PREPARATION AND QUALITY EVALUATION OF PROTEIN-RICH CONCENTRATE USING BUFFALO (*BUBALUS BUBALIS*) LIVER AND GERMINATED SOYBEAN *(GLYCINE MAX*)

by

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Preparation and Quality evaluation of Protein-Rich Concentrate using Buffalo *(Bubalus bubalis*) Liver and Germinated Soybean (*Glycine max)*

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Approval Letter

The ***dissertation*** entitled ***Preparation and Quality Evaluation of Protein-Rich Concentrate using Buffalo (Bubalus Bubalis) Liver and Germinated Soybean (Glycine max)*** presented by **Mamata G.C** has been accepted as the partial fulfillment of the requirements for **B. Tech. degree in Food Technology**

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Abstract

Protein concentrate was prepared from buffalo liver and germinated soyabean. Soybean seeds were sprouted, dehulled, ground into a slurry, filtered to obtain soy milk, coagulated, then curd was dried to a moisture content of around 6% to make soy protein concentrate (SPC). Similarly, buffalo liver dried (40-50ºC) for 120-180 hours to get buffalo liver powder (BLP). Both powders were blended for improved functionality property varying from 10:90 to 50:50 (SPC: BLP) to make five formulations using Design Expert software which were subjected to the sensory analysis (as soup). The formulation containing 20:80 (SPC: BLP) was selected as the best which was aseptically packed in sterilized Polyethylene plastic pouch with zipper and then stored for 49 days at 2 different storage temperature conditions; room temperature (25±3°C), refrigeration temperature (5±1°C) for the study of storage stability of the product in the interval of 7 days. During the storage period changes in the microbial count, moisture, peroxide value (PV) and pH of the product was evaluated.

Evaluation of the final product revealed that it contained 5.5% of moisture, 72.5% of Protein, 13.5% of fat, 2.5 % of fiber, 5.3% of ash and 6.2% of carbohydrate and contain all essential amino acids. The moisture, PV, pH, Total Plate Count (TPC) of freshly prepared concentrate were found to be 5.5%, 4 meq/kg sample, 6.1 and 5×10^2 CFU/ml respectively but coliform was found to be absent. During total storage of 49 days, the weekly chemical analysis showed there was an increase in moisture by 1.3% which was 6.5 times faster as compared to sample stored at refrigeration. Peroxide value and total plate count were increased by 17 meq/Kg sample and 2.9×10^9 CFU\ml respectively at room temperature which were increased by 11.33 times and a thousand times faster rate as compared to refrigeration temperature. In contrast pH value decreased by 1.3% at room temperature which was 2.6 times faster as compared to refrigeration temperature. PV and Total plate count were beyond the acceptable limits stored at room temperature after 21 days and 28 days respectively. Hence, Protein concentrate stored at the refrigeration temperature (5±1°C) was significantly better in retaining the desired quality attributes than the sample stored at room temperature (25±3°C) throughout 49 days of storage.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
LSD	Least significance difference
PCA	Plate count agar
TPC	Total plate count
PE	Polyethylene
PV	Peroxide value
CFU	Colony Forming Unit
Meq/Kg	Milli-equivalent per Kilogram
AA	Amino Acids
DM	Dry Matter

PART I

Introduction

1.1 Background

Protein concentrate is an animal or human dietary supplement that has a very high protein content and is extracted or prepared from vegetable or animal matter. Protein comes from two sources: animal foods and plant foods(Gorska- Warsewicz *et al.*, 2018). Animal sources of protein are considered "high-quality proteins" because they contain all the essential amino acids and are easier for the body to digest and utilize. Most plant sources do not contain all of the essential amino acids, so it is important to eat a variety of plant proteins every day (Petsko and Ringe, 2004). Protein and amino acid supplements are widely marketed for athletes and habitually active consumers as muscle growth and performance-enhancing products, and high-protein, low-carbohydrate diets are traditionally applied for weight-loss purposes. However, the knowledge about the nutritional significance and effects of dietary protein and sports supplement products varies greatly among sportspeople and lifestyle users, especially in relation to individual sports activity levels and overall diet and metabolic state. Protein is an essential nutritional component in the human diet throughout life, as it secures growth in infancy, supports muscle and bone metabolism, ensures the maintenance and development of a normal nervous system, and helps to sustain muscle mass and physical performance in older ages, for instance, (Kårlund *et al.*, 2019). Food for extreme conditions is mainly based on concentrates, for example, soups, porridge, buck-wheat pudding and other cereal puddings. These are dry product mixtures that differ from traditional foods due to low moisture content, high concentrations of nutrients, as well as long shelf life. Food concentrates for special purposes, such as consumption in extreme conditions, must provide a good taste, high calorific content and a high satiety index. Nowadays, the main protein ingredient in food concentrate recipes is dried meat or minced meat, usually beef or chicken (Kalenik *et al.*, 2017).

Different types of protein concentrate and isolate are: whey protein concentrate/isolate, casein concentrate/isolate, soy protein concentrate/isolate, pea protein concentrate/isolate, egg protein concentrate/isolate, combined beef liver and soy protein concentrate/isolate, Hemp protein concentrate isolate, etc. Concentration and isolation of proteins from different sources is primarily aimed at providing a satisfactory solution for protein

malnutrition/undernutrition and effective utilization of the underutilized protein sources. Several sources ranging from algae to soybeans have been studied for this purpose. Utilization of protein concentrates/isolates from human food purposes has used several foods including spaghetti, macaroni, pasta, bread, and cookies. Other modes of utilization include meat extenders, high protein beverages, and ration diets for mass feeding programs. Cereals appear to be the most commonly used vehicle for this nutrient propagation (Sathe and Salunkhe, 2007).

This research is mainly focused on proposing a production method and chemical analysis of protein concentrate prepared from the combination of different proportions of soyabean protein concentrate and protein-rich buffalo meat byproduct (liver) protein concentrate. This protein concentrate is obtained from both plant sources and animal sources which can be one complete source of protein. Protein concentrate from both sources will be prepared separately. Finally, different proportions of them will be mixed and evaluated to obtain the best combination of protein concentrate.

1.2 Statement of the problem

It is well known and well-accepted today that the function of dietary protein is to supply the various amino acids needed for tissue growth and maintenance (Joint and Organization, 2007). An insufficient amount of protein in the diet is held to be at the heart of the problem of persistent and widespread malnutrition in developing countries. However, when one examines the available data, the conclusion is clear that what diets lack is not protein but energy foods to enable the body to utilize the protein people actually do eat (Fieldhouse, 2013). The shortage of foods containing protein of good quality in the developing countries, particularly those suitable for the vulnerable groups, especially young children, pregnant women are mostly affected world widely. Protein malnutrition in infancy and early childhood resulted in poor physical and, possibly, mental development in later life. Inequitable distribution of food containing high-quality protein such as meat and fish, which were comparatively expensive compared with cereals and starchy roots, was found in all developing countries and also inside families. Unlike carbohydrates and fat, there is no mechanism to store excess amino acids that are consumed in the diet, a continuous supply of amino acids is needed especially all nine essential amino acids(Khan *et al.*, 2017). Protein intake is 0.9 g/kg/day from 3 to 18 years of age for boys and from 3 to 15 years of age for

girls. Between 15 and 18 years of age, the level decreases slightly for girls to 0.8 g/kg/day (Wu, 2016).

Animal-source foods (*e.g.*, meat, dairy products, egg, poultry, seafood, and other products) contain higher quantities and more balanced proportions of amino acids relative to human tissues, than plant-sourced foods. In this research two ingredients which are germinated soybean (plant based) and buffalo liver which is animal based more precisely its by-product are used in order to complement each other with complete essential amino acids. Soybeans are deficient in one of the most essential amino acids i.e methionine (0.5-0.6%), also it consists of heat resistant protein due to which it takes longer time to cook and digest (65-75%) than buffalo liver (96-98% of digestibility) which contains about 3-4% of methionine but also contains cholesterol and toxic substances which are absent in soybeans. Fiber present in soybean can help to balance digestion with liver. Liver contains high amount of saturated fats. Buffalo liver is an important edible meat byproduct. However, in developing countries, it has a low commercial value and is underutilized. The efficient utilization of this edible meat byproduct is essential to support an economical and viable buffalo meat production system. Liver and liver products are a rich and economical source of essential nutrients that are more readily available to man in the form of animal products than non-meat products (S. Devatkal *et al.*, 2004b). Similarly Germination of soybeans has been known to be beneficial for the reduction of anti-nutrients like trypsin inhibitor, phytic acid, flatulent, etc (Murugkar, 2014). Fulfilling each other's deficiencies buffalo liver and germinated soybeans can make protein-rich concentrate with complete essential amino acids.

1.3 Objectives of the study

1.3.1 General objectives

To prepare protein-rich concentrate using buffalo (*Bubalus bubalis)* liver and germinated soybean (*Glycine max*).

1.3.2 Specific Objectives

- To formulate and evaluate protein concentrate using the varying proportion of buffalo liver and soyabean protein powder using Experiment Design-Expert software.
- To determine the sensory and nutritional properties of prepared protein concentrate.
- To study the amino acid profile of the final product.
- To study the storage stability of best-formulated protein concentrate for 49 days.

1.4 Significance of the study

This dissertation will use the knowledge from other kinds of literature and research done in the field of protein concentrate making. In this study Protein concentrate will be prepared from soybean and buffalo liver which will contain a very high amount of protein (>70%). Soybean contains about 43% of protein with all essential amino acids but deficient in sulfur containing amino acids methionine(0.5-0.6%) and cysteine (Gupta, 2013). Whereas buffalo liver contains 18.44 ± 0.56% of protein with all essential amino acids especially methionine of about 3-4% which will complement to soybean (S. Devatkal *et al.*, 2004b). Germinated soybean (3 to 6 days of germination) contains 8.9% to 22.4% more essential amino acids. They are good sources of ascorbic acid, riboflavin, choline, thiamine, tocopherols and pantothenic acid and also have increased availability of minerals such as copper, sodium, potassium, iron, phosphorus, calcium, manganese and magnesium (Kayembe, 2011). Germination of soybeans has been known to be beneficial for the reduction of anti-nutrients like trypsin inhibitor, phytic acid, flatulent, etc (Murugkar, 2014). This combination of buffalo liver and soybean can give complete essential amino acids needed for the human body which can be used in soup making, incorporation of different foods and vegetables to make them more nutritious. This research aspired to introduce protein concentrate powder

based on both animal (especially bi-product i.e liver) and plant source with complete essential amino acids which is primarily aimed at providing a satisfactory solution for protein malnutrition/undernutrition and effective utilization of the underutilized protein sources. Similarly, there is a need to develop new foods for participants of expeditions in extreme conditions, which must be self-sufficient and this protein concentrate can solve this problem by being light to carry, with a long shelf life, tasty and with high nutrient density. Currently, protein sources are limited mainly to dried and canned meat so in this situation it can play a very important role as a protein supplement too. Other modes of utilization include meat extenders, high protein beverages, and ration diets for mass feeding programs (Kalenik *et al.*, 2017). Hence it also has the potential to get introduced in different food industries fulfilling protein requirements.

1.5 Limitations of the study

- The rheological properties of protein concentrate were not studied.
- Quantitative analysis of amino acids could not be performed due to insufficient lab equipment.
- Toxicity and cholesterol of liver and anti-oxidant properties of germinated soybean were not determined.

Part II

Literature review

2.1 Soybean

The soybean, soy bean or soya bean (*Glycine max*) (Modgil *et al.*, 2021) is a species of legume native to East Asia widely grown for its edible bean, which has numerous uses. Traditional unfermented food uses of soybeans include soy milk, from which tofu and tofu milk are made. Fermented soy foods include soybean, fermented bean paste, natto, and tempeh. Fat-free (defatted) soybean meal is a significant and cheap source of protein for animal feeds and many packaged meals. For example, soybean products, such as textured vegetable protein (TVP), are ingredients in man meat and dairy substitutes (Riaz, 2005).

Soybeans contain significant amounts of phytic acids, dietary minerals and B vitamins. Soy vegetable oil, used in food and industrial applications, is another product of processing the soybean crop. It is the most important protein source for feeding farm animals (that in turn yields animal protein for human consumption). Together, protein and soybean oil content account for 56% of dry soybeans by weight (36% protein and 20% fat). The remainder consists of 30% carbohydrates, 9% water and 5% ash. Soybeans comprise approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ (Heuze V., 2020). Similarly, Etiosa *et al.* (2017) reported that soybean contains 37.69% of protein, 28.20% of crude fat, 4.29% of ash, 8.07% of moisture, 5.44% of fiber, 16.31% of carbohydrate. Similarly, Contents of amino acids in soybean are given in Table 2.1. Most soy proteins are relatively heat-stable storage protein. This heat stability enables soy food products requiring high-temperature cooking, such as tofu, soy milk and textured vegetable protein (soy flour) to be made (Derbyshire *et al.*, 1976). Soy protein products can be good substitutes for animal products because, unlike some other beans, soy offers a 'complete' protein profile. Soy protein products can replace animal-based foods which also have complete proteins but tend to contain more fat, especially saturated fat without requiring major adjustments elsewhere in the diet (Food and Administration, 1999).

Table 2.1 Content of essential amino acids of soybean

Amino acids	Soybean seeds, (% of Dry matter)
Histidine	1.0-1.22
Isoleucine	1.76-1.98
Methionine	0.5-0.67
Arginine	2.45-3.1
Lysine	2.5-2.66
Phenylalanine	1.6-2.08
Valine	1.5-2.44
Tryptophan	0.51-2.44
Leucine	2.2-4.0

Source: Banaszkiewicz (2011)

2.2 Nature of Buffalo Liver and its composition

Bubalus bubalis, or Asian buffalo, is a species of buffalo raised in the Mediterranean region (Borghese and Moioli, 2016). The major attractive features of buffalo meat are red color, reduced fat, and cholesterol with poor marbling, low connective tissue, desirable texture, high protein, water-holding capacity, myofibrillar fragmentation index, and emulsifying capacity (Kandeepan *et al.*, 2013). Buffalo liver is an important edible meat byproduct. By-

product is an incidental or secondary product made in the manufacture or synthesis of something. However, in developing countries, it has a low commercial value and is underutilized. The efficient utilization of this edible meat byproduct is essential to support an economical and viable buffalo meat production system. Liver and liver products are a rich and economical source of essential nutrients that are more readily available to man in the form of animal products than non-meat products (S. Devatkal *et al.*, 2004b). Proximate composition of Buffalo meat and Liver with its minerals content and Physiological properties are given in Table 2.2 and Table 2.3 respectively.

