PREPARATION AND QUALITY EVALUATION OF MUFFIN PREPARED FROM AVOCADO SEED AND SOYBEAN FLOUR

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Approval Letter

This *dissertation* entitled *Preparation and Quality Evaluation of Muffin Prepared from Avocado Seed and Soybean Flour* presented by **Hari Kumar Pokharel** has been accepted as the partial fulfillment of the requirement for the **B. Tech. degree** in **Food Technology**

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Abstract

The purpose of this study was to prepare and evaluate quality of muffin prepared from avocado seed and soybean flour. The study looked into the effects of various substitution amounts on the physicochemical properties and sensory aspects of muffins. Avocado seed flour and soybean flour were used to prepare the muffin batter in varying proportions to replace wheat flour (70-100). The physicochemical study comprised moisture, protein, fat, and ash content measurements, while sensory evaluation included appearance, color, taste, texture, aroma, and overall acceptability. Proximate analysis was performed on soybean flour, wheat flour, and avocado seed flour (de-pitted and homogenized). The avocado seed flour and soybean flour were incorporated at the level of 0 -10 parts and 0-20 parts with 70-100 parts wheat flour. Design Export 13 was used to make the experimental design with 16 number of samples.

The results demonstrated that substituting avocado seed and soybean flour for wheat flour had a substantial impact on the nutritional composition and sensory aspects of the muffins. The moisture content, protein, fat, crude fiber, ash and carbohydrate of wheat flour were found to be 11.21%, 10.28%, 1.14%, 0.36%, 0.47% and 87.45% respectively and 14.51%, 7.74%, 0.71%, 4.93%, 2.81%, 4.18% and 68.9% respectively for avocado seed flour and 10.45%, 35.93%, 19.68%, 4.33%, 5.82% and 34.18% respectively or soybean flour. Increased substitution levels resulted in increased protein content and potassium content, as well as changes in texture and flavor. From the analysis, an effect of avocado seed, soybean and wheat flour on the specific loaf volume of muffins (2.39) incorporated with 3.88 parts avocado seed flour, 0 parts soybean flour and 96.12 parts wheat flour samples were superior in comparison to all other muffin formulations. These findings add to our understanding of the use of alternative flours in composite products and provide insights for making nutritious and appealing baked goods for people on a diet or looking for healthier options.

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Abbreviations	Full form
ANOVA	Analysis of variance
Wb	Wet basis
Db	Dry basis
GI	Gastrointestinal
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
BMI	Body Mass Index
SD	Standard deviations
LSD	Least significant difference

List of Abbreviations

Part I

Introduction

1.1 General introduction

Muffins are a sweet, high-calorie baked delicacy that customers adore for their flavor and soft texture (Martínez-Cervera *et al.*, 2012). Muffins are rapid bread because "quick-acting" chemical leavening agents are employed in place of yeast, which is a "longer-acting" biological leavening agent (M. Araújo *et al.*, 2015; Hui *et al.*, 2007). Muffins' main ingredients are flour, sugar, oil, and egg, all of which play an essential role in the final product's structure, looks, and eating quality (Garcia *et al.*, 2017).

The main component of muffins is flour (Bhaduri, 2013). Flour contains carbohydrates as well as the proteins glutenin and gliadin, which work together to keep other ingredients together and give the completed baked product structure. When starch is heated and hydrated, it gelatinizes, breaking hydrogen bonds and expanding the starch granules, giving the batter a more solid structure (Bena, 2005).

Due to its nutritional importance for human health, avocados (*Persea americana*), a tropical and subtropical fruit native to Mexico and Central America, are becoming more and more well-known throughout the world (R. G. Araújo *et al.*, 2018). The avocado tree is a large, evergreen tree that can reach a height of 20 meters(Majid *et al.*, 2020). Currently, there are more than 500 different varieties of avocados. Each avocado variety has its own shape, weight, size, and flavor, but the difference that stands out the most as it ripens is the color of the bark(Yahia, 2011).

Avocado varieties have been called by their botanical classifications: Mexican (*Persea americana var. drymifolia*), Guatemalan (*Persea nubigena var. guatemalensis*), and West Indian (*Persea americana var. americana*). Avocado is regarded as the world's healthiest fruit due to its high nutrient content, which includes Vitamin K, dietary fiber, potassium, folic acid, Vitamin B6, Vitamin C, and copper at a low-calorie count (Cheeke *et al.*, 2006).

Soybeans are high in nutrients and provide a wide range of health advantages. They supply healthy lipids, including heart-protective polyunsaturated and monounsaturated fats, as well as omega-3 fatty acids, and are a good source of complete protein, having all

required amino acids (Morton, 1984). Furthermore, soybeans are high in dietary fiber, vitamins (such as B vitamins, vitamin K, and folate), important minerals (such as iron, magnesium, and zinc), antioxidants, and isoflavones, all of which have potential health benefits. Soybeans are a nutritious alternative for a variety of diets because they are low in saturated fat and cholesterol-free, but it is vital to address any anti-nutrients through suitable cooking methods (Refstie *et al.*, 1999). Incorporating soy products into a well-balanced diet can have a major impact on overall health and well-being.

