclusions and recommendations

5.1 Conclusions

Based on the physico-chemical, sensory and statistical analysis of the lab prepared *sargayangma* of different formulations, following conclusions were drawn.

- The water holding capacity was found to be increased significantly with increase in *yangben* amount and decrease in blood amount. High interaction between blood and *yangben* was observed.
- The variation in fat content did not affect the water holding capacity of *sargayangma* significantly.
- The processing yield of *sargayangma* was not significantly affected by variation in fat, blood and yangben amount in the formulation.
- The variation in formulation had significant effect on all the sensory attributes of *sargayangma*.
- The sample F with 17.5% fat, 30% blood and 12.5% *yangben* was found to be best among all samples by sensory analysis.
- The processing yield and water holding capacity of sensory optimized sample was found to be 95.28% and 67.74% respectively.

5.2 Recommendations

Based on the present study following recommendations for further study can be made:

- Shelf-life study of the product can be carried out.
- Optimization based on other functional properties can be carried out.
- Comparative study of the market samples from different parts of country can be carried out.

- Comparative study of binding capacity of *yangben* and different commercial binders can be performed.
- Since optmized product from sensory analysis and response analysis were different, an approach to maintain proper balance between the consumer preference and functional properties of product is highly recommended prior to the commercialization of *sargayangma.* .

PART VI

Summary

Sargayangma is a valuable indigenous product of Limbu communities still under the shade of western sausage. The present study was conducted to obtain the optimum formulation for fat, blood and *yangben* based on sensory and chemical analysis. The recipe for the *sargayangma* was extracted from the previous study and variation in the formulation to be done were fixed based on that recipe given in Table 3.2. Experimental design with fat content 10-25%, blood content 10-30% and *yangben* content 5-20% as independent variables at three levels produced twenty combinations with six centre points that were studied using central composite face centered design of response surface mothodology (RSM). The effect of these variables on the responses processing yield and water holding capacity was investigated. The data were anayzed using Design Expert.

For the lab preparation of *sargayangma* meat, blood, offals, *yangben*, spices and casings were brought from local market of Dharan. All of the ingredients were weighed out as per the formulations. Meat, offals, fat, *yangben* and spices were chopped and all the ingredients were mixed together with blood and salt. The prepared sausage mass was stuffed in intestinal casing. The weight of sausage before cooking was noted and then cooked at 85^{o}C for 45 min. The cooked sausage was again weighed for final weight and left overnight for sensory and chemical analysis. The sensory analysis was conducted for aroma, color, blood taste, *yangben* taste, texture, juiciness and overall acceptability of *sargayangma*. From sensory analysis, sample with 17.5% fat, 30% blood and 12.5% *yangben* was found to be best one. But the numerical optimization by RSM gave an optimum formulation with 10% fat, 10% blood and 20% yangben which was different from sensory optimized product. Giving sensory optimized product a major consideration proximate analysis of optimized sample was done. The moisture content, crude fat, crude protein, crude fibre, total ash and total carbohydrate of *sargayangma* was found to be 149.5%, 52.27%, 35.3%, 2.22%, 3.89% and 6.32% on dry basis respectively.

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Appendices

Appendix A

Table A.1 Experiment design for samples and their responses

S.N.	Fat	Blood	Yangben	WHC	PY
1	17.5	20	12.5	75.7	92.13
2	17.5	20	12.5	73.36	89.8
3	10	10	5	68.89	86.49
4	25	30	20	77.31	91.89
5	17.5	20	5	65.51	85.8
6	25	10	20	86.53	85.58
7	25	10	5	66.33	91.18
8	17.5	20	20	74.19	95.38
9	17.5	10	12.5	72.41	96.49
10	10	30	5	69.37	91.85
11	17.5	30	12.5	67.74	95.28
12	17.5	20	12.5	74.19	88.72
13	17.5	20	12.5	72.25	94.18
14	25	20	12.5	75	91.37
15	10	10	20	90.32	88.89
16	17.5	20	12.5	73.26	90.6
17	17.5	20	12.5	722	93.35
18	10	20	12.5	70.46	94.09
19	25	30	5	75.86	89.15
20	10	30	20	69	92.27

Appendix B

Specimen card for sensory evaluation

Hedonic rating test

Name of panelist…………… Date ……………..

Product: Sargayangma

Please taste the sample and check out how much you like or dislike. Use the appropriate scale to show your attitude by giving the point that best describes your feeling about the sample.

Parameters	A	B	C	D	E	F	G
Aroma							
Color							
Blood taste							
Yangben taste							
Texture							
Juiciness							
Overall acceptability							

Give points as follows:

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

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

Like moderately **7** Dislike slightly **4** Dislike extremely **1**

Comments………………………………………………………………………………

Signature………………………..

Appendix C

Table C.1 Mean sensory scores for aroma of *sargayangma*

Samples	Mean scores ± S.D.
A	6.5 ± 0.53
B	6.6 ± 0.69
C	6.4 ± 0.69
D	6.5 ± 0.53
E	6.1 ± 0.32
F	5.9 ± 0.57
G	6.3 ± 0.82
H	6.2 ± 0.92
I	6.8 ± 0.79
J	6.8 ± 0.42
K	6.7 ± 0.48
L	7.0 ± 0.47
M	6.4 ± 0.52
N	6.2 ± 0.42
O	6.9 ± 0.74

Table C.2 Two way ANOVA for aroma

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	13.9733	0.9981	2.92	<.001
Panelist	9	8.4067	0.9341	2.73	0.006
Residual	126	43.0933	0.3420		
Total	149	65.4733			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.5176.