Table 2.2 Proximate composition of Buffalo meat and Liver

Parameters	Buffalo meat	Buffalo liver	Mineral content of buffalo liver (mg/100 g)	
Moisture (%)	74-78	71.92 ± 0.37	Na	60.04 ± 7.78
Protein (%)	20.2-24.2	18.44 ± 0.56	K	274 ± 17.57
Fat (%)	0.9-1.8	5.60 ± 0.30	Ca	5.60 ± 0.32
Carbohydrate	Less than 1%	2.72 ± 0.12	Mg	6.20 ± 0.37
Total ash (%)	1.0	1.32 ± 0.04	Fe	20.86 ± 1.39
Total Energy(kcal/100g)	131	135.05 ± 1.88	Zn	5.16 ± 0.28
			Cu	5.60 ± 0.32

Source: S. Devatkal *et al.* (2004b)

Table 2.3 Physiological properties of Buffalo Liver

pH	6.42 ± 0.02
WHC (%)	38.00 ± 5.59
Cooking yield (%)	73.15 ± 0.72
Glycogen (mg/g)	7.07 ± 0.88
Total pigments (mg/g)	8.49 ± 0.95
Cholesterol (mg%)	283.88 ± 10.1

Source: S. Devatkal *et al.* (2004a)

2.2.1 Technology for preparation of Buffalo Liver powder

2.2.1.1 Boiling

Boiling meat in water can reduce harmful organisms, effective in destroying several classes of meat borne pathogens such as bacterial spores, fungi, cysts, worms which will help in preservation for long period.

2.2.1.2 Drying

The main aim of drying food products is the removal of moisture up to a certain level at which microbial spoilage and deterioration chemical reactions are greatly minimized. In addition to preservation, the reduced weight and bulk of dehydrated products decrease packaging, handling, and transportation costs. Furthermore, most food products are dried for improved milling or mixing characteristics in further processing. In contrast, with literally hundreds of variants actually used in the drying of particulates, solids, pastes, slurries, or solutions, it provides the most diversity among food engineering unit operations. Flow sheet of the preparation of dried liver powder is given in Figure 2.1.

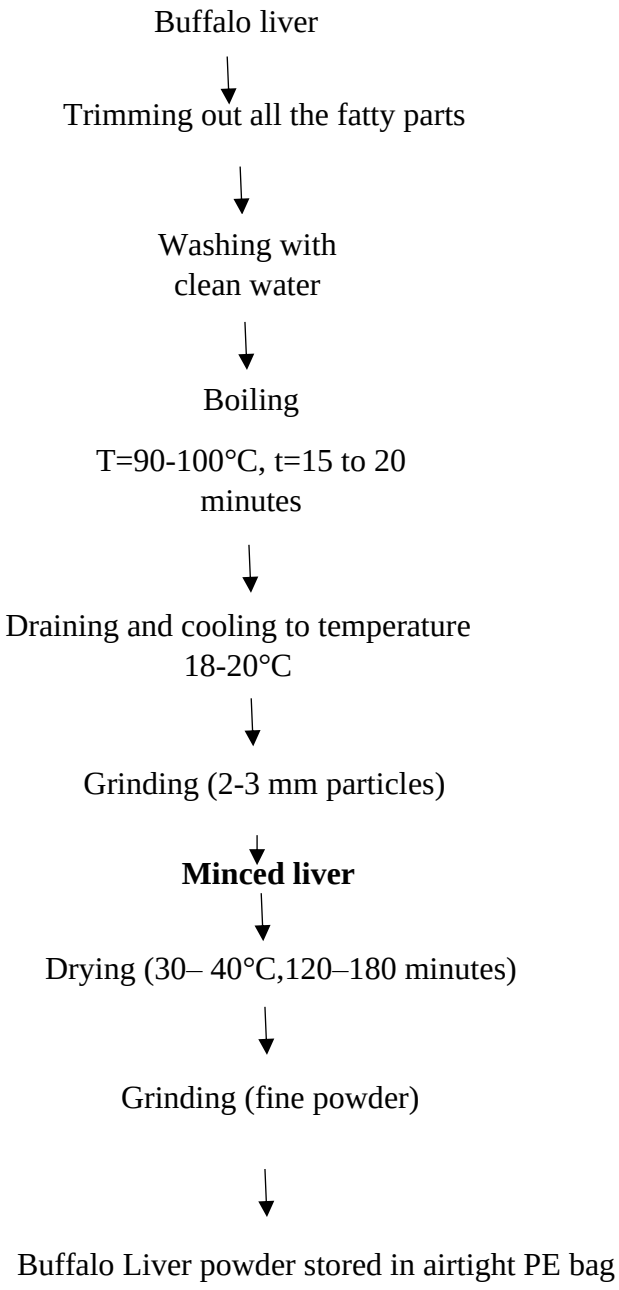


Fig. 2.1 Flow sheet of the preparation of dried liver powder

Source: Kalenik *et al.* (2017)

2.3 Protein Concentrate

Protein concentrate is a food powder having protein between 40% and 89% and a protein isolate is a powder having a minimum protein content of 90% (Alimentarius, 1989). Concentrated protein powder is commonly used as dietary supplements and in food processing, are available in a variety of flavors and forms, including ready to drink shakes, bars, bites, oats, gels and powders manufactured using soy, milk, peas, or eggs as the source of the protein. Protein concentrates are created by pushing the protein source through a very small filter that allows water, minerals, and other organic materials to pass through. The proteins, which are too big to pass through the filter, are collected, resulting in protein powder or protein concentrate. Concentrates can have substantial amounts of carbohydrates and fat. Further purification uses additional filtration or a technique called ion exchange or cross-flow microfiltration. Results in the formation of the protein isolate. Isolates have very low levels of carbohydrates and fat and are almost exclusively pure protein (Kalman, 2014). In order to understand more clearly about differences between protein concentrate, protein isolate and protein hydrolysate some points are given in Table 2.4.

Table 2.4 Difference between protein concentrate, protein isolate and protein hydrolysate

Protein concentrate (PC)	**Protein isolate (PI)**	**Protein Hydrolysate (PH)**
1. It is obtained by first step of purification. So, manufacturing cost is lower than protein isolate	**1.** It is obtained by double purification, with method named ion exchanges or cross flow microfiltration. So, it is more expensive in terms of its manufacture than concentrate.	**1**. Protein hydrolysate is obtained by breaking down PC and PI from complex protein into smaller fragments for easy and faster digestion.
2. Protein powder contains 30-80% of protein per serving.	2. Protein powder contains up to 90% of protein per serving.	2. It contains up to 90% protein per serving but more effective due to high absorption rate.

3. concentrate protein contain high amount of carbs and fat. Example: whey concentrate contains 3.5 g carb and 1.5 g fat.	3. Isolate contains very low amount of carbs, fats. Example: whey isolate contains 1 g carbs and almost nil fat.	3. contains almost negligible amount of carbs and fat.
4. mixes well but requires vigorous shake or blend	4. mixes well and consumed within one hour to avoid dispersion of protein in beverage.	4. mixes best and is advised to consume quick.

Source: Manninen (2009)

2.3.1 Soybean Protein Concentrate

Soy protein concentrate is about 70% soy protein and is basically defatted soy flour without the water-soluble carbohydrates. It is made by removing part of the carbohydrates (soluble sugars) from dehulled and defatted soybeans. Three basic processes are used for carbohydrates removal: 1. acid leaching (isoelectric pH 4.5), 2. aqueous ethanol (60-80%) extraction, and 3. moist heat-water leaching (M. Guo, 2009). Soy protein concentrate retains most of the fibre of the original soybean. It is widely used as a functional or nutritional ingredient in a wide variety of food products, mainly in baked foods, breakfast cereals, and some meat products. Soy protein concentrate is used in meat and poultry products to increase water, fat retention and to improve nutritional values (more protein, less fat). Procedure for the preparation of soy protein concentrate using acid wash method and alcohol wash method is given in Fig. 2.2 and Fig. 2.3 with their chemical composition in Table 2.5.

2.3.1.1 Technology for preparation of soybeans protein concentrate

Traditional treatments such as soaking, cooking, germinating have been used to improve the nutritional quality of the cereals and legumes. Sprouting of soy has been known to be beneficial for the reduction of anti-nutrients like trypsin inhibitor, phytic acid, flatulent etc. Soymilk prepared by the traditional method presents some problems and many workers have tried to improve the quality by eliminating of off-flavour, inhibiting the anti-nutritional factors, reducing the phytic acid content .

2.3.1.1.1 Sorting and Cleaning

Soybean seeds were cleaned thoroughly and made free from dust, dirt, stubbles and foreign matter. Damaged seeds with cracked hull etc. were discarded and the seeds were surface sterilized with 0.1% (w/v) potassium permanganate solution for 5 min. They were then rinsed with distilled water to remove any traces of potassium permanganate.

2.3.1.1.2 Soaking or steeping

Soaking or steeping is a pretreatment for decertification of grain which facilitates the removal of the husk or skin. Non- corticated grains are soaked in water for a short time which leads them to easy husk removal. The soaking process increases hydration coefficient, seed weight, total protein, ash, fat, fibre of cereals and legumes. The malting process begins when the cereal grain is steeped in water. Steeping is arranged so that sufficient moisture enters the grain to initiate germination. The period for steeping depends on temperature and degree of aeration of the steep water. A temperature of 10- 12°C is recommended with steeping times of 40- 60 hours. A temperature of 20- 25°C is recommended with steeping times of 16- 20hours for legumes (Dhital, 2021).

2.3.1.1.3 Germination of Soybeans

Sprouting is a period in the life cycle of plants when they start emerging from the seed. This natural process is also known as germination. The germination process resulted in a marked increase in the relative contents of both essential and non-essential amino acids. The rate of the relative increase in essential amino acids was 8.9% after 3 days of germination, 22.4% after 6 days of germination. The corresponding relative increases in non-essential amino acids were 17.6% and 17.5% after 3 and 6 days of germination, respectively. The levels of sulphur amino acids in germinated seeds remained almost constant, whereas aspartic acid increased 51.9% compared to ungerminated seeds. Available lysine decreased gradually and significantly during the germination process; after 5 days of germination, the decrease reached 54.9% compared to that of ungerminated seeds (from 5.85 g per 16 g N to 2.64 g per 16 g N). During germination reserve nutrients (lipids and carbohydrates) degrade whose essential purpose is to provide the energy required for protein synthesis in plant growth. The total protein content and the non-protein nitrogen increased significantly after 5 days of etiolated germination. The total crude protein content of more than 21% increases in dehulled germinated soya beans compared to ungerminated. Germination causes increases in the number of vitamins in soya beans (Ahmad and Pathak, 2000).

Germinated grains are good sources of ascorbic acid, riboflavin, choline, thiamine, tocopherols and pantothenic acid and also have increased availability of minerals such as copper, sodium, potassium, iron, phosphorus, calcium, manganese and magnesium (Kayembe, 2011). Germination of soybeans has been known to be beneficial for the reduction of anti-nutrients like trypsin inhibitor, phytic acid, flatulent, etc (Murugkar, 2014).

2.3.1.1.4 Soy milk preparation

This is prepared by the traditional method in which the whole bean is soaked in water and then extracted with water; the water extract is then boiled and filtered through a cheesecloth. The product thus obtained can be taken as such or flavored with syrup and taken as a drink. The soymilk produced can also be further processed into various forms of soybean curd. Soymilk is rich in protein (>3.0%, except for the amino acid, methionine). Generally low in fat (<2.0%), have moisture >93%, 1.1% of crude fiber and is the source of minerals like calcium, Iron, Zinc with vitamins like Thiamin, Riboflavin, Niacin, etc (Mazumder and Begum, 2016).

2.3.1.1.5 Coagulation of soy milk

Coagulation is essentially the formation of a gel by destabilizing the casein micelles causing them to coagulate and form a network that partially immobilizes the water and traps the fat globules in the newly formed matrix (Abeykoon *et al.*, 2016).

This may be accomplished with:

- Enzymes
- Acid treatment
- Heat-acid treatment

2.3.1.1.6 Separation of Soy coagulated mixture from whey and drying to powder form

The mixture coagulated and the tofu was separated from the whey using a muslin cloth after which it was dried to moisture content <9.8% (Kalenik *et al.*, 2017).

2.3.1.2 Different methods of preparation of soy protein concentrate

a. Procedure for the preparation of soy protein concentrate using acid wash method

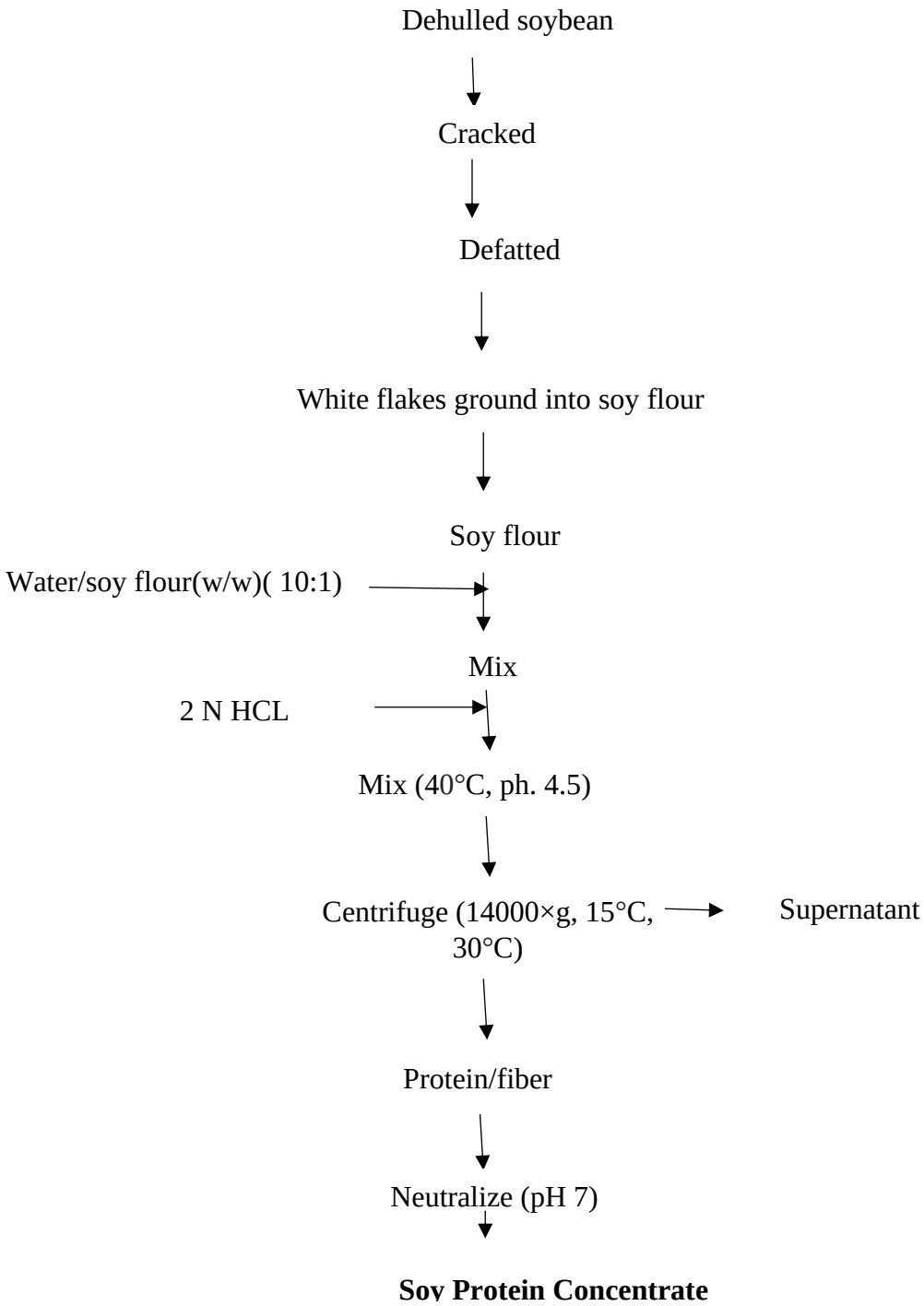


Fig. 2.2 Flowsheet of preparation of soy protein concentrate by acid wash metho

Source: Wang *et al.* (2004)

b. Procedure for the preparation of soy protein concentrate by alcohol wash.