Composite flours, which substitute grain or bean flour for some of the wheat, as well as other useful elements such as protein sources are frequently utilized in the creation of baked goods. Composite flours have reportedly been observed to give products functional and therapeutic value in addition to increasing the texture and nutritional qualities of food products (Goldberg, 2012; Sheehy and Morrissey, 1998). Millions of tons of processed plant byproducts are produced annually by the food industry, particularly in the processing of fruits and cereals. Fruit and cereal processing byproducts are extensively treated as a waste of environmental concern and only partially valorized at various levels of value addition. This has a detrimental impact on the overall sustainability of the food-processing business. New technologically feasible methods for converting processing byproducts into high-value foods would prevent their disposal as waste and significantly boost the sustainability and competitiveness of the global food industry. The treatment and disposal of processing byproducts are a serious environmental problem (Lafka *et al.*, 2007).

1.2 Statement of problem

Wheat is a dominating food grain that plays an important part in human diets because it supplies roughly 20% of total food calories and is a national staple in most nations (Anonymous, 2005). Wheat contains phytic acid, which binds minerals such as calcium, iron, magnesium, and others, altering their solubility, functioning, digestion, and absorption, rendering these nutrients unavailable to the body. This has an impact on the skin and digestive tract, as well as the brain and central nervous system, immunological, skeletal, and reproductive systems. Wheat fiber may also cause people to consume too much vitamin D, contributing to insufficiency. Wheat significantly elevates tiny, dense LDL cholesterol, and high levels of LDL (the "bad") cholesterol are linked to an increased risk of heart disease (Doom and Gunnar, 2013).

Due to the low fiber content of wheat flour, various digestive disorders such as constipation and irregular bowel movement develop, leading to weight gain. Obesity has been related to a plethora of health problems (Apovian *et al.*, 2019). Obesity among adults was 42.4% in 2017-2018, with no significant differences between men and women across all individuals or age groups. Obesity rates continue to rise year after year(Ogden *et al.*, 2020). Similarly, celiac disease is caused by wheat protein (gluten) (Hui *et al.*, 2007). Gluten-allergic people should avoid avocado seed flour (Cheeke *et al.*, 2006). Avocado seeds are high in soluble fiber, which is beneficial to overall heart health. It contains potassium and phosphorus, which help to maintain a regular heartbeat and fluid balance in the body, as well as relax the blood vessels and arteries, decreasing high blood pressure (Cheeke *et al.*, 2006). They suffer digestive issues such as diarrhea, gas, and constipation. Other symptoms include bone joint or stomach discomfort, nausea, anemia, bone density loss, weight loss, and heartburn (Nijhawan and Goyal, 2015).

Muffins frequently include additional sugars and harmful fats, which can contribute to weight gain and obesity. Excess calorie intake from muffins, along with a sedentary lifestyle and poor dietary choices, can result in an energy imbalance, in which the calories taken exceed the calories burnt. This energy imbalance can lead to weight gain and an increased risk of obesity-related health problems such as diabetes mellitus(Ludwig *et al.*, 1999).

Avocado seeds are currently underutilized since they are wasted by the majority of countries. Avocado processors have also reported waste. Avocado seeds that are tossed carelessly are exacerbating the problem of hazardous waste, particularly in cities. This waste could be an environmental or human pollutant (Blakeney, 2019).

According to Ashwini (2011), protein-energy malnutrition is a fundamental nutrition problem in developing countries. A lack of appropriate quality food has resulted in several nutritional concerns, including a lack of micronutrients. Avocado seed flour and soya flour can be added as a supplement to improve the nutritional profile of muffins and supply essential amino acids in adequate amounts. Avocado seed flour and soy flour are both high in protein and contain key amino acids like lysine and tryptophan that wheat flour lacks. We can boost the overall protein level and improve the balance of key amino acids in muffin recipes by combining avocado seed flour and soya flour. This can contribute to the creation of a more nutritionally balanced product.

As a result, new ingredients must be discovered in order to address this problem in the baking sector. However, knowledge of the physicochemical properties that influence baking quality and, as a result, consumer acceptance is limited. Hence, developing standards for wheat replacement in bakery items using other grain products is problematic. As a result, locally available raw materials are employed to prepare higher-quality local agricultural food items in a cost-effective manner(Shahzadi, 2004).

Thus, the current work is only focused on the creation and quality evaluation of wheat, avocado seed flour and soya flour cakes, which aid in replacing the missing minerals and proteins found in wheat, namely potassium, and the fiber content found in avocado seed flour and protein found in soya flour. In addition to nutritional benefits, soya-supplemented wheat flour improves the functional features of the end products by increasing moisture retention and decreasing oil absorption.

1.3 Objectives

1.3.1 General objectives

The general objective of this work was the preparation and quality evaluation of muffin prepared from avocado seed and soybean flour.