Appendix D

Table D.1 Mean sensory scores for color of *sargayangma*

Samples	Mean scores ± S.D.
A	6.5 ± 0.97
B	6.5 ± 1.27
C	7.3 ± 0.67
D	7.4 ± 0.52
E	5.7 ± 0.67
F	5.7 ± 0.67
G	6.7 ± 0.67
H	6.7 ± 1.34
I	6.7 ± 0.67
J	6.7 ± 0.67
K	5.8 ± 0.79
L	7.1 ± 0.32
M	6.9 ± 0.79
N	6.3 ± 0.48
O	6.7 ± 0.48

Table D.2 Two way ANOVA for color

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	38.8400	2.7743	4.93	<.001
Panelist	9	8.8067	0.9785	1.74	0.087
Residual	126	70.8933	0.5626		
Total	149	118.5400			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.6639.

Appendix E

Table E.1 Mean sensory scores for blood taste of *sargayangma*

Samples	Sensory scores ± S.D.
A	6.1 ± 0.7
B	6.2 ± 0.4
C	7.8 ± 0.4
D	7.9 ± 0.3
E	5.4 ± 0.48
F	6.0 ± 0.63
G	6.3 ± 0.9
H	6.3 ± 1.1
I	7.0 ± 0.77
J	6.5 ± 0.5
K	5.8 ± 0.6
L	7.2 ± 0.4
M	7.1 ± 0.53
N	6.6 ± 0.66
O	6.4 ± 0.49

Table E.2 Two way ANOVA for blood taste

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	69.6933	4.9781	12.40	<.001
Panelist	9	8.4267	0.9363	2.33	0.018
Residual	126	50.5733	0.4014		
Total	149	128.6933			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.5607.

Appendix F

Table F.1 Mean sensory scores for *yangben* taste of *sargayangma*

Samples	Mean scores ± S.D.
A	6.2 ± 0.63
B	6.3 ± 0.48
C	5.8 ± 0.63
D	5.9 ± 0.57
E	5.5 ± 0.53
F	5.6 ± 0.52
G	6.2 ± 0.79
H	6.4 ± 1.07
I	6.7 ± 0.48
J	7.3 ± 0.48
K	6.6 ± 0.84
L	7.8 ± 0.63
M	6.1 ± 1.1
N	6.5 ± 0.7
O	6.8 ± 0.79

Table F.2 Two way ANOVA for *yangben* taste

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	53.0400	3.7886	7.50	<.001
Panelist	9	4.6733	0.5193	1.03	0.421
Residual	126	63.6267	0.5050		
Total	149	121.3400			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.6289.

Appendix G

Table F.1 Mean sensory scores for texture of *sargayangma*

Samples	Mean scores ± S.D.
A	6.3 ± 0.82
B	6.5 ± 0.53
C	6.2 ± 0.63
D	6.3 ± 0.82
E	5.9 ± 0.74
F	6.1 ± 1.19
G	5.6 ± 0.7
H	5.8 ± 0.63
I	6.7 ± 0.48
J	6.8 ± 0.63
K	7.0 ± 0.94
L	7.2 ± 0.63
M	6.3 ± 0.82
N	5.9 ± 0.56
O	7.2 ± 0.79

Table C.5 Two way ANOVA for texture

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	35.5733	2.5410	4.58	<.001
Panelist	9	6.1067	0.6785	1.22	0.287
Residual	126	69.8933	0.5547		
Total	149	111.5733			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.6592.

Appendix H

Table H.1 Mean sensory scores for juiciness of *sargayangma*

Samples	Mean scores ± S.D.
A	5.6 ± 0.52
B	5.9 ± 0.57
C	5.9 ± 0.57
D	6.3 ± 0.48
E	7.3 ± 0.48
F	7.6 ± 0.97
G	7.6 ± 0.84
H	7.9 ± 0.73
I	6.7 ± 0.48
J	7.2 ± 0.63
K	6.6 ± 0.69
L	7.2 ± 0.63
M	6.0 ± 0.67
N	7.4 ± 0.52
O	7.0 ± 0.82

Table H.2 Two way ANOVA for juiciness

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	74.5733	5.3267	14.07	<.001
Panelist	9	10.5067	1.1674	3.08	0.002
Residual	126	47.6933	0.3785		
Total	149	132.7733			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.5445.

Appendix I

Table I.1 Mean sensory scores for overall acceptability of *sargayangma*

Samples	Mean scores ± S.D.
A	5.9 ± 0.57
B	6.2 ± 0.42
C	6.5 ± 0.53
D	6.8 ± 0.42
E	5.5 ± 0.53
F	5.6 ± 0.52
G	6.2 ± 0.79
H	6.6 ± 1.07
I	7.2 ± 0.42
J	7.4 ± 0.52
K	6.3 ± 0.48
L	7.9 ± 0.57
M	6.2 ± 0.42
N	6.5 ± 0.53
O	7.3 ± 0.82

Table C.7 Two way ANOVA for overall acceptability

Source of variation	d.f.	s.s.	m.s.	v.r.	Fpr.
Sample	14	64.5600	4.6114	14.11	<.001
Panelist	9	7.5267	0.8363	2.56	0.010
Residual	126	41.1733	0.3268		
Total	149	113.2600			

Since, Fpr < 0.05, significant difference was observed between the samples at 5% level of significance. The Least Significant Difference (LSD) value was calculated to be 0.5059.