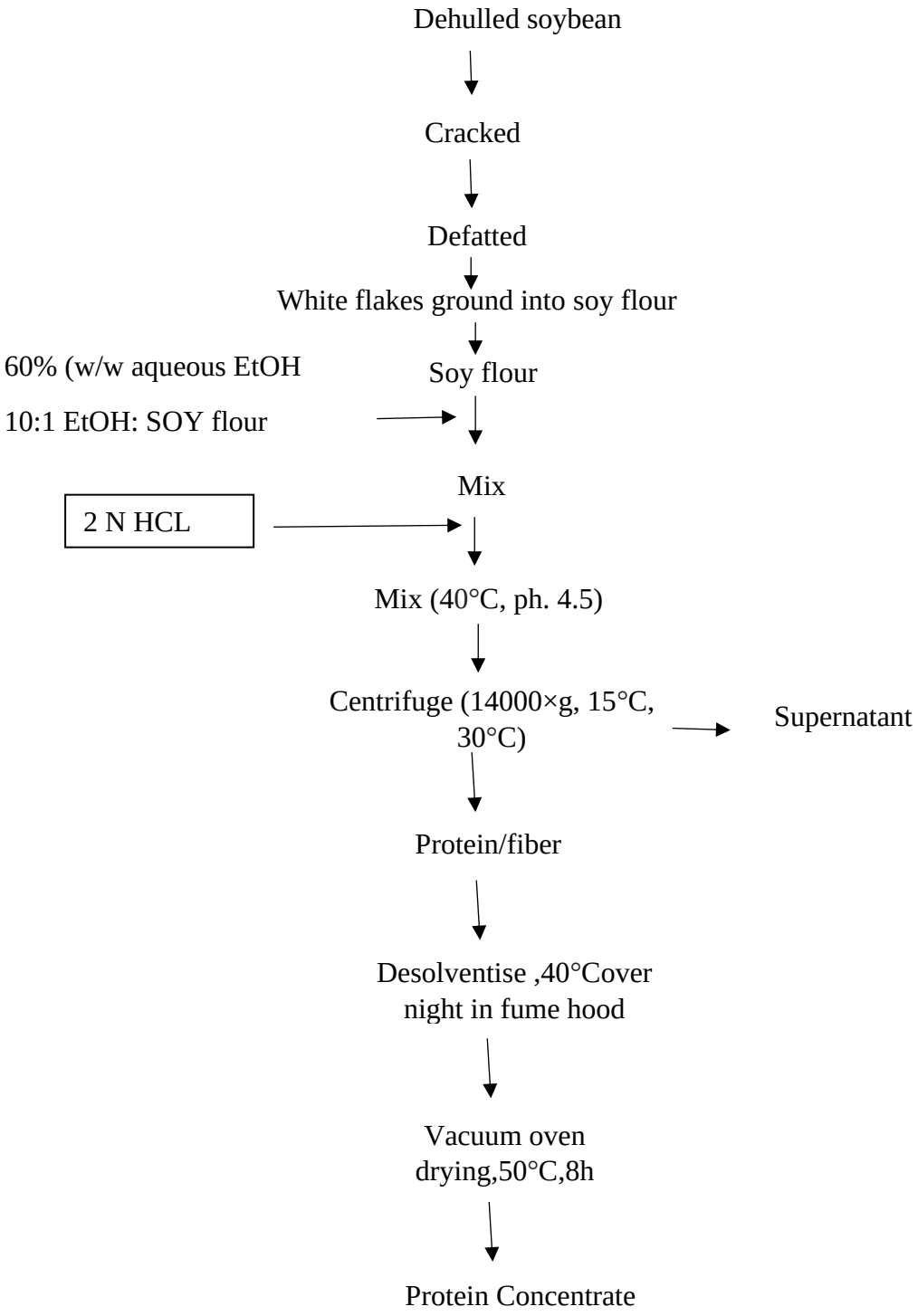


Fig. 2.3 Flowsheet of preparation of soy protein concentrate by alcohol wash method

Source: Wang et al. (2004)

Table 2.5: Chemical composition of soy protein concentrate made by three extraction processes

Component	Alcohol Washing	Acid Washing	Hot-water Washing
Protein $(N \times 6.25)_b$	71.0	70.0	72.0
Protein	67.0	66.0	68.0
Moisture	6.0	6.0	5.0
Fat	0.3	0.3	0.1
Crude fibre	3.5	3.4	3.8
Ash	5.6	4.8	3.0
Carbohydrate	17.6	19.5	20.1

Source : A.Deak *et al.* (2008)

Table 2.6: Amino Acid Composition of Soy Protein Concentrates, Soy Soluble, and Soy Flour

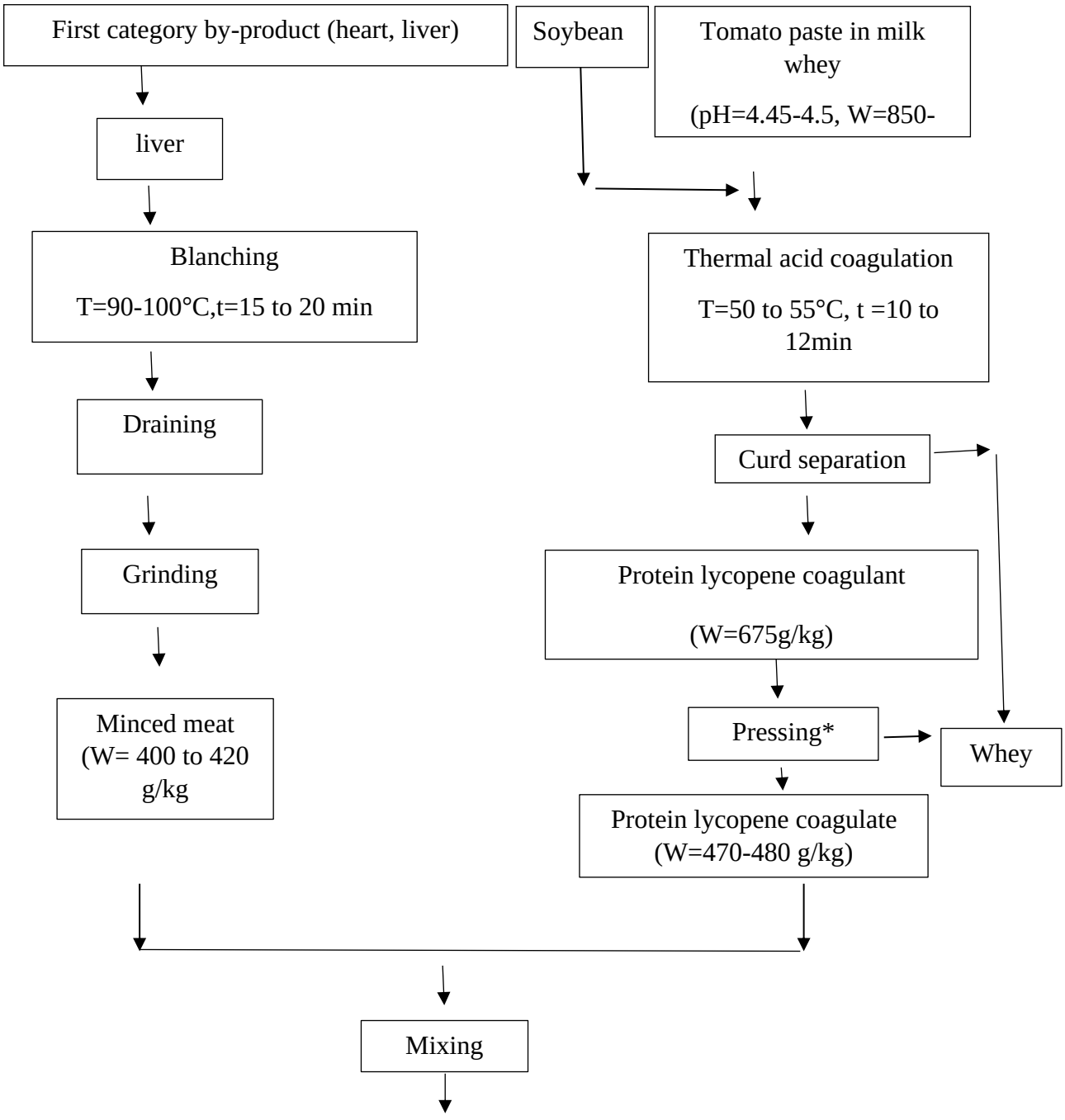
Amino acids	**Soy flour**	**Alcohol Washed**	**Acid Washed**	**Soy Soluble from Alcohol Washing**
Alanine	4.00	4.86	4.03	3.94
Arginine	6.95	7.98	6.46	7.36
Aspartic acid	11.26	12.84	11.28	15.0
Half-cystine	1.45	1.40	1.36	4.14
Glutamic acid	17.18	20.20	18.52	20.7

Glycine	3.99	4.60	4.60	3.47
Histidine	2.60	2.64	2.59	2.50
Isoleucine	4.80	4.80	5.26	2.11
Leucine	6.50	7.90	8.13	3.17
Lysine	5.70	6.40	6.67	3.53
Methionine	1.34	1.40	1.40	3.60
Phenylalanine	4.72	5.20	5.61	5.65
Proline	4.72	6.00	5.32	3.48
Serine	5.00	5.70	5.97	3.38
Threonine	4.27	4.46	3.93	3.36
Tryptophan	1.80	1.60	1.35	7.00
Tyrosine	3.40	3.70	4.37	5.47
Valine	4.60	5.00	5.57	2.12

Source: A.Deak *et al.* (2008)

2.3.2 Technological development of protein rich concentrates using soybean and beef biproduct (liver, heart)

Protein concentrates were developed using minced beef/buffalo liver and heart, dehydrated and mixed with a soya protein-lycopene coagulate (SPLC) obtained from a solution prepared with germinated soybeans and mixed with tomato paste in milk whey, and finally dried. The technological parameters of pressing SPLC and of drying the protein concentrate were optimized using response surface methodology(Kalenik *et al.*, 2017).



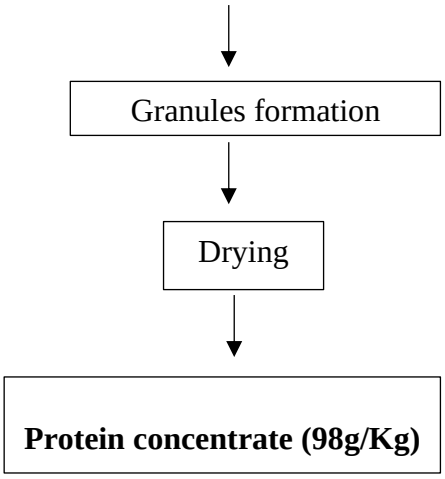


Fig. 2.4 Flow sheet of development of protein-rich concentrate using soybean and meat by-products (beef liver/heart)

Source : Kalenik *et al.* (2017)

Table 2.7 Chemical composition and energy value of the protein concentrate prepared with minced meat and SPLC ratio of 70:30.

Protein concentrate based	Content, g/kg						
	water	protein	fat	carbohydrate	fibre	ash	Energy Value kcal/100g
Heart	98	641	87	58	42	74	376.1
Liver	98	644	88	56	39	75	376.2

Source: Kalenik *et al.* (2017)

Table 2.8 Essential amino acid composition of the protein concentrates prepared with minced meat and SPLC ratio of 70:30.

	Essential amino acid, g·kg-1								
Product	Val	Ile	Leu	Lys	Met+Cys	Thr	Trp	Phe + Tyr	Cmin,%
FAO standard	40	30	61	48	23	25	6.6	60	100
Beef liver based	62	48	82	71	36	41	13	85	128
Beef heart based	57	47	90	74	32	40	12	60	100

Source: Kalenik *et al.* (2017)

2.4 Amino acids

Amino acids often referred as the building blocks of protein

- They're needed for vital processes like the building of proteins and the synthesis of hormones and neurotransmitters

Amino acids are categorized as

1. Essential. Essential amino acids cannot be made by the body. As a result, they must come from food which are: Phenylalanine, Valine, Threonine, Methionine, Leucine, Isoleucine, Lysine, Tryptophane and Histidine.

2. Non-essential amino acids: The body produces them even if the body doesn't obtain them from the food eaten which are: Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Glutamic acid, Glutamine, Glycine, Proline, Serine and Tyrosine

3. Conditional amino acids: They are usually not essential, except in times of illness and stress which are: Arginine, Cysteine, Glutamine, Tyrosine, Glycine, Ornithine, Proline and Serine

2.5 Chromatography

Chromatography is a separation process that depends on the differential distributions of the components of a mixture between a mobile bulk phase and an essentially thin-film stationary phase (Block *et al.,* 2016). Chromatography is the most powerful technique to separate chemically closely related substances into individual components on the basis of their physicochemical properties. These compounds are separated on the basis of their partition coefficients between two immiscible phases. The static phase may be solid or liquid while the mobile phase may be a solid, liquid or gas. Depending upon the static and mobile phases, a variety of chromatographic techniques are available. These include chromatography on paper, thin layer gel, ion-exchange resin etc. Although modern instrument facilities such as high-performance liquid chromatography (HPLC) are available for the separation of chemical substances, the classical techniques- paper chromatography and thin-layer chromatography are still easy, can be set up even in an ordinary laboratory without much expenditure (Smith, 2013).

2.5.1 Principle of paper chromatography

The separation of the solute (amino acids) is based on the liquid-liquid partitioning of amino acids in paper chromatography. The partitioning takes place between the water 24 molecule (static phase) adsorbed to the cellulosic matter of the paper and the organic (mobile) phase(Smith, 2013).

2.5.2 RF value

RF value (in chromatography) is the distance travelled by a given component divided by the distance travelled by the solvent front. For a given system at a known temperature, it is a

characteristic of the component and can be used to identify components. The constant RF is indicated by:

$$\text{RF value} = \frac{\text{Distance(cm) moved by the solute from origin}}{\text{Distance(cm) moved by the solvent from origin}}$$

Distance cm moved by the solvent from the origin The amino acid present in the sample are then identified by comparing the RF values with that of the authentic amino acids, a co-chromatographed (Sadasivam, 1996).

2.6 Packaging Product

Product damage is usually caused by either climatic conditions or physical environments (Maskey *et al.*, 2021). Packaging's main function is to protect its content and 30% of all food produced worldwide is lost or wasted along the supply chain, optimized packaging may be one of the solutions to reduce this staggering amount. Developing countries struggle with losses in the supply chain before food reaches the consumer. Here, appropriate packaging may help to protect food and prolong its shelf life so that it safely reaches these households. In developed countries, food tends to be wasted rather at the household's level due to wasteful behaviour. Knowing which product group spoils easiest, at what point along the chain they spoil the most, what brings about the food loss and last but not least, can losses be avoided or not, are specific concerns along the value chain, with high implications on packaging (Wohner *et al.*, 2019).