1.3.2 Specific objectives

- 1. To carry out the chemical analysis of raw material.
- 2. To make muffins, use a variable proportion of avocado seed flour and soybean flour.
- 3. To determine the best formulation muffin through sensory and chemical characteristics of muffin.
- 4. To perform cost evaluation.

1.4 Significance of the study

Avocado seed and soybean flour can boost the nutritional content of muffin recipes. Avocado seeds are high in antioxidants, fiber, and good fats, but soybean flour is heavy in protein and vital amino acids. These additives can increase muffins' overall nutritious profile, providing consumers with a better snacking option. The study addresses the growing demand for allergy-free food by substituting avocado seed and soybean flour for wheat flour, a major allergen. Individuals with wheat allergies or gluten intolerances can now enjoy muffins without compromising their dietary needs. Because avocado seeds are generally discarded, using them in baking helps decrease food waste and supports sustainability. Furthermore, because the study focuses on texture and sensory evaluation, the muffins have a pleasant taste and feel despite the substitutions, making them more enticing to customers. As a result, adding avocado seed and soybean flour has important economic ramifications, as these alternative ingredients can diversify product offerings, broaden markets, and contribute to the creation of a sustainable and lucrative food sector.

It is suggested that food manufacturers support the use of composite flour technology in order to make economic use of locally accessible raw materials and generate nutritious products for consumers. As a result, the production of locally available, nutritious, and underutilized avocado seed will grow. The current work will be an instructive document for food processing practitioners and entrepreneurs because it provides insights into the process parameters of muffin preparation technology. Hence, the manufacture of composite flour muffins in Nepal will benefit not only industries but also farmers and consumers.

1.5 Limitations of the study

• Vitamins were not determined due to a lack of facilities.

Part II

Literature review

2.1 Muffin

Muffins are individual-sized baked delicacies. Muffins are porous cereal-based baked goods with a spongy texture and high volume (Martínez-Cervera *et al.*, 2012). A muffin is a baked item that comes in individual serving sizes. Muffins are sweet baked goods that are popular with customers because of their soft texture and distinct flavor (Ramya and Anitha, 2020). They're similar to cupcakes, only they're usually less sweet and lack icing. Savory muffins, such as cornbread muffins and cheese muffins, are also available. Outside of the United Kingdom, the word refers to an English muffin, a disk-shaped muffin bread. Muffin mace comes in a variety of flavors, including low fat and flavors with a specific ingredient baked into the muffin, such as blueberries, chocolate chips, raspberry, cinnamon, pumpkin, date, nut, lemon, banana, orange, peach, strawberry, almond, and carrot. Muffins are typically eaten for breakfast, although they can also be served with tea or other meals (Limbachiya and Amin, 2015).

A muffin's top should be uniform, nicely shaped, free of peaks, without cracks, and large in comparison to its weight. The surface should be pebbly or slightly rough and polished, with a consistent golden-brown tint on the outside. With an even, round-holed grain, the inner texture should be wet, delicate, and light. Inside, the color will be creamy white or slightly yellow, and streaks will be nonexistent. Fruits, nuts, herbs, cheese, chopped meats, and spices can be added to the batter to form a variety of muffins. Customers demand a crumb that is soft, spongy, and sensitive, with some resistance to disintegration (Öztürk and Mutlu, 2019).

2.1.1 The method of mixing

Muffin compositions are distinguished by a complex blend of interacting components, primarily sugar and different degrees of fat, flour, eggs, and baking powder, which results in the porous structure and huge volume typical with muffins (Baixauli *et al.*, 2008). The muffin method and the standard method are the two main methods for mixing muffins. The muffin method combines two mixtures: a wet mixture (eggs, soft or liquid fat, milk, and sugar) and a dry ingredient (flour, baking powder, and salt) (flour, leavening, and

flavorings such as cocoa powder). They're blended and stirred briefly before being panned and baked after they've been readied and the oven has been preheat (Miller, 1971).

The chemical composition of muffin is seen in Table 2.1.

Constituents	Values
Moisture, %	20.33
Fat, %	14.37
Protein, %	17.64
Carbohydrate, %	44.28
Total dietary fibre, %	2.22
Ash, %	1.21

 Table 2.1 Chemical composition of muffin

Source: Rahman et al. (2015)

2.2 Ingredients and their role in muffin making

2.2.1 Wheat flour

Wheat (*Triticum spp.*) originates in the eastern Mediterranean, Near East, and Middle East. Wheat grows best in cool, dry, temperate climates. Wheat flour (WF) contains all of the anatomical components of the grain, such as endosperm, bran, and germ, in the same proportions as occur in the intact form. Thus, WF includes far more fibre, vitamins, minerals, and phytochemicals than refined flour (RF). As a result, it is regarded as a wonderful source of nutritional and functional elements for human health, with numerous associated benefits, including the prevention of diseases such as diabetes, cardiovascular disease, obesity, and cancer (Liu, 2007).