2.6.1 Packaging materials used for protein concentrate powders

Selecting the suitable material for packaging a certain type of food depends on the functions that the package is supposed to fulfil. These functions include shielding the foods against moisture, temperature variations, oxygen, light, and biological microorganisms. Also, damage protection, permeability, food identification, and chemical and optical properties play a significant role in the material selection (Galanakis, 2018). Several types of packaging are approved for dry dairy powders. The more durable and most frequently used type is a multi-wall Kraft paper bag with an inner low-density polyethylene (LDPE) bag liner, both of which are heat-sealed. This type of bag construction is referred to as a "bag within a bag". Packaging differs in the thickness and number of layers of Kraft paper and the thickness and

material of the bag liner. The combination of a 3-4 ply multi-wall Kraft paper bag and 3-4 mil thickness LDPE bag liner offers good protection during storage and handling. The LDPE bag liners have vents to allow air to escape from the bag. This prevents rupture during handling and storage. There are different technologies available to reduce the amount of air and moisture getting into the dry product through these vents; the technology varies by bag manufacturer (Council, 2010). The HP bag was made of a plastic liner (Kraft paper, 0.127 mm) thick while the Std bag made up of the same paper with 0.076 mm thickness were used to store Whey Protein Concentrate (Ukuku *et al.*, 2017).

2.7 Quality Changes During Storage of Dairy dry powders

Ideal storage conditions for dry dairy products are temperatures below 25°C with relative humidity below 65%. The greatest loss of product quality during storage is often the result of moisture uptake by the powder which may result in chemical, physical and bacteriological changes. The lactose in dairy ingredients is hygroscopic and readily takes up water. Packaging that is more robust and water-resistant can help extend shelf life, especially in more extreme environments (Council, 2010).

a) **Clumping**

This is typically caused when the lactose component in a dry dairy ingredient begins to absorb moisture. Ingredients with higher lactose content will be affected more than those with lower lactose levels (Roos, 2002).

b) **Flavour**

Dry dairy products are known for having a neutral, mild dairy flavor. Storage at high temperatures and high relative humidity may increase the potential development of off-flavors (Burgain *et al.*, 2016) (Wright *et al.*, 2009). In many cases, significant changes in product flow and solubility will occur before any changes in flavor and aroma become evident. Flavor changes develop more quickly in agglomerated or "instant" ingredients.

c) **Functionality**

The solubility, foaming and emulsification properties of dairy ingredients are fairly stable during storage. Changes in solubility may occur and are most often related to colour changes, such as browning(Anema *et al.*, 2006).

d) **Hardening**

This is typically caused when a dry dairy product has been exposed to high storage temperatures and high storage relative humidity (>85%) for an extended period of time (Burgain *et al.*, 2016). The amount of time before hardening occurs will vary depending on the specific storage conditions, product type and packaging.

e) **Nutritional**

Storage at higher temperatures may reduce the amount of the amino acid lysine in the product (Li-Chan, 1983). Exposure to moisture may increase the amount of loss (Uppu, 2001).

f) **Powder Structure**

Powders with higher lactose levels can have the following undesirable changes during storage: stickiness and caking, lactose crystallization, lipid release from powder particles, increased browning; and lipid oxidation (Roos, 2002. If dry whey ingredients become caked they can be ground with the resulting powder remaining free-flowing unless exposed to high humidity.

2.8 Physical and chemical changes in whey protein concentrate stored at elevated temperature and humidity

The liquid whey generated during cheesemaking may be ultrafiltered and dried to produce whey protein concentrate (WPC) powder with a recommended shelf life of 9 to 12 months (Sithole *et al.*, 2005), which may be extended to 24 months under refrigeration. WPC sent overseas are usually stored without refrigeration, exposing the product to elevated temperature and humidity. The shelf life of WPC under these conditions must be known to prevent the product from being rejected.

Maillard reactions and color changes have been observed in shelf-life studies of sweet whey powder (Sithole *et al.*, 2005), as have lipid oxidation (Wright *et al.*, 2009), volatile compound formation (Lee *et al.*, 1996), and reduction of lysine (Li-Chan, 1983). Absorption or desorption of moisture can significantly affect the shelf life of foods. This is particularly the case for dry, powdery products such as milk powders. The main purpose of packaging is to protect the powder from moisture ingress to preserve the product characteristics. When they gain moisture, powdery products become lumpy or cake. In addition, the moisture may lead to deleterious changes such as structural transformations, enzymic reactions, browning, and oxidation, depending on temperature and the availability of the O2 (Roos, 2002). Moisture or water vapor ingress in combination with light, O2, and an elevated temperature can result in physical loss of texture and caking due to lactose crystallization, microbial spoilage, nonenzymic reactions (such as Maillard browning), and fat oxidation (Uppu, 2001). There was an increase of a_w and yeast and mould populations up to 6 months, and then this association declined at 9 and 12 months and slightly increased again at 18 months during storage of Whey Protein Concentrate in HP bag made of a plastic liner (Kraft paper, 0.127 mm) thick while the Std bag made up of same paper with 0.076 mm thickness (Ukuku *et al.*, 2017). The effectiveness of a package can be determined during shelf life testing or by combining information from break-point testing (holding at increasing humidity) and knowledge about the characteristics of the moisture permeability of the packaging material (Brown and Williams, 2003). Microorganisms that grow optimally at pH less than 5.55 is called acidophiles(F. Guo *et al.*, 2020). pH decreases more rapidly in high temperatures than in low temperatures. This may be due to microbial growth with an increase in moisture content (Ukuku *et al.*, 2017). Bacteria in cold environments stop their relentless growth, limiting the number of food-borne illness-causing bacterial cells and preventing other ravenous bacteria from eating our food before we do (Wiebe *et al.*, 1992)

2.9 Sensory evaluation

Quality is the ultimate criterion of the desirability of any food product to the consumer. Overall quality depends on quantity, nutritional and other hidden attributes, and sensory quality. Hedonic rating relates to pleasurable or unpleasurable experiences. Part of food product development and the launching of new products in the market require some measure of whether the products are liked or not by the appropriate consumers. There have been many rating scales developed for measuring degree of liking of which the Labeled Hedonic Scale,

sometimes called the LIM scale, and the LAM scale are more recent developments. The hedonic rating test is used to measure the consumer acceptability of food products. The 9-point hedonic scale has been used routinely in food science, the same way for 60 years. Now, with advances in technology, data from the scale are being used for more and more complex programs for statistical analysis and modeling. Accordingly, it is worth reconsidering the presentation protocols and the analyses associated with the scale, as well as some alternatives. How the brain generates numbers and the types of numbers it generates has relevance for the choice of measurement protocols. There are alternatives to the generally used serial monadic protocol, which can be more suitable. Traditionally, the 'words' on the 9-point hedonic scale are reassigned as 'numbers', while other '9-point hedonic scales' are purely numerical; the two are not interchangeable (Wichchukit and O'Mahony, 2015). Parametric statistical analysis of scaling data is examined critically and alternatives discussed. The potential of a promising alternative to scaling itself, simple ranking with a hedonic R-Index signal detection analysis, is explored in comparison with the 9-point hedonic scale. Semi trained panels in smaller number are used to screen a number of products for selecting a few for consumer preference studies. The samples are served to the panelist at one session. The panelist is asked to rate the acceptability of the products on the scale, usually of 9 points, ranging from 1 point usually given for "like extremely" to 9 points given for "dislike extremely" The scores received by each samples are then averaged and compared with the average score received by other sample in the series (Ranganna, 1986)

PART III

Materials and method

3.1 Material

3.1.1 Soybeans

Soybeans were collected from the Dharan market. It is locally known as ‘Bhatmas’. Its variety *was ‘Glycine max’*

3.1.2 Buffalo Liver

Buffalo liver was collected from the Dharan market. It is locally known as ‘Bhaisiko kalejo’.

3.2 Chemical and equipment required

The equipment, instruments and chemicals used for the analysis (Kjeldahl distillation and digestion set, weighing machine, hot air oven, digital moisture meter (Wile-55 moisture meter), Soxhlet apparatus, chromatography chamber, mill, colourimeter was available at the Central Campus of Technology, Dharan.

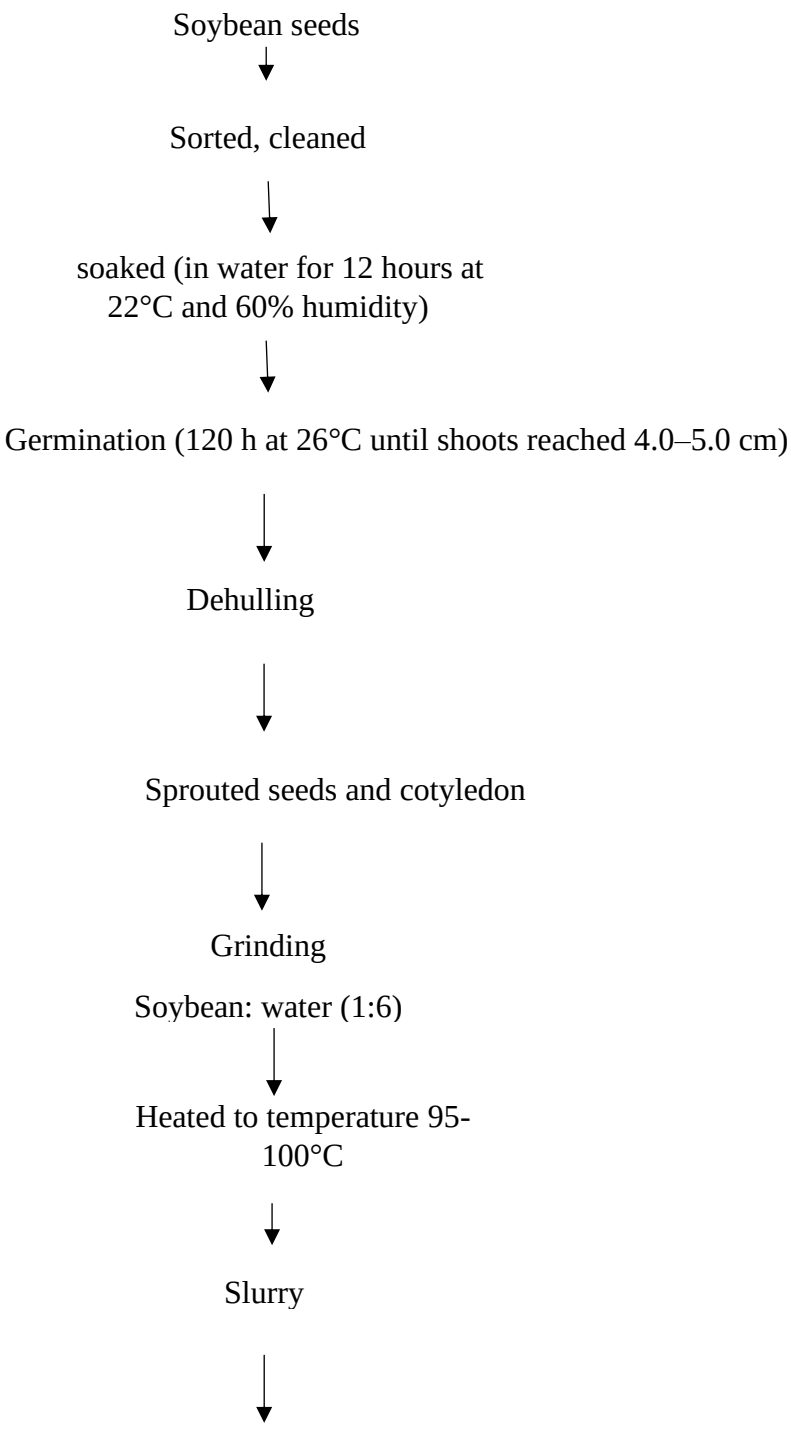
3.3 Methods

3.3.1 Processing of raw materials

3.3.1.1 Soybeans protein concentrate

Soybeans were sorted, cleaned and soaked in water for 12 hours at 22°C and 60% humidity and then drained. It was spread on a wetted muslin cloth and covered by a wetted muslin cloth. The soybean seeds were germinated, crushed and subjected to extraction. Germination was achieved over 120 h at 26°C until shoots reached 4.0–5.0 cm. Germinated soybeans were washed, soaked in water for swelling for 8 hours, then washed to remove all the deed coats and milled in water at a proportion of 1:6 (soybeans: water), heated to 95–100°C for 1–2 minutes and, finally, separated into the soluble (suspension) and insoluble fractions. The suspension was the soybean ingredient used in the next stages (Kalenik *et al.*, 2017). Milk

obtained from the above process was cooled to around 80 °C subsequently 3% calcium sulphate was added (of the total milk obtained). The mixture coagulated and the coagulated protein complex (similar to tofu) was separated from the whey using a muslin cloth after which it was dried to a moisture of about 5-6% at temperature 40-50ºC for 120-180 minutes. The dried protein complex was milled into powder and packed in airtight plastic bags.



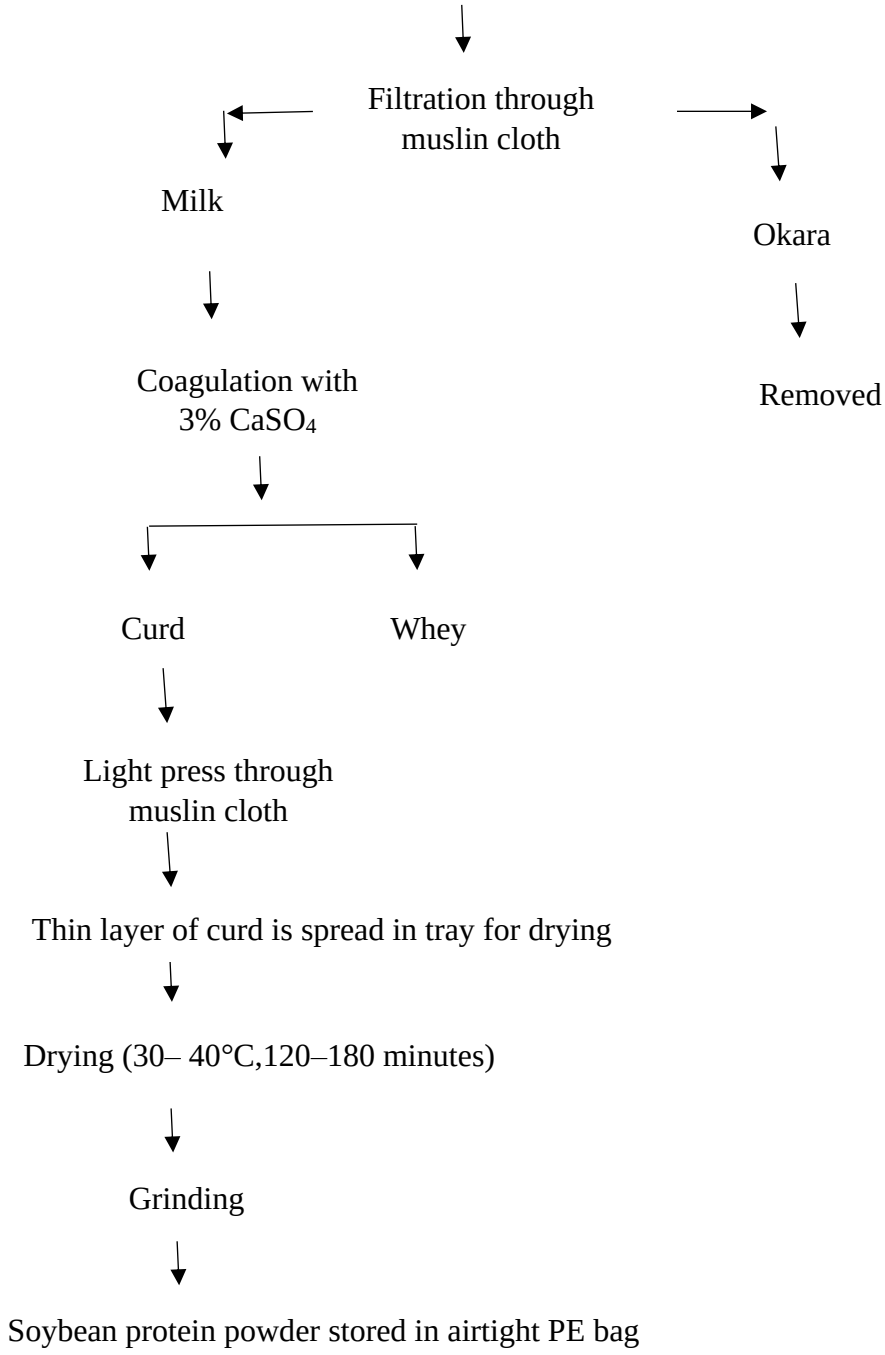


Fig. 3.1 Flow sheet of preparation of soy protein concentrate

Source: Murugkar (2014)

3.3.1.2 Buffalo Liver powder

Buffalo liver was boiled (cooked) in water at a temperature between 90–100°C for 15–20 minutes and drained for 3–5 min at a temperature between 18–20°C until water from the

surface of the offal pieces had evaporated, to achieve a reduction of the water content from 600–700 g·kg-1 to 400–420 g·kg-1. Then the meat was ground into 2–3 mm particles cloth after which it was dried to a moisture of about 5-6% at temperature 40-50ºC for 120-180 minutes. Dried liver chunks were milled into powder and packed in airtight plastic bags (Kalenik *et al.,* 2017).

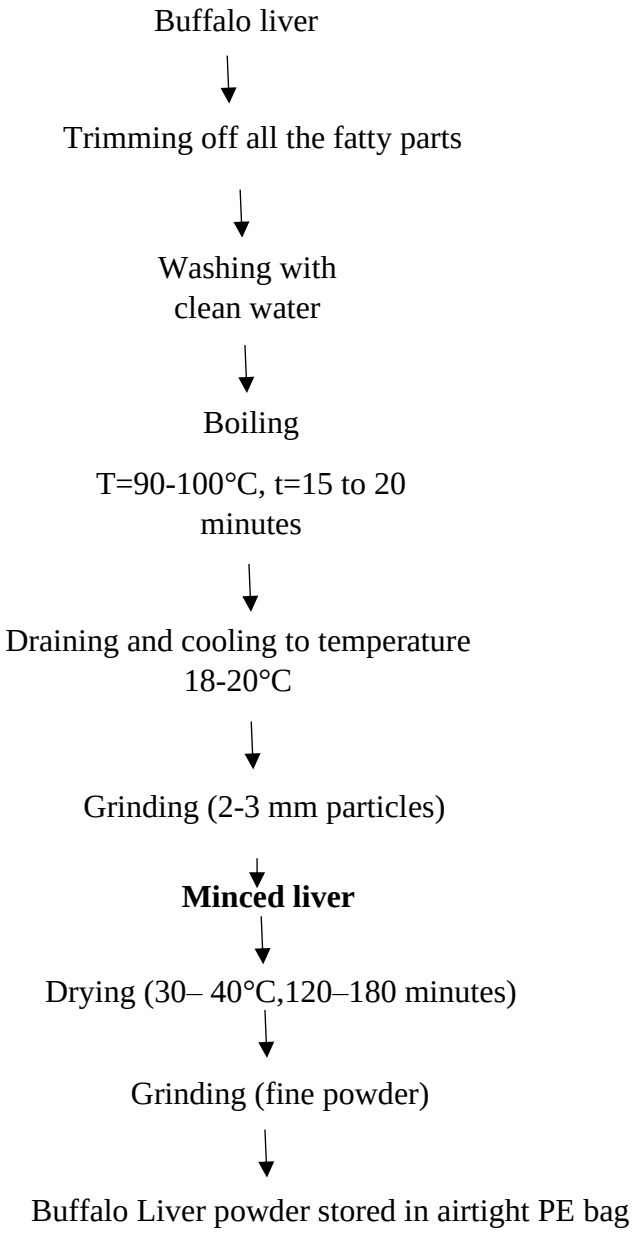


Fig. 3.2 Flowsheet of the preparation of dried liver powder.

Source: Kalenik *et al.* (2017)

3.4 Formulation of the final product

For the formulation of protein concentrate, the amounts of ingredients were calculated on a dry weight basis. Soybean was taken as plant-based main source of protein which was rich in all essential amino acids except Methionine. To obtain a food product that is high in protein content with complete essential amino acids buffalo liver which is also a byproduct is used. Soybeans were sorted, cleaned, soaked in water, germinated, extracted soy milk, coagulated, protein complex was dried and ground into powder. Similarly, the liver was boiled, cut into pieces, dried and ground into powder. Both powders were stored in airtight plastic separately. The stored soybean protein powder and liver were then taken in the required amount as per the formulations. Then they are mixed uniformly to make protein-rich concentrate as shown in Fig. 3.3

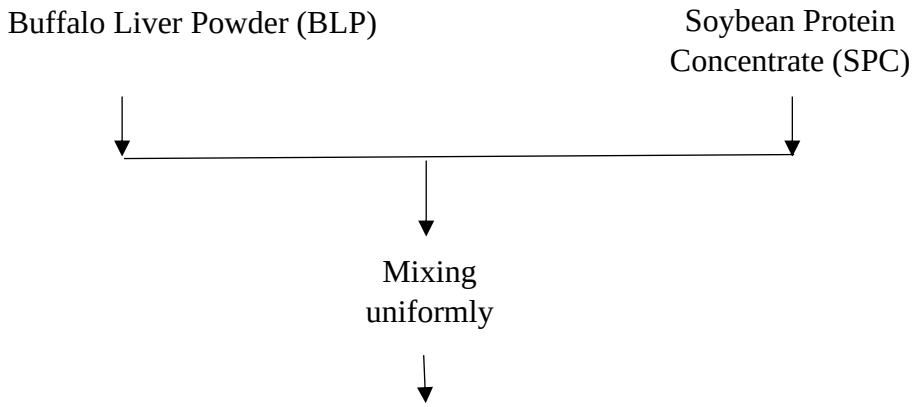


Fig. 3.3 Flow sheet of preparation of final product

3.5 Research Design

Design-Expert v10.0.0 software was used to create the samples with ratios varying from 90:10 to 50:50 as done by Kalenik *et al.* (2017). “User-defined mixture design” was used to formulate the variations as given in Table 3.1. Similar formulations were excluded to keep the work less complicated and result oriented. Five samples were made which were then coded alphabetically as given in Table 3.1.

Table 3.1 Sample code for different formulations

Code	Liver dried powder	Soy protein powder
A	90	10
B	80	20
C	70	30
D	60	40
E	50	50

3.6 Sensory evaluation

The prepared five formulations were selected as done by Kalenik *et al.* (2017). Five formulations i.e. A, B, C, D and E as per the Design of Expert were cooked as soup. Contents of ingredients of soup were formulated as given by Kalenik *et al.* (2017) using food concentrate(different proportion) with constant weight of tomato paste, onion paste, ginger and garlic paste in the ratio of 2:2:1. Five soup samples were provided to 14 panelists i.e. teachers and students of the Central Campus of Technology. Sensory evaluation was performed by a 9-point hedonic scoring test (9 = like extremely, 1=dislike extremely) for appearance, aroma, flavor, mouthfeel and overall acceptance. The panelists were requested to provide scores in the score sheets as per their perception. Data were analyzed statistically and the best product was found.

Analytical methods

3.7 Proximate analysis

3.7.1 Determination of moisture content

The moisture contents of the raw samples and the final product were determined by the hot air oven method as described by KC and Rai (2007). The results were expressed in terms of percentage.

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3.7.2 Determination of protein

The protein content of the raw, dried and final product was determined by Kjeldahl nitrogen method as described by KC and Rai (2007). The calculated data were presented per 100 gm on a dry basis.

3.7.3 Determination of crude fat

The fat content of the samples was determined by solvent extraction method as described by KC and Rai (2007). The calculated data were presented as gm per 100 gm on a dry basis.

3.7.4 Determination of total ash

The total ash of the samples was determined by incinerating the samples in a muffle furnace at a temperature not exceeding 525℃ for 5-6 hours as described by KC and Rai (2007). The calculated data were presented as gm per 100 gm on a dry basis.

3.7.5 Determination of crude fiber

The crude fiber of the samples was determined by the recovery of ash-free residue after sequential treatment of solid sample(ground) with 1.25% sulfuric acid and 1.25% of sodium hydroxide each under standardized conditions. The calculated data were presented as gm per 100 gm on a dry basis as described by KC and Rai (2007).

3.7.6 Determination of carbohydrates

The total carbohydrates content of the samples was determined by the difference method as described by KC and Rai (2007).

Carbohydrates (%) = 100- [sum of protein, total ash, fiber and fat]

3.8 Chemical analysis

3.8.1 Determination of pH

It will be directly measured by using a pH meter which will be standardized by using buffer solution of pH 7 and 4 at the temperature required according to KC and Rai (2007).

3.8.2 Determination of PV

The peroxide value of the sample was determined by the volumetric method according to KC and Rai (2007).

3.9 Microbiological analysis

Harrigan and McCance (1976) procedure was followed to measure the total plate count using agar plate count and distilled water as a diluent by the pour plate process. 1 mL of the sample from 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilution will be withdrawn with the help of a sterile pipette and transferred to each sterile Petri plate. 10 mL of molten Plate Count Agar will be added to every plate at 45 °C and the medium and inoculum will be mixed immediately by a combination of to-and-fro shaking and circular movements for 5–10 s. The plates will be allowed to be set and incubated at 37 °C for 24–48 h and the analysis will be performed. The results of the mean counts will be expressed as CFU/g.

3.10 RF value calculation

- Sample preparation for Amino acid estimation was done according to procedure provided by Sadasivam (1996) in his book "Biochemical Methods" in chapter three.

For estimating the amino acid composition of foodstuff, feed or any protein, it has to be first hydrolyzed.

- **Materials required**
    1. Ethanol
    2. 0.01 N HCl
- **Procedure**

- Sufficient quantity of sample was weighted which should have 2 to 6µ moles of each amino acids.
- Sample was extracted with warm (60°C) 70% ethanol, three to six times Extractant was used five times the weight of sample for each extraction.
- Extract was pooled after centrifugation and evaporated in rotary vacuum evaporator to dryness.
- Residue was taken in 1-10ml of 0.01 N HCl.

❖ Paper Chromatography was performed for the qualitative analysis of essential amino acid profile of final product according to procedure given by Sadasivam (1996) in his book "Biochemical Methods" in chapter 12.

➢ **Materials**

a. Whatman No. 1 filter paper
b. Chromatography chamber
c. Hair-dryer or spot-lamp
d. Atomizer
e. Micro syringe or micropipette

➢ **Mobile Phase (Solvent System)**

- n-butanol, glacial acetic acid and water in the ratio 4:1:5 were mixed in a separating funnel and stood to equilibrate for 30 min. Lower aqueous phase was drained off into a beaker and placed it inside to saturate the chromatography chamber. The upper organic phase was saved and used it for developing the chromatogram.
- Different individual amino acids were dissolved in distilled water at a concentration of 1 mg/ml. Very dilute (0.05 N) HCI was used to dissolve the phenylalanine. Tryptophan was dissolved in very dilute (0.05 N) NaOH.
- Ninhydrin reagent: 100 mg of ninhydrin was dissolved in 100 mL acetone.

➢ **PROCEDURE**

- Chromatography sheet was cut carefully to a convenient size (40 x 24 cm). A line was drawn with pencil across the sheet about 5 cm away from one end. A number of points were marked at intervals of 3 cm.
- A small volume (say, 25 µL) of each amino acid were applied as a separate small spot using a micro-syringe. A stream of hot air from a hair-dryer facilitated fast drying of spot. The spot should be as small as possible for better resolution.
- After spotting, the sheet in a stainless-steel trough was placed in the chromatography chamber, firmly held it by placing a long steel rod over the sheet. The spot-end of the sheet should be in the trough (descending chromatography). Otherwise, the sheet may be rolled as a cylinder, tied together with fine thread and placed upright with the spots as the bottom in a large petri dish for upward movement of solvent (ascending chromatography).

- The organic (phase) solvent was added to the trough/petri dish and the chamber airtight was closed. The chromatogram was developed, preferably overnight or longer, until the solvent moves almost to the other end.
- Solvent front was noted and dried the chromatogram free of solvent in a fume chamber.
- The chromatogram was sprayed with the ninhydrin reagent using an automizer. The paper was dried for about 5 min at room temperature followed by at 100°C in an oven for 2-3 min. Amino acids appear as purple spots.

➢ All the spots were marked and calculated their R_f values by the formula

$$R_f \text{ value} = \frac{\text{Distance(cm) moved by the solute from origin}}{\text{Distance(cm) moved by the solvent from origin}}$$

➢ The amino acids present in the sample were then identified by comparing the R_f values with that of the authentic amino acids, co-chromatographed.

3.11 Storage studies

The films that are traditionally used for MAP are patented and generally too costly to be used. So, for economical convenience, polypropylene plastic bags (PP) of dimensions 12 cm × 12 cm were brought from the local market (Tunick *et al.*, 2016). Each sample was subdivided into sachets of 51 ± 3 g. For convenience and easy accessibility during the whole work. The storage environment of the low store was recreated in a laboratory refrigerator (Model: LG-GL-V292RVBN). Eight samples for each storage environment; room temperature (25±3°C), refrigeration temperature (5±1°C) were prepared and stored for 50 days. All of the bags in the study remained sealed until their analysis times when they were opened, sampled, and discarded, so the outside atmosphere did not come in contact with the material during storage except by penetration through the liner (Tunick *et al.*, 2016). Samples were drawn at intervals of 7 days and evaluated for chemical properties (Moisture, peroxide value, pH) and microbiological qualities (TPC and coliform count) (Ukuku *et al.*, 2017).

3.12 Data analysis

Analysis of variance (ANOVA) was carried out for data from sensory evaluation. The R-programming was used for graphical outputs and multivariate analysis (Principal Component Analysis). The significance test among the mean of the changes in parameters (moisture, PV, pH and Total plate count were done by subjecting data to a one-way analysis of variance (ANOVA) and posthoc analysis was performed using Tukey's honesty test to compare the significant differences among means at a level of $p<0.05$.

PART IV

Results and discussion

4.1 Proximate analysis of raw samples

The proximate analysis gives inexpensive yet very important information, particularly from the nutritional and biochemical points of view. The results are normally expressed in percentages and because of the fairly general nature of the test employed for the determination, the term crude is usually used as a modifier; for an instant, crude protein, crude fat and crude fibre, etc. Therefore, proximate constituent represents only a category of compounds present in the biological material (Acharya and Karki, 2008).

Table 4.1 The proximate and ultimate composition of the soybeans and liver on a dry basis.