Regardless of the health benefits, WF may change the structural and sensory qualities of food, resulting in decreased consumer acceptance. As a result, it is difficult to produce WF food with the same functionality and quality as refined grain goods. In addition to the qualitative aspects of the finished product, WF provides for different changes in dough properties and processing parameters. The particle size of WF has a considerable impact on the quality and utility of the flour (Kihlberg *et al.*, 2004).

Moder *et al.* (1984) reported that lowering coarse bran particle size improved bread volume marginally. Noort *et al.* (2010) reported when the size of the bran particles was reduced, it had a greater negative influence on baking quality. Cai also discovered that when wheat flour bread with smaller particle size bran is stored, the bread volume decreases and the degree of starch retro gradation increases (Cai *et al.*, 2014).

2.2.1.1 Requirements of flour characteristic

The flour should be free flowing, dry to the touch, creamy in appearance, and free of visible bran particles. It should also have a distinct flavor and be free of musty and rotten flavors. The needed flour qualities as shown in table

Characteristics	Requirements
Moisture content	13.0% max
Gluten content in dry basis	7.5% min
Total ash on dry basis	0.5% max
Acid insoluble ash on dry basis	0.05% max
Protein (N×7.5) on dry basis	9%
Alcohol acidity as H ₂ SO ₄ in 90% alcohol	0.1%
Water absorption	5%
Sedimentation	22%
Granularity	To satisfy the taste

Table 2.2 Requirements of flo	our characteristic
-------------------------------	--------------------

Source: Kaur et al. (2015)

2.2.2 Shortening

Shortening contributes to the eating attributes of tenderness, flavor, texture, and a distinct mouth feel. Fat keeps the crumb and crust supple and helps retain moisture, contributing to the keeping qualities or shelf-life. Fat improves the flavor of baked goods because flavor components dissolve in fat (Pérez-Nieto *et al.*, 2010). The principal activity of the fat or shortening during mixing is to keep the gluten-forming protein molecules from coming

into contact with water by insulating the gluten-forming protein molecules due to their hydrophobic nature. As a result, less difficult dough with the optimum amount of gluten formation is possible. Thus, shortened baked goods have a less hard, crisper texture and can readily melt in the tongue. Without the addition of antioxidants, the fat (butter) should have a reasonable shelf life.

However, because to their high fat content, bakery products are frequently avoided and referred to by health-conscious and obese people. The inclusion of functional ingredients to bakery products has grown in popularity due to its capacity to minimize the risk of chronic diseases in addition to basic nutritional function (Eswaran *et al.*, 2013).

2.2.3 Sweeting agents

Sugar is one of the most regularly used components in muffins. Sugar is hygroscopic, which means it attracts and retains water molecules. This property of sugar contributes in the moistening of baked foods (Gray). Sugar is present in almost all baked goods, from chemically leavened sweets to yeasted pastries. It is very significant in baking systems, including:

- i. During fermentation, yeast creates alcohol and CO₂ gas, which is used to leaven dough.
- ii. Sweetener
- iii. Increases shelf life by attracting free water and decreasing water activity (natural preservative).
- iv. Humidify (through its hydroscopic nature)
- v. Tenderizing/creaming agents (aerator in batter systems)
- vi. Texturizer (mouth feel improver)
- vii. Color and taste enhancer (through browning reactions like Millard and caramelization)
- viii. In sponge muffins, foaming agents (together with egg whites)
 - ix. Depression of the freezing point.
 - x. Bulking agents.

Source: Hui et al. (2008)

2.2.4 Leavening agents

When carbon dioxide gas produced by yeast fermentation or chemical leavening processes, or the effect of heat, expands air bubbles that have been integrated into the dough or batter during mixing, baked goods are leavened. The air bubbles expand, causing the batter or dough to expand in size and the cell structure to expand, increasing the final baked product's taste and texture. The three principal leavening gases are air, steam, and carbon dioxide gas. Air is injected during the mixing procedure. Steam is produced as the water in the dough or batter evaporates during baking. The most common carbon dioxide donors in chemical leavening reactions are sodium bicarbonate, potassium bicarbonate, and ammonium bicarbonate. Ammonium bicarbonate decomposes during baking to produce ammonia gas, carbon dioxide gas, and water. These three ingredients are all leaveners (Caballero *et al.*, 2015).

The following are the chemical equations for the reaction of soda and regularly used acidulants:

 $NaHCO_3 + H^+ \longrightarrow Na^+ + CO_2 + H_2O$

One advantage of ammonia bicarbonate is that it does not produce salts, which can interfere with dough rheology.

 $NH_4HCO_3 \longrightarrow NH_3 + CO_2 + H_2O$

Source: (Neeharika et al., 2020)

Practically, the following considerations control the usage of chemical aerating agents:

- i. They should generate an adequate amount of CO₂.
- ii. The leftover salts should not have a negative impact on the quality of the finished goods.
- iii. They should be safe.
- iv. They should not respond too quickly until the products are placed in the oven.