Parameters	Soybeans	Liver
Moisture (%)	10.5±0.5	71.5±0.61
Crude protein (%)	42.02±0.65	64.91±0.37
Crude fat (%)	27.46±0.87	19.29±0.35
Crude fibre (%)	6.78±0.2	-
Ash (%)	5.36±0.4	4.5±0.2
Carbohydrates (%)	18.38± 0.18	11.3±0.94

(Each value is the average of three replicates expressed on a dry weight basis and the figures in parentheses are the standard deviations.)

The proximate and ultimate composition of soybean seeds was found to contain 10.5% moisture, 42,02% protein, 27.46% fat, 5.36% ash, 6.78% fiber and 18.38% carbohydrate. Similarly, buffalo liver was found to contain 71.5% moisture, 64.91% protein, 19.29% fat, 4.5% ash and 11.3% carbohydrates on a dry basis in this study. These data were bit different to that of data reported by Etiosa *et al.* (2017) which contained 40.99% of protein, 30.67%

of crude fat, 4.66% of ash, 8.07% of moisture, 5.91% of fiber, 17.77% of carbohydrates and by Heuze V. (2020) which contained 39.56% of protein, 21.97% of fat, 9% water, 5.49% ash. This may be due to the variation of species, variety of the Soybeans. Similarly, the proximate and ultimate composition of buffalo (Bubalus bubalis) liver was found to be similar to that of data reported by S. Devatkal *et al.* (2004a) which contained 71.92% moisture, 65.66% protein, 19.94% fat, 4.7% total ash and 9.7% carbohydrates. All the results were converted into a % dry basis for the comparison.

Table 4.2 The proximate analysis of dried soybean protein powder and dried liver (% dry basis)

Parameters	Soy protein Powder	Dried liver powder
Moisture (%)	5.5±0.1	6±0.17
Crude protein (%)	70.73±0.08	74.46±0.45
Fat (%)	24.35±0.11	9.5±0.34
Crude fiber (%)	1.2±0.13	-
Ash (%)	1.3±0.06	5±0.17
Carbohydrates (%)	2.42±0.16	11.04±0.64

(Each value is the average of three replicates expressed on a dry weight basis and the figures in parentheses are the standard deviations).

The proximate and ultimate composition of soybean protein powder in this study was found to contain 5.5% moisture, 70.73% protein, 24.35% fat, 1.3% ash content, 1.2% crude fiber and 2.42% carbohydrates on a dry basis. Similarly, the liver powder was found to contain 6% moisture, 74.46% crude protein, 9.5% crude fat, 5% ash and 11.04% of carbohydrates on a dry basis. These results were found to be similar to the data reported by A.Deak *et al.* (2008) in soy protein concentrates made by three extraction processes where all proximate parameters including protein, moisture, fat, crude fiber, ash and carbohydrates were found to be 71%, 6%, 0.3%, 3.5%, 5.6% and 17.6% respectively by alcohol washing process.

Likewise,70% of protein, 6% of moisture, 0.3% of fat, 3.4% of crude fat, 4.8% of ash and 19.5% of carbohydrate by the acid washing process. Similarly, 72%, 5%, 0.1%, 3.8%, 3% and 20.1 % respectively by hot water washing process. Fat content was found to be very high but carbohydrates were found to be very low than the data obtained by A.Deak *et al.* (2008). This may be due to the use of defatted soybeans in their study.

Comparing results of Table 4.1 (Proximate of raw soybean and liver) and Table 4.2 (Proximate of soy protein concentrate and buffalo liver powder)

A significant difference ($p<0.05$) was observed between all the proximate parameters of raw soybean and dried soy protein concentrate. Similarly, a significant difference ($p<0.05$) was observed between all the proximate parameters of raw buffalo liver and dried liver powder but there was no significance difference ($p<0.05$) on the content of carbohydrate.

4.2 Sensory evaluation of different formulations of protein concentrate

The prepared five formulations were selected as done by Kalenik *et al.* (2017). Five formulations i.e. A, B, C, D and E as per the design of the expert were cooked as soup. Contents of ingredients of soup were formulated as given by Kalenik *et al.* (2017) using food concentrate(different proportion) with constant weight of tomato paste, onion paste, ginger and garlic paste in the ratio of 2:2:1. Five soup samples were provided to 10 panelists i.e. teachers and students of the Central Campus of Technology. The panelists evaluated various parameters of the product namely appearance, aroma, flavor, mouthfeel and overall acceptability. The panelists were requested to provide scores in the score sheets as per their perception. Data were analyzed statistically and the best product was found. The average sensory scale for 5 sensory parameters of different samples is represented in Table 4.3. Highlighted portion is sensory scale for sample B which was found to be best compared to rest of samples after sensory evaluation.

Table 4.3 The average sensory scale for 5 sensory parameters of different samples

Sample	Mean score				
	Appearance	Aroma	Flavor	Mouthfeel	Overall Acceptance
A	7.2±0.78 [ab]	7.2±1.03 [a]	6.8±0.94 [ab]	7.15±1.10 [ab]	7.1±0.69 [b]
B	**7.7±0.67 [a]**	**7.7±1.35 [a]**	**7.5±0.81 [a]**	**7.7±0.67 [a]**	**7.9±0.56 [a]**
C	7±0.66 [ab]	6.7±0.82 [a]	6.7±0.63 [ab]	6.6±1.17 [b]	6.8±0.63 [bc]
D	6.8±0.78 [ab]	6.3±0.67 [a]	6.3±0.48 [b]	6.5±0.51 [b]	6.3±0.67 [c]
E	6.6±0.96 [b]	6.3±0.67 [b]	6.3±0.67 [b]	6.4±0.52 [b]	6.3±0.42 [c]

4.2.1 Appearance

The average sensory scale for appearance was 7.2, 7.7, 7, 6.8 and 6.6 for A, B, C, D and E respectively. The analysis of variance showed that in terms of appearance there was no significant difference ($p>0.05$) between the formulations A, B, C and D. Samples B and E were found to be significantly different. However, sample E was not significantly different from A, C and D. In terms of appearance sample B was found to score higher (7.7) among the samples. In comparison to the other formulations, the formulations with higher buffalo liver powder content had an appealing color. The color of all formulation soup was nearly the same because of the presence of other added ingredients. Among all formulation samples, B had the best appealing color. The color is a very important parameter for selecting any food, as man eats with his eyes.

4.2.2 Aroma

The average sensory scale for aroma was 7.2, 7.7, 6.7, 6.3 and 6.3 for A, B, C, D and E respectively. The analysis of variance showed that there was no significant difference ($p>0.05$) between all the formulations i.e. A, B, C, D and E in terms of aroma. This may be

due to the strong aroma of buffalo liver in all formulations. Sample B was found to score higher (7.7) among samples.

4.2.3 Flavor

The average sensory score for flavor was 6.8, 7.5, 6.7, 6.3 and 6.3 for samples A, B, C, D and E respectively. The analysis of variance showed that there was no significant difference ($p>0.05$) between the formulations A, C, D and E. Sample B was not significantly different with sample A and sample C but was significantly different with sample D and E. Sample B was found to score higher (7.5) among samples.

In comparison to the other formulations, the formulation having a higher amount of buffalo liver powder had an acceptable flavor. This may be due to the pleasant flavor of buffalo liver.

4.2.4 Mouthfeel

The average sensory scale for the mouthfeel of five samples A, B, C, D and E were found to be 7.15, 7.7, 6.6, 6.5 and 6.4 respectively. The mouthfeel of sample B was reported to be higher by the sensory panelist compared to the other 4 samples. The statistical analysis at a 5% level of significance showed that samples A, D, C and E were not significantly different to each other in terms of mouthfeel. But sample B was significantly different from C, D and E. Similarly, there was no significant difference between sample B and sample A.

In this study, formulation B had the best acceptable mouthfeel in comparison to the other formulation. This may be because of the smooth texture of concentrate with high fine liver powder and less soybean protein granules. The other formulations had a higher amount of soybean protein granules which made the texture of soup rough with an unpleasant mouthfeel. The mouthfeel of formulation B was pleasing and acceptable to the sensory panelist.

4.2.5 Overall acceptance

The overall acceptability of the protein concentrate can be seen in Table 4.3. The mean sensory score value for the overall acceptability of samples A, B, C, D and E were found to be 7.1, 7.9, 6.8, 6.3 and 6.3 respectively. Statistical analysis showed that the mean score

value for overall acceptability of samples A and C were not significantly different from each other but sample A was significantly different from samples B, D and E. Similarly, samples C, D and E were not significantly different from each other. Sample B had the highest overall acceptability mean score (7.9) This may be due to the better eye-appealing appearance, better aroma, flavor and mouthfeel of the formulation.

4.3 Chemometric analysis

Principal Component Analysis (PCA) was conducted to select the best variety of protein concentrate among five samples. The first principal component was responsible for 50.6% of the variation while the second principal component was reported for 23.8% of the variation. So, together, they accounted for about 75% of the total variation as shown in **Fig 4.1**

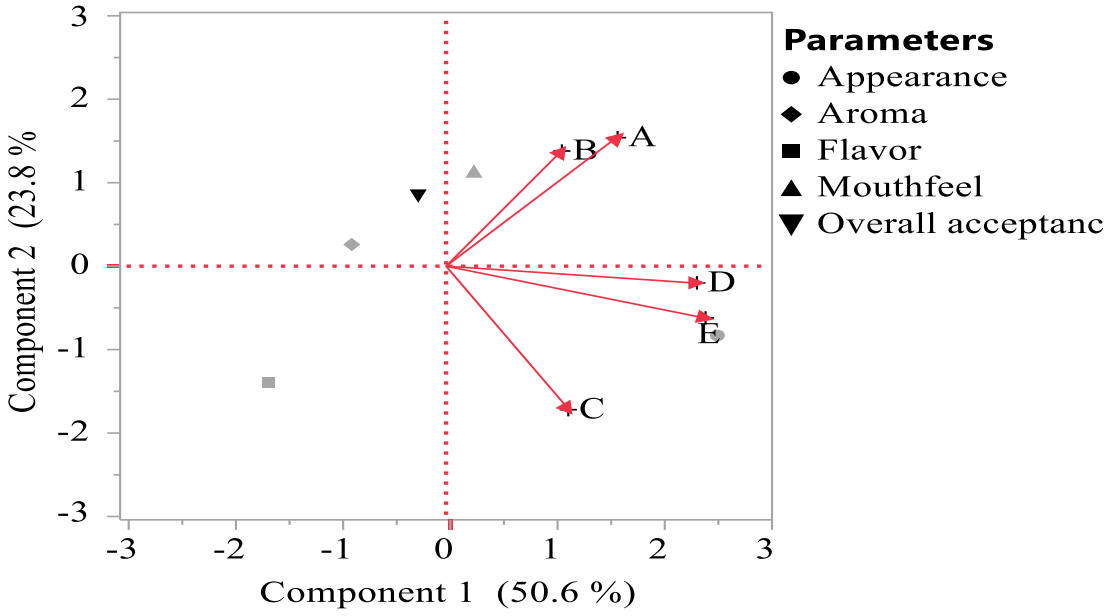

Fig. 4.1 Bi-plot distribution of component 1 and component 2 for selecting the best variety of protein concentrate.

In terms of appearance sample, E was superior of all followed by D and C. Likewise in terms of mouthfeel sample B is superior of all followed by sample A. Similarly, in terms of flavour sample B was superior of all followed by sample A and in terms of aroma sample C was superior followed by sample E. Sample D was similar to E with a correlation of 0.9661 in overall parameters. Hence in terms of overall acceptance sample B was superior followed by sample A.

4.4 Dendogram analysis of different sensory samples

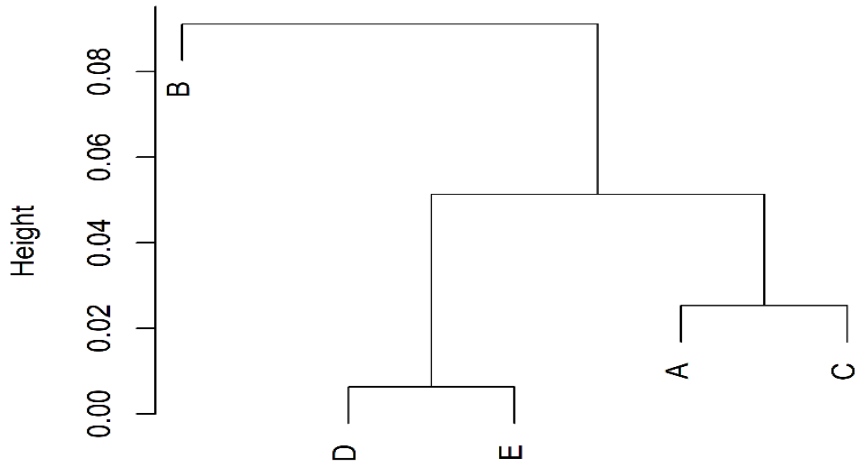

Fig. 4.2 Cluster dendogram of different sensory samples

Cluster dendogram showed that groups A and C were similar to each other and better than D and E which were similar to each other but significantly worse than the rest of the samples. Sample B with 20 parts of soy protein powder and 80 parts of Buffalo liver powder had maximum height which showed it was entirely different from rest of clusters. Hence, sample B was better, different and superior to the rest of the samples.

4.5 Choosing of best sample

The consistency and overall acceptance were highest in the sample containing 20 parts of soybean protein granules and 80 parts of buffalo liver powder i.e sample B according to Principal Component Analysis (PCA) and Cluster Dendogram. This may be due to the better eye-appealing appearance, better aroma, flavor and mouthfeel of the formulation.

4.6 Analysis of the best product

Analysis of sample B which contained 20 parts of soybean protein powder and 80 parts of buffalo liver powder (20:80) was carried out which was selected as best from the sensory analysis.

Table 4.4 Proximate Analysis of the final product (% dry basis)

Parameters	Amount
Moisture (%)	5.5±0.26
Crude protein (%)	72.5±0.79
Fat (%)	13.5±0.1
Crude fiber (%)	2.5±0.2
Ash (%)	5.3±0.1
Carbohydrates (%)	6.2±1.13

(Each value is the average of three replicates expressed on a dry weight basis and the figures in parentheses are the standard deviations.)

Evaluation of the final product revealed that it contained 5.5% of moisture, 72.5% of protein, 13.5% of fat, 2.5 % of fiber, 5.3% of ash and 6.2% of carbohydrates. Similar nutritional value was reported by Kalenik *et al.* (2017) where analysis of protein-rich concentrate from soybean and beef liver (30:70) showed moisture 9.8%, protein 71.39%, fat 9.64%, carbohydrates 6.34%, fiber 4.32%, ash 8.31%. Slight differences may be seen because of the difference in proportions of the two main ingredients in the two studies. All the results were converted into a % dry basis for the comparison.

4.7 Determination of amino acids in the best product

The best product i.e sample B which was selected from the sensory analysis was then analyzed for the presence of essential amino acid. Thus, supposing the essential amino acids present in the sample as Q, R, S, T, U, V, W, X and Y. RF values of these amino acids were calculated as given in table 4.5 concerning known essential amino acids.

Table 4.5 Calculation of RF value of the known and unknown amino acids of best product

Amino acids	Solvent distance(cm)	Spot distance(cm)		RF value	
		Known essential AA	Unknown essential AA	Known essential AA	Unknown essential AA
Histidine	12	1.4	1.5	0.11	0.12
Isoleucine	12	9	8.8	0.72	0.73
Methionine	12	6.9	6.8	0.55	0.56
Threonine	12	4.2	4.1	0.35	0.34
Lysine	12	1.8	1.7	0.14	0.14
Phenylalanine	12	8.5	8.5	0.68	0.7
Valine	12	7.8	7.4	0.62	0.61
Tryptophan	12	8.3	8.3	0.66	0.66
Leucine	12	9.1	9.1	0.73	0.73

Thus, comparing the RF values of the unknown amino acids with that of known amino acids, we found that the RF values of the unknown amino acids were similar to that of the known amino acids. From table 4.5 the RF values of unknown amino acid Q was the same as that of histidine, R was the same as isoleucine, S was the same as methionine, T was the same as arginine, U was the same as lysine, V was the same as phenylalanine, W was same as valine, X was same as tryptophan and Y was same as leucine.

Hence, we can conclude that all the known essential amino acids were present in the best sample i.e. sample B and were a product with complete protein. A similar result was reported by Kalenik *et al.* (2017) where all essential amino acids were quantitatively analyzed in protein concentrate prepared from soybean and beef liver.

4.8 Storage stability of the best product

Eight samples for each storage environment; room temperature (25±3°C) and refrigeration temperature (5±1°C) were prepared and stored for 50 days. Two different samples were taken and coded as A and B where A was for protein concentrate in PE at room temperature, B was for protein concentrate in PE at refrigeration. All of the bags in the study remained sealed until their analysis times when they were opened, sampled, and discarded, so the outside atmosphere did not come in contact with the material during storage except by penetration through the liner (Tunick *et al.*, 2016). Samples were drawn at intervals of 7 days and evaluated for chemical properties (moisture, peroxide value, pH) and microbiological qualities (TPC and coliform count) (Ukuku *et al.*, 2017).

4.8.1 Microbial analysis

In microbiological analysis, TPC and coliform count were performed and the changes in Total Plate Counts (TPC) during storage was observed. The trend of change in Total Plate Count (TPC) is presented in Fig. 4.3

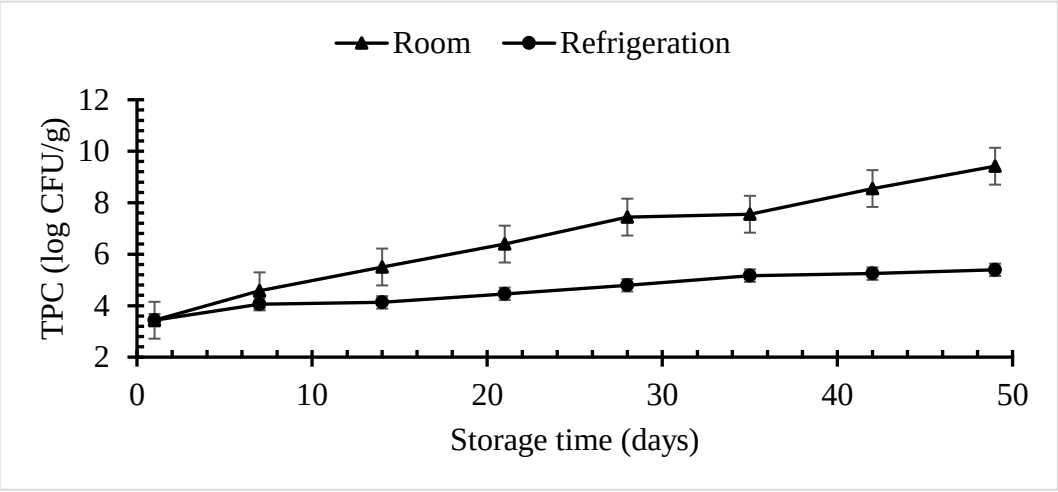

Fig. 4.3 Effect of storage time and temperature on TPC of best protein concentrate

*Values are the mean of three determinations.

During microbial analysis, the freshly prepared sample was free from the coliform count on day zero. During storage increase in Total Plate Count (TPC) took place at higher rate at room temperature which ranged from 5×10^2 to 3×10^9 CFU\g as compared to the sample stored at refrigeration temperature which seemed to increase at slow rate ranging from 5×10^2 to 3×10^6 CFU\g during 49 days of storage period. A significant difference ($p<0.05$) was observed between two samples stored at refrigeration temperature and room temperature on the 21st day onwards. Total plate count was beyond the acceptable limits stored at room temperature after 28 days ($>10^7$ CFU/g). The threshold of microbial spoilage is $>10^7$ CFU/g (S. K. Devatkal *et al.*, 2014).

4.8.2 Chemical changes during storage of protein concentrate

Two samples were taken which were stored at room temperature and refrigeration temperature. They were coded as A and B respectively. Chemical characteristics of protein concentrate were analyzed in terms of moisture, peroxide value and pH at 7 days intervals and the result is shown in Fig. 4.4 to 4.6

4.8.2.1 Changes in moisture during storage

Microorganisms need water in an available form to grow in food products but respond differently to water activity depending on a number of factors. For example, microbial growth, and in some cases, the production of microbial metabolites may be particularly sensitive to alterations in water activity (Jay *et al.*, 2008). During storage period changes in moisture content of best product was observed. The trend of change in moisture content is presented in Fig. 4.4

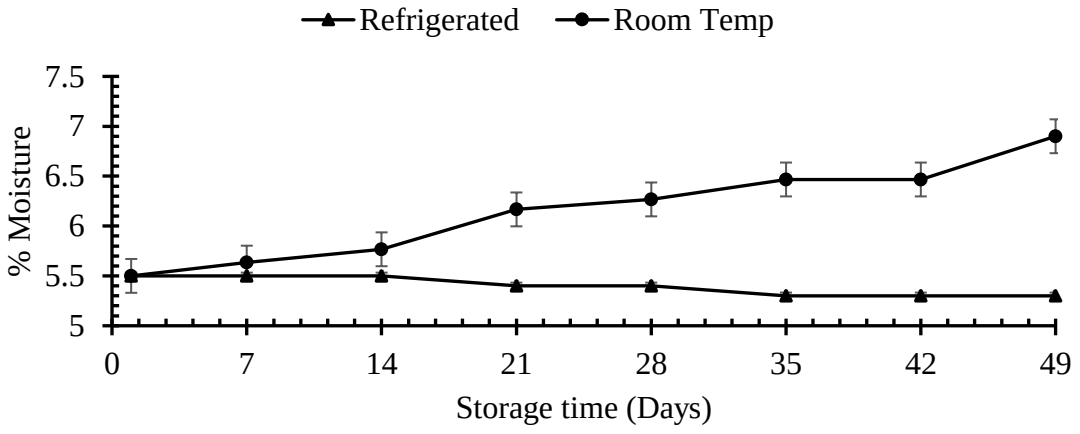

Fig.4.4 Effect of storage time and temperature on the moisture of best protein concentrate

During storage moisture increased significantly ranging from 5.5% to 6.8% at room temperature but decreased slightly at refrigeration temperature ranging from 5.5% to 5.3 % for 49 days. A significant difference ($p<0.05$) was observed between two samples stored at refrigeration temperature and room temperature from the 7th day onwards during total storage period. A slight increase in moisture of protein concentrate(A) stored at room temperature may be due to the result of microbial activities inside the packaging material that catalyzed the release of organic acid. The packaging material (PE) which had moderate moisture barrier properties may also cause the fluctuation of the moisture content (Zubair *et al.*, 2019). A similar result was reported by Ukuku *et al.* (2017) where a_w of Whey Protein Concentrate was found to be increased with an increase in total plate count in High-Performance bags (HP) and Standard bags during storage at 25°C at 70% Relative Humidity (RH) for 18 months. A decrease in moisture content at refrigeration temperature may be due to dehydration that took place inside the refrigerator.

4.7.2.2 Changes in peroxide value during storage

Peroxide value is the degree to which oils and fats have been initially oxidized, which is affected by both applications of antioxidants and time of storage(Pokorny *et al.*, 2001). Peroxide value measures the content of hydroperoxides and is often used as an indicator of the primary products of lipid oxidation (Mariod *et al.*, 2017). The trend of change of peroxide value is presented in Fig. 4.5.

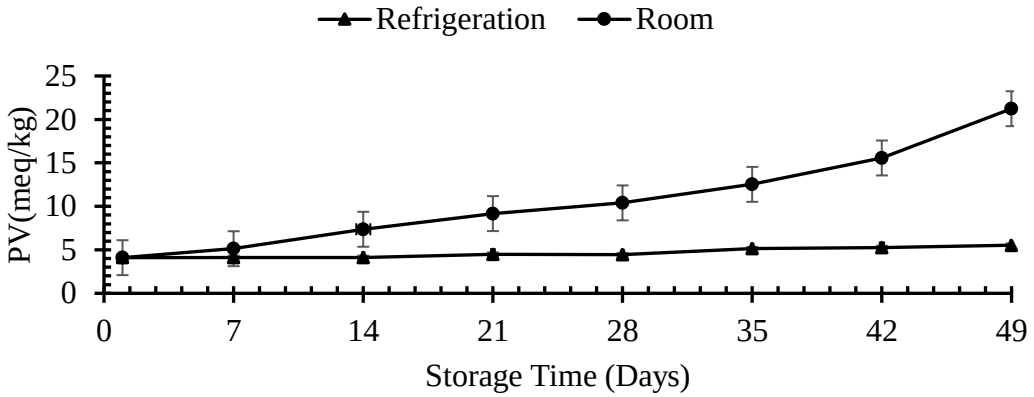

Fig. 4.5 Effect of storage time and temperature on peroxide value of best protein concentrate

*Values are the mean of three determinations.

Change in peroxide value of protein concentrate during 49 days of storage at two different storage condition was observed. The PVs of the sample stored at room temperature increased significantly at higher rate ranging from 4 meq/Kg of sample to 21 meq/Kg compared to refrigeration temperature ranging from 4 to 5.5 meq/Kg. There was only a slight change in PV in the sample stored at refrigeration temperature. There was a significant difference between products stored at two different conditions after 7^{th} day onwards. The PV was beyond the acceptable limits stored at room temperature after 21 days of storage time i.e. >10 meq/Kg fat (Esfarjani *et al.*, 2019). Similar results had been reported by Liu *et al.* (2019) where the PVs of walnuts were 15 and 32 meq/kg sample after storage for 12 months at 4°C and 20°C, respectively and also Lipid oxidation was affected greatly by storage temperature, with a maximum level of free radicals being detected after 47 days at 45 °C, and with the highest level in low-heat powder, irrespective of water activity in the report reported by Stapelfeldt *et al.* (1997).

4.7.2.3 Changes in pH during storage

The pH scale is used to measure the acidity or alkalinity of a sample and describes how many hydrogen ions or hydroxides are present in the sample. The change of pH will lead to the ionization of amino acids atoms and molecules, change the shape and structure of proteins, thus damaging the function of proteins (F. Guo *et al.*, 2020). A microorganism that grows optimally at pH less than 5.55 is called acidophiles(F. Guo *et al.*, 2020). Bacteria in cold environments stop their relentless growth, limiting the number of food-borne illness-causing bacterial cells and preventing other ravenous bacteria from eating our food before we do (Wiebe *et al.*, 1992). A change in pH value of protein concentrate during 49 days of storage at two different storage conditions was observed. The trend of change in pH is presented in Fig. 4.6

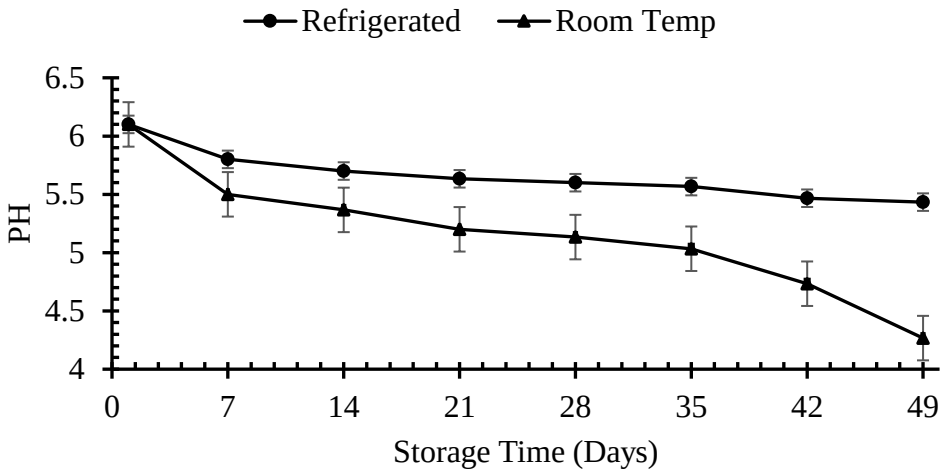

Fig. 4.6 Effect of storage time and temperature on pH value of best protein concentrate

During storage pH was decreasing gradually at room temperature ranging from 6.1 to 4.8 whereas at refrigeration temperature pH value seemed to decrease very slowly ranging from 6.1 to 5.6 as compared to room temperature. A significant difference ($p<0.05$) was observed between two samples stored at refrigeration temperature and room temperature from 21st day onwards during total storage period. This may be due to microbial growth with an increase in moisture content. A similar result was reported by Ukuku *et al.* (2017) where pH value of Whey Protein Concentrate decreased with an increase in the water activity and total plate count.

Part V

Conclusions and recommendation

5.1 Conclusions

Based on the results and discussion of the study, the following conclusions were drawn:

1. Among five protein concentrate formulations; formulation with 80 parts of buffalo liver powder and 20 parts of soybean protein powder were found to be superior from sensory evaluation.
2. The final product was found to contain all essential amino acids with 72.5 % of protein content.
3. The moisture, PV and microbial load increased while pH decreased during storage.
4. The changes in different parameter during storage was slower at refrigeration temperature as compared to at room temperature. The best product was beyond the acceptable limits stored at room temperature after 21 days of storage time.
5. Protein concentrate stored at the refrigeration temperature (5±1°C) retained the desired quality attributes better than protein concentrate stored at room temperature(22-27°C) throughout the 49 days of storage.

5.2 Recommendations

The experiment can be further continued with the following recommendations.

1. Different food industries can incorporate this protein concentrate (20:80 ratio of soyabean and buffalo liver powders) with various nutritious food stuffs in order to replace expensive protein powders in future.
2. Study on the quantitative analysis of essential amino acids in protein concentrate prepared from buffalo liver and soybeans.
3. A study can be carried out on the instantiation of fortified protein concentrate.

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Part VI

Summary

Protein concentrate is very popular among health-conscious people. There are numerous types of protein concentrate made from a wide variety of sources. Protein concentrate made from germinated soybean and buffalo life is not introduced in Nepal. Though it has several uses, it is not being utilized commercially. The blending of soy protein powder and buffalo liver powder improved nutritional value as well as appealing value than every single ingredient. In this study, soybean and buffalo liver was taken from the local market of Dharan. And other essential materials, chemicals and apparatus were taken from the Central Campus of Technology laboratory respectively. First soybeans and buffalo liver were subjected to preliminary operations for powder production separately. The chemical composition (DB) of raw soybean was found to be 10.5% moisture, 42.02% protein, 27.46% fat, 5.36% ash, 6.78% fiber and 18.38% carbohydrate. similarly buffalo liver was 71.5% moisture, 64.91% protein, 19.29% fat, 4.5% ash and 11.3% carbohydrates on a dry basis respectively.