2.2.5 Whole Egg

The egg is one of the most significant ingredients in maintaining the nutritional and physical properties of muffins. Eggs are vital because their proteins interact with the other

ingredients to form the structure of the muffin. The emulsifiers in the yolks also aid in the mixing of components that would normally separate, such as water and oil. When heated during baking, those same proteins cause the muffin to create a gorgeous golden-brown hue. The lipids included in egg yolks enhance the flavor of any muffin (Lin *et al.*, 2017).

The carbon dioxide generated by the baking powder during baking acts as a nucleus, causing the bubbles formed during the mixing process to grow in size. Egg solids, and to a lesser extent egg white, are important foam stabilizers that prevent air bubbles from coalescing (Singh *et al.*, 2017).

2.2.6 Water

Water, a simple chemical ingredient, is equally as important as flour in the cooking and baking process. It serves a number of purposes in baking processes, some of which are unknown, while others are decided by the qualities of the finished product. To be used in baking activities, the water must be drinking independent of its source. The mineral makeup of water can have an effect on dough characteristics. Water can play a role in producing the ideal dough and final product qualities. Three factors of water quality that must be examined are discussed. They are as follows: flavor, chemical composition, and mineral content (Sinani *et al.*, 2014).

Water serves the following purposes:

- i. Provide moisture to the products.
- ii. Mix together all of the dry ingredients.
- iii. Creates the structure of baked goods.
- iv. Control batter and dough temperature and increases storage quality
- v. Controls the consistency of the dough and batter, as well as the size and texture of the muffins.

2.3 Wheat Flour

Triticum vulgare is the botanical name for wheat. Wheat flour for muffins is collected from the endosperm as particles large enough to pass through a flour screen, typically 100 mesh per linear inch (D. Kent-Jones *et al.*, 1958). Wheat flour is unique among cereal flours in that it creates an elastic mass when mixed with the proper proportion of water. This unusual feature is owing to the presence of insoluble proteins known as gluten. Gluten-

forming proteins (glutenin and gliadin) account for approximately 75-80% of total flour proteins (Mukhopadhyay, 1990).

Glutenin gives the product firmness, whereas gliadin is the binding factor that gives the gluten its soft, sticky texture. Gliadin is 70% alcohol soluble and can be isolated from flour, whereas glutenin is soluble in both alcohol and water (Gorinstein *et al.*, 2002). Gluten is an elastic, cohesive, and rubbery substance that keeps the dough's various elements together. It has the ability to contain the gases released during fermentation and baking. It hardens in the oven to provide the firm, porous, open texture required for biscuits and crackers. Thus, gluten serves as the required framework, producing the sustaining wall of the entire baked food structure (Bohn, 1957).

Wheat flour for muffins should be obtained by milling cleansed hard or soft wheat, or a combination of the two. The percentage of protein in the flour is commonly used to describe flour strength. Weak flour is commonly defined as flour with a low protein content. Typically, this protein is assumed to be gluten, which becomes extremely extensible under tension when flour is mixed with water, but does not entirely return to its previous dimensions after the stress is removed. Furthermore, the amount of stress necessary to form the dough piece is smaller than that required under same conditions when strong flour is utilized (Smith, 1972).

The strong flour protein contains long linkages and few bonds, whereas the weak flour protein has short links and many bonds. Soft wheat flour is preferable for producing cakes that are weak and easy to stretch (Kim and Kim, 1999). Aside from the natural quality of the flour, several treatments can be used to change the strength of the flour. Sulphur dioxide treatment decreases the flour strength. Heat-treated flour added to untreated flour is said to strengthen the flour. According to D. W. Kent-Jones and Amos (1967), improvers have an effect on the nature and character of the gluten, causing it to behave like stronger wheat gluten during fermentation.

The flour should be free flowing and dry to the touch, with a creamy color and no visible bran particles. It should also have a distinct flavor and be free of musty and rotten flavors (Cauvain and Young, 2006).

Characteristics	Requirements
Moisture (g)	13.3
Protein (g)	11
Fat (g)	0.97
Carbohydrate (g)	85.27
Minerals (g)	0.6
Fiber (g)	0.3
Energy (Kcal)	348
Calcium (mg)	23
Phosphorous (mg)	121
Iron (mg)	2.7
Carotene (µg)	25
Vitamin C (mg)	0
Thiamine (mg)	0.12
Riboflavin (mg)	0.07
Niacin (mg)	2.4

 Table 2.3 Chemical composition of wheat flour

Source: DFTQC(2017)

2.4 Soybean

Soybean (*Glycine max*) is a commercial crop in many nations. It belongs to the family Leguminosae, the subfamily Papilionoideae, the tribe Phaseoleae, the genus Glycine Willd, and the subgenus Soja (Moench). It is an erect, bushy herbaceous annual that can grow up to 1.5 m tall. It is also known as the "King of Legumes," and it is produced largely for seed production. It has a wide range of uses in the food and industrial sectors, and it is a key source of edible vegetable oil and proteins for cattle feed (Neupane, 2018).

The biggest soybean producers in the world are the United States, China, North and South Korea, Argentina, and Brazil. Soybean is often referred to as 'Bhatmas' in Nepal. The agricultural farms of Khumaltar, Kakani, and Rampur collected 138 samples of soybeans from different districts with elevations ranging from 500 to 1800 meters, and the conclusion was that the most dominant varieties of soybeans in Nepal are white, brown, grey, and black in hue. It is known by several local names based on the variety, color of the seeds, and region, such as Nepale, Hardi, Saathiya, Darmali, Maily, Kalo, Seto, and so on (Lama, 2009).

Soybean is one of the world's most important protein sources and a good provider of all essential amino acids. It is dubbed "the protein hope of the future" because it includes 3% lecithin, which is beneficial for brain development. It also contains calcium, phosphate, and the vitamins A, B, and D (Orunaboka *et al.*, 2023). Consumption of soybean products appears to prevent cancer, blood serum cholesterol, osteoporosis, chronic renal disease, heart disease, oxidative stress, and other diseases (Ekor *et al.*, 2010).

2.4.1 Historical background

China is the birthplace of soybean agriculture. During the first part of the twenty-first century, China was the world's greatest soybean producer and exporter. The annual wild soybean (*Glycine soja*), a close relative of the cultivated soybean (*Glycine max*), can be found across Northeast China. In 2007, China's soybean cultivated area was 8.90 ha, total production was 13.80 million, and yield per unit area was 1550 kg ha⁻¹. For ages, China has used soybean as a human food (Qiu LiJuan and Chang RuZhen, 2010).

Soybeans were introduced to the Western world in the 18th century. Soybeans were initially grown largely as a fodder crop and an alternate source of protein-rich animal feed (Kumudini, 2002). However, it wasn't until the late 1800s, with the introduction of industrialization and better milling techniques, that soybeans began to be processed into numerous forms, including soybean flour (Lee *et al.*, 2007). Archer Daniels Midland (ADM), a major American agricultural processing firm, was instrumental in the industrialization and mass manufacture of soybean products. ADM constructed the first soybean processing factory in the United States in 1918, representing an important step forward in the production of soybean flour (Kumudini, 2002).

Soybean flour's nutritional properties have contributed to its increasing popularity throughout time. Protein, vital amino acids, dietary fiber, vitamins, and minerals are all abundant in soybeans. As a result, soybean flour has become a popular ingredient in vegetarian and vegan diets, as well as in a variety of food applications such as baking, cooking, and as a fortifier in processed goods (Muthukumarappan and Karunanithy, 2015). Thus, soybean flour has a long and varied history, beginning in ancient China and expanding to Japan, Korea, and, eventually, the Western world. Cultural habits, agricultural developments, and the recognition of its nutritional worth all influenced its growth and use.

2.4.2 Nutritional importance of soybean flour

Soybean flour has substantial nutritional value as a flexible plant-based protein source. It is high in complete protein, which contains all of the essential amino acids needed for growth and development (Messina *et al.*, 2022). Soybean flour is high in dietary fiber. Fiber is essential for digestive health, reducing constipation, and maintaining bowel regularity. Soybean flour's fiber content promotes satiety, which can be good for weight management and blood sugar control (Anderson *et al.*, 2009). It contains beneficial fats, including as omega-3 and omega-6 fatty acids, which are essential for brain function and cardiovascular health. Soybean flour is also high in vitamins (such as B vitamins) and minerals (such as iron, calcium, and potassium), which are required for a variety of body activities. It also includes isoflavones, which have been linked to potential health advantages. Because of these characteristics, soybean flour is a valuable nutritional ingredient for people who follow vegetarian, vegan, or health-conscious diets (Messina *et al.*, 2022).

The chemical composition of soyabean as shown in Table 2.4.

Parameters (g/100g)	Value
Moisture % (wb)	10.33±0.37
Protein % (db)	37.72±3.73
Lipid % (db)	20.53±3.73
Ash % (db)	4.56±0.45
Fibre % (db)	5.75±0.67
Carbohydrate % (db)	31.41±0.74

Table 2.4 Proximate composition of soybean

Source: Ojha et al. (2014)

The soybean flour contains mineral, which are required for variety of body activities. The mineral content of soybean flour as shown in Table 2.5.

Table 2.5 Mineral content of soybean flour

Element	mg/100g
Potassium	1797
Magnesium	258.24
Calcium	300.36
Zinc	2.7
Phosphorous	695.20
Sodium	3.0

Source: Etiosa et al. (2018)

2.4.3 Preparation of soybean flour

Soybean seeds were served as a raw material for this study. To make roasted soybean flour, start with good-quality soybeans and inspect them for contaminants or broken beans. To remove any dirt or debris, thoroughly rinse the soybeans under running water. Preheat the oven at 60°C for 6 hours. Roast the soybeans in a preheated oven at 110°C for 10 minutes,

stirring regularly, until golden brown and emitting a nutty scent. Over-roasting might cause the flour to taste harsh. Allow the roasted soybeans to cool completely before crushing them into a fine powder in a blender or food processor. location the roasted soybean flour in an airtight container and keep it in a cool, dry location



Fig. 2.1 Preparation of soyabean flour

Source: Agume et al. (2017)

2.4.4 Benefits of soybean flour

Soybean flour is made by pulverizing soybeans into a fine powder. It has grown in popularity as a healthful and adaptable component in a variety of food products. Here are some of benefits of using soybean flour:

- 1. High protein content: Soybean flour is a high-quality plant-based protein source. It is a complete protein source since it contains all important amino acids. This makes it particularly useful for vegetarians, vegans, and anybody trying to improve their protein intake (Fukushima, 1991).
- Rich in dietary fiber: Soybean flour is high in dietary fiber, which is important for digestive health. Fiber aids in the maintenance of regular bowel movements, the reduction of constipation, and the promotion of a healthy gut environment (Azizah and Zainon, 1997).

- 3. Low saturated fat: Soybean flour is low in saturated fat and cholesterol. A diet low in saturated fat is favorable to heart health and can help reduce the risk of cardiovascular. Soy products may help lower LDL cholesterol levels, lessening the risk of heart disease (Elsharawy *et al.*, 2013).
- 4. Nutrient rich: Iron, calcium, phosphorus, potassium, magnesium, and B vitamins (such as folate and vitamin B6) are all found in soybean flour. Soybean flour contains calcium and magnesium, both of which are necessary for strong and healthy bones (Campos *et al.*, 2022).
- Gluten free option: Soybean flour is naturally gluten-free, making it an excellent choice for people who have gluten sensitivities or celiac disease (Milde *et al.*, 2012).
- 6. Weight management: Soybean flour's high protein and fiber content may aid with weight management and hunger control (Paddon-Jones *et al.*, 2008).
- Blood sugar regulation: Soybean flour's fiber and protein content can help regulate blood sugar levels, making it advantageous for people with diabetes or at risk of developing diabetes (Paddon-Jones *et al.*, 2008).

2.5 Avocado

Avocado (*Persea americana*), a tropical fruit originated from Mexico is classified as a functional food due to the nutraceutical components in the fruit. The wide range of avocado variety found worldwide are from Hass avocado (Pahua-Ramos *et al.*, 2014). Avocados have a calorie density of 1.7 calories per gram, with 114 calories, 4.6 grams of fiber, 345 mg of potassium, 19.5 mg of magnesium, 1.3 mg of vitamin E, and 57 mg of phytosterols in a half unit (68g) (Dreher and Davenport, 2013).

The avocado is regarded as the healthiest fruit in the world due to its high vitamin content. such as dietary fiber, potassium, folic acid, vitamin B6, vitamin C, copper, and vitamin K at calorie-efficient levels. One of the fruits that is most frequently advised as a nourishment for the body building and medication for illnesses caused with high cholesterol. Avocado is a fruit with a pear-like shape and a delicious, creamy flavor. Its significant proportion of healthy fats and remarkably low cholesterol levels have attracted attention in the health community. Although there are many different kinds of avocados, the creamy Hass kind is the most often consumed. Due to their form and the leathery look of their skin, avocados are sometimes known as alligator pears. Avocados are a "super

food" that are loaded with vitamins, minerals, and other nutrients. They are where monounsaturated fatty acids are found (Cheeke *et al.*, 2006).

A medium or good-sized California Hass avocado contains around 22.5 g of fat, according to the California Avocado Commission (CAC). Above all else, avocados contain a distinctive assortment of organic substances like phytosterols, carotenoids, and flavonoids. The 2 to 2.5-inch avocado seed, which is usually thrown away as garbage, is a nutrient-rich alternative. The avocado seed, or pit, is the plant's storage organ and is packed with vital nutrients. A lesser amount of magnesium and calcium, which are necessary for blood coagulation, muscle contraction, and healthy teeth and bones, is also present in avocado seeds. Avocado seeds also have a 2.5%–5% protein content. The avocado seed has a number of byproducts that could be produced. For instance, seeds could be used to make flour (Gorinstein *et al.*, 2002).

2.5.1 Historical background

The avocado (*Persea Americana*) is a tree, long thought to have originated in South Central Mexico, classified as a member of the flowering plant family Lauraceae. The fruit of the plant, also called an avocado (or avocado pear or alligator pear), is botanically a large berry containing a single large seed. The fruit grows on trees that can reach 60 feet or higher, but typically stand about 30 feet tall. Most varieties are pear-shaped and can range in skin color from yellow-green to green to a dark purple that is almost black in appearance. Skin texture ranges from smooth to bumpy while the flesh of the fruit is typically a buttery yellow or yellow-greenish color depending on variety. In the middle sits 17 a single seed, from 2 to 2.5 inches long, which can vary in shape but is usually hard and heavy with ivory coloring beneath papery-thin brown seed coats.

Avocado seed (2-2.5inches), which is usually discarded as garbage, is actually a highnutrient source. The avocado seed, or pit, is the plant's storage organ and contains a plethora of vital nutrients. Avocado seeds also have a reduced concentration of magnesium and calcium, which are necessary for blood coagulation, muscle contraction, and healthy teeth and bones. Avocado seeds also contain 2.5% - 5% protein. Avocado seed byproducts could be developed in a variety of ways. Flour, for example, could be made from seeds (Gorinstein *et al.*, 2002). Avocados have more soluble fibre than other fruit and contain a number of useful minerals such as iron, copper and potassium and are a good source of the B vitamin, folate. Eating avocado can improve heart disease risk factors like total, "bad" LDL and "good" HDL cholesterol, as well as blood triglycerides. People who ate avocados regularly also weighed less, had a lower BMI and significantly less belly fat. They also had higher levels of "good" HDL cholesterol. Eating avocado or avocado oil with vegetables can dramatically increase the number of antioxidants you take (Umoh *et al.*, 2019).

The local administration has declared Dhankuta, in the eastern hill district, an avocado capital, due to the rising popularity of the fruit. Commercial avocado farming has become a key income generating sector in the district. Similarly, the Municipal Council under the Mayor"s Agro Tourism programme has officially declared Dhankuta as a special area for avocado. Now, Dhankuta will be developed as a commercial avocado producing area. The municipality had discussed the potential of establishing Dhankuta as commercial producers of these two fruits with several agro experts. Avocado is very nutritious and contains a wide variety of nutrients, including 20 different vitamins and minerals. They have a green-skinned, fleshy body that may be pear-shaped, egg-shaped, or spherical. These days, avocado has become a popular food among health-conscious individuals, according to agro expert of Dhankuta Municipality Jeevan Rai. He said that avocado has a history of more than 30 years in Dhankuta. Among the top region where avocado farming has been getting popular, Dhankuta stands at the top. According to statistics, more than 26,000 saplings of avocado have been planted in Dhankuta in the last few years (Adhikari, 2018).

Avocado (*Persea americana*) is a tropical fruit, which is nutritive and richest natural fruit known and produced annually. Although avocado pulp is consumed, it's seed is not utilized in commercial way in context of Nepal. Avocado seeds display antioxidant activity because they contain carotenoids, minerals, phenolics, vitamins, fatty acids, saponins, tannins, flavonoids, glycoside, alkaloids, phenols, and steroids. Avocado seed flour is rich in fiber, protein, and healthy fats, and can potentially contribute to the development of healthier baked goods.

2.5.2 Nutritional importance of avocado seeds

Avocado seeds include phytochemicals that are responsible for its color and organoleptic qualities as well as for the treatment and prevention of a wide range of illnesses, including

as cancer, heart disease, diabetes, and high blood pressure (Doyle *et al.*, 2006). Because they contain a lot of tannins, which you may be familiar with from wine, avocado seeds have a slightly bitter flavor and are crimson in color. Despite the taste, seeds contain tannins in acceptable amounts that are safe for people to consume. The presence of tannins in the body can increase hunger, enhance breathing, and alleviate circulatory issues like high blood pressure and cholesterol levels (Cheeke *et al.*, 2006).

Studies have shown that avocado seed contains more soluble fiber than oat powder and nearly every other food, and that it contains 70% of the antioxidants present in an avocado whole. Avocado seed oil is also rich in antioxidants, lowers cholesterol, and aids in the prevention of disease. Lowering cholesterol and preventing strokes are all benefits of avocado seed. Additionally, avocado seeds are excellent for treating diarrhea and GI tract irritation.

In fact, dysentery and other GI tract issues are treated with avocado seeds in South America. Avocado seeds contain a lot of phenolic chemicals that protect against bacterial and viral infections as well as gastric ulcers. A flavanol found in avocado seeds inhibits the formation of tumors. Avocado seeds strengthen the immune system and protect against contracting crippling illnesses. An additional benefit of antioxidants is that they prevent free radicals and slow down aging, which helps to maintain a strong immune system. Avocado seeds appear to be effective at reducing inflammation, which benefits conditions like arthritis and other joint ailments. Studies have demonstrated that avocado seed oil increases skin's collagen levels, keeping skin appearing young and wrinkle-free. In addition to making hair lustrous, avocado seed oil can be used to remove dry, dead skin (Fulgoni *et al.*, 2013).

The chemical composition of avocado seed as shown in Table 2.6.

Parameter(g/100g)	Avocado seed
Moisture (wb)	15.10±0.14
Protein (db)	5.55±0.36
Lipid (db)	7.90±4.48
Ash (db)	2.26±0.23
Fibre (db)	7.1±1.23
Carbohydrate (db)	49.03±0.02

 Table 2.6 Proximate composition of avocado seed

Source: Ejiofor et al. (2018)

The avocado seed contains mineral which helps for blood clotting, muscle contraction and maintains a steady heart beats and fluid balance in the body and strong bones. The mineral contains of avocado seed flour as shown in Table 2.7.

Table 2.7 Mineral content of avocado seed flour

Element	mg/100g
Potassium	4.160±0.13
Magnesium	0.100 ± 0.01
Calcium	0.820 ± 0.01
Zinc	0.177±0.02
Phosphorous	0.097 ± 0.01
Sodium	