Preparation of soy protein powder was done from germinated soybeans. Soybeans were germinated, dehulled, ground to make a slurry, heated to a temperature(95ºC-100ºC), filtered to get soy milk, milk was coagulated using 3% $CaSO_4$, precipitated curd was separated from the whey, pressed a little through muslin cloth, dried and ground to fine powder simultaneously. The chemical composition of soy protein concentrate was found to be 5.5% moisture, 70.73% protein, 24.35% fat, 1.3% ash content, 1.2% crude fiber and 2.42% carbohydrates on a dry basis. Similarly, the liver powder was prepared by washing, boiling, draining, mincing, drying and finally ground into fine powder simultaneously. The chemical composition of buffalo liver powder was found to be 6% moisture, 74.46% crude protein, 9.5% crude fat, 5% ash and 11.04% of carbohydrates on a dry basis. Five samples of protein concentrate were made by varying proportions of soy protein powder (SPC) and buffalo liver powder (BLP) coded as A (10:90 parts), B (20:80 parts), C (30:70 parts), D (40:60 parts) and E (50:50 parts) using Experiment Design Expert software respectively. These samples were subjected to sensory analysis. The statistical analysis and LSD showed that sample B with 20 parts of soy protein powder and 80parts of buffalo liver powder was found to be superior to other samples. Evaluation of the final product revealed that it contained 5.5% of

moisture, 72.5% of Protein, 13.5% of fat, 2.5 % of fiber, 5.3% of ash and 6.2% of carbohydrates with all essential amino acids. The moisture, PV, pH, Total Plate Count (TPC) of freshly prepared concentrate were found to be 5.5%, 4 meq/kg sample, 6.1 and 5×10^2 CFU/g respectively but coliform was found to be absent. The best-formulated product was filled in a pre-sterilized PE plastic pouch and stored for 49 days in two different conditions. Sample coded as A was stored at refrigeration temperature(5±1°C) and sample coded as B was stored at room temperature (25±3°C). The sample was analyzed weekly for changes in moisture, peroxide value, pH and total plate count. Peroxide value and total plate count were found to be increased at higher rate at room temperature as compared to refrigeration temperature whereas in contrast pH value was found to be in decreasing order. Protein concentrate stored at the refrigeration temperature (5±1°C) was found to be significantly better in quality attributes than protein concentrate stored at room temperature (25±3°C).

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Appendices

Appendix A

SENSORY EVALUATION SCORECARD FOR PROTEIN CONCENTRATE

Name of the panelist: ……

Date: ……

Product: Protein concentrate (buffalo liver and soybean)

Observe the product by tasting. Use appropriate scale to show your attitude by checking at the point best described your feeling of products. Write any of defects present described below. An honest expression of your personnel feeling will help me;

Sample Code	Parameters				
	appearance	aroma	Flavor	Mouthfeel/	Overall
A					
B					
C					
D					
E					

Judge the characteristics on the 1-9 scale as below:

Dislike extremely – 1 Dislike very much – 2 Dislike moderately – 3

Dislike slightly – 4 Neither like nor dislike – 5 Like slightly – 6

Like moderately – 7 Like very much – 8 Like extremely – 9

Any comments: Signature:

Appendix B

- ❖ Independent t-test to compare the results of proximate between raw soybean and buffalo liver with soy protein concentrate and dried buffalo liver.

A. Between Soybean and Soy-powder

1. Moisture

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	37.500000	37.5000	288.4615	<.0001*
Error	4	0.520000	0.1300		
C. Total	5	38.020000			

2. Crude Protein

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	1236.3962	1236.40	5646.934	<.0001*
Error	4	0.8758	0.22		
C. Total	5	1237.2720			

3. Crude fat:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	14.508150	14.5081	36.9399	0.0037*
Error	4	1.571000	0.3927		
C. Total	5	16.079150			

4. Crude Fiber

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	46.704600	46.7046	1624.508	<.0001*
Error	4	0.115000	0.0287		
C. Total	5	46.819600			

5. Ash

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	24.725400	24.7254	301.7132	<.0001*
Error	4	0.327800	0.0820		
C. Total	5	25.053200			

6. Carbohydrate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	382.08240	382.082	12547.86	<.0001*
Error	4	0.12180	0.030		
C. Total	5	382.20420			

B. Between Buffalo Liver and Dried buffalo Liver:

1. Moisture

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	6435.3750	6435.38	31476.52	<.0001*
Error	4	0.8178	0.20		
C. Total	5	6436.1928			

2. Crude Protein

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	136.80375	136.804	795.1395	<.0001*
Error	4	0.68820	0.172		
C. Total	5	137.49195			

3. Crude fat:

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	143.76615	143.766	1174.080	<.0001*
Error	4	0.48980	0.122		
C. Total	5	144.25595			

4. Ash

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	0.37500000	0.375000	10.7143	0.0307*
Error	4	0.14000000	0.035000		
C. Total	5	0.51500000			

5. Carbohydrate

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	1	0.1872667	0.187267	0.3116	0.6065
Error	4	2.4042667	0.601067		
C. Total	5	2.5915333			

Appendix C

ANOVA result for sensory analysis of protein concentrate

Table B.1 Analysis of Variance for the appearance of protein concentrate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	4	7.120000	1.78000	2.8917	0.0326*
Error	45	27.700000	0.61556		
C. Total	49	34.820000			

Connecting Letters Report

Level		Mean
B	a	7.7000000
A	a b	7.2000000
C	a b	7.0000000
D	a b	6.8000000
E	b	6.6000000

Levels not connected by same letter are significantly different.

Table B.2 Analysis of Variance for aroma of protein concentrate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	4	11.600000	2.90000	3.2302	0.0206*
Error	45	40.400000	0.89778		
C. Total	49	52.000000			

Connecting Letters Report

Level		Mean
B	a	7.5000000
A	a	7.2000000
C	a	6.7000000
D	a	6.3000000
E	a	6.3000000

Levels not connected by the same letter are significantly different.

Table B.3 Analysis of Variance for the flavour of protein concentrate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	4	9.680000	2.42000	4.5565	0.0036*
Error	45	23.900000	0.53111		
C. Total	49	33.580000			

Connecting Letters Report

Level			Mean
B	a		7.5000000
C	a	b	6.8000000
A	a	b	6.7000000
D		b	6.3000000

Level		Mean
E	b	6.3000000

Levels not connected by same letter are significantly different.

Table B.4 Analysis of Variance for the mouthfeel of protein concentrate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	4	11.980000	2.99500	4.1565	0.0060*
Error	45	32.425000	0.72056		
C. Total	49	44.405000			

Connecting Letters Report

Level			Mean
B	a		7.7000000
A	a	b	7.1500000
D		b	6.6000000
C		b	6.5000000
E		b	6.4000000

Levels not connected by the same letter are significantly different.

Table B.5 Analysis of Variance for the overall acceptance of protein concentrate

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Samples	4	17.680000	4.42000	11.9819	<.0001*

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Error	45	16.600000	0.36889		
C. Total	49	34.280000			

Connecting Letters Report

Level		Mean
B	a	7.9000000
A	b	7.1000000
C	b c	6.8000000
D	c	6.3000000
E	c	6.3000000

Levels not connected by same letter are significantly different.

Table B.6 Principal Component analysis

Correlations

Samples	**A**	**B**	**C**	**D**	**E**
A	1.0000	0.3825	-0.0718	0.4582	0.4272
B	0.3825	1.0000	0.0987	0.2077	0.1833
C	-0.0718	0.0987	1.0000	0.2511	0.4855
D	0.4582	0.2077	0.2511	1.0000	0.9661
E	0.4272	0.1833	0.4855	0.9661	1.0000

Appendix D

Storage stability analysis

- **TPC change:**

1. One- way analysis of Day 1:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-1.5125	-1.5125
Room Temp	-1.5125	-1.5125

Positive values show pairs of means that are significantly different.

2. One- way analysis of Day 7:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.3724	-1.1867
Refrigerated	-1.1867	-1.3724

Positive values show pairs of means that are significantly different.

3. **One- way analysis of Day 14:**

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.3381	-0.2965
Refrigerated	-0.2965	-1.3381

Positive values show pairs of means that are significantly different.

4. **One- way analysis of Day 21:**

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.3698	0.5642
Refrigerated	0.5642	-1.3698

Positive values show pairs of means that are significantly different.

5. One- way analysis of Day 28:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.2806	1.5827
Refrigerated	1.5827	-1.2806

Positive values show pairs of means that are significantly different.

6. One- way analysis of Day 35:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.2322	0.8585
Refrigerated	0.8585	-1.2322

Positive values show pairs of means that are significantly different.

7. **One- way analysis of Day 42:**

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.2398	1.8092
Refrigerated	1.8092	-1.2398

Positive values show pairs of means that are significantly different.

8. One- way analysis of Day 49:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-1.4384	1.5883
Refrigerated	1.5883	-1.4384

Positive values show pairs of means that are significantly different.

- **Moisture change:**

1. One- way analysis of Day 1:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	0	0
Room Temp	0	0

Positive values show pairs of means that are significantly different.

2. One- way analysis of Day 7:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.09255	0.04079
Refrigerated	0.04079	-0.09255

Positive values show pairs of means that are significantly different.

3. One- way analysis of Day 14:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.09255	0.17412
Refrigerated	0.17412	-0.09255

Positive values show pairs of means that are significantly different.

4. One- way analysis of Day 21:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	Room Temp	Refrigerated
Room Temp	-0.09255	0.67412
Refrigerated	0.67412	-0.09255

Positive values show pairs of means that are significantly different.

5. One- way analysis of Day 28:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	Room Temp	Refrigerated
Room Temp	-0.09255	0.77412
Refrigerated	0.77412	-0.09255

Positive values show pairs of means that are significantly different.

6. One- way analysis of Day 35:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.0925	1.0741
Refrigerated	1.0741	-0.0925

Positive values show pairs of means that are significantly different.

7. One- way analysis of Day 42:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.0000	1.2000
Refrigerated	1.2000	-0.0000

Positive values show pairs of means that are significantly different.

8. One- way analysis of Day 49:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.1603	1.2397
Refrigerated	1.2397	-0.1603

Positive values show pairs of means that are significantly different.

- **PV change:**

1. One- way analysis of Day 1:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.22670	-0.22670
Refrigerated	-0.22670	-0.22670

Positive values show pairs of means that are significantly different.

2. One- way analysis of Day 7:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.20694	0.79306
Refrigerated	0.79306	-0.20694

Positive values show pairs of means that are significantly different.

3. One- way analysis of Day 14:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.6139	2.6194
Refrigerated	2.6194	-0.6139

Positive values show pairs of means that are significantly different.

4. One- way analysis of Day 21:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.2449	4.4218
Refrigerated	4.4218	-0.2449

Positive values show pairs of means that are significantly different.

5. One- way analysis of Day 28:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.1851	5.7816
Refrigerated	5.7816	-0.1851

Positive values show pairs of means that are significantly different.

6. One- way analysis of Day 35:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.1309	7.2691
Refrigerated	7.2691	-0.1309

Positive values show pairs of means that are significantly different.

7. One- way analysis of Day 42:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.2927	9.9740
Refrigerated	9.9740	-0.2927

Positive values show pairs of means that are significantly different.

8. **One- way analysis of Day 49:**

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Room Temp**	**Refrigerated**
Room Temp	-0.207	15.493
Refrigerated	15.493	-0.207

Positive values show pairs of means that are significantly different.

- **pH change:**

1. One- way analysis of Day 1:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.22670	-0.22670
Room Temp	-0.22670	-0.22670

Positive values show pairs of means that are significantly different.

2. One- way analysis of Day 7:

Comparisons for each pair using Student's t

Confidence Quantile

t	Alpha
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.18510	-0.05176
Room Temp	-0.05176	-0.18510

Positive values show pairs of means that are significantly different.

3. One- way analysis of Day 14:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.26177	-0.06177
Room Temp	-0.06177	-0.26177

Positive values show pairs of means that are significantly different.

4. One- way analysis of Day 21:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.48972	0.07695
Room Temp	0.07695	-0.48972

Positive values show pairs of means that are significantly different.

5. One- way analysis of Day 28:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.79613	-0.59613
Room Temp	-0.59613	-0.79613

Positive values show pairs of means that are significantly different.

6. One- way analysis of Day 35:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.29266	0.07400
Room Temp	0.07400	-0.29266

Positive values show pairs of means that are significantly different.

7. One- way analysis of Day 42:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.76317	-0.26317
Room Temp	-0.26317	-0.76317

Positive values show pairs of means that are significantly different.

8. One- way analysis of Day 49:

Comparisons for each pair using Student's t

Confidence Quantile

t	**Alpha**
2.77645	0.05

LSD Threshold Matrix

Abs(Dif)-LSD	**Refrigerated**	**Room Temp**
Refrigerated	-0.61390	0.38610
Room Temp	0.38610	-0.61390

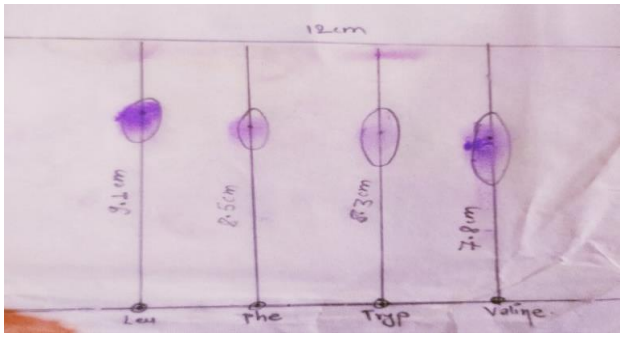
Positive values show pairs of means that are significantly different.

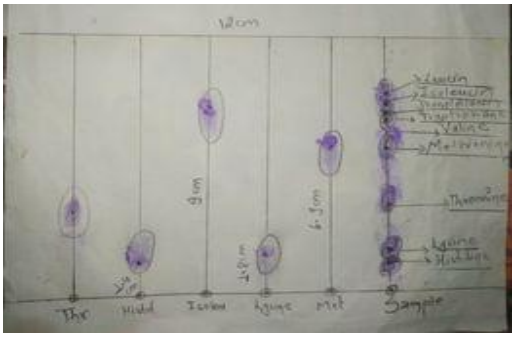
Colour Plates

P1 Germinated Soybeans

P2 final products

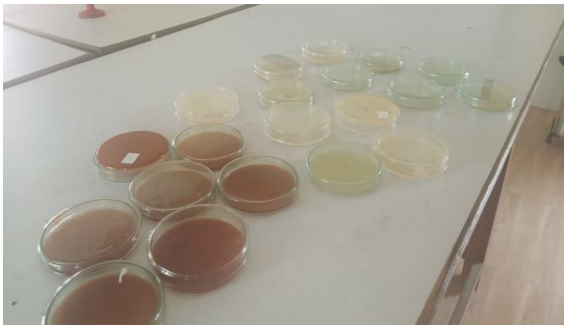
P3. Qualitative analysis of essential amino acids by paper chromatography





P4. Result of paper chromatography

P5. Samples were stored at two different conditions (at refrigeration temperature and room temperature)



P6. results of microbial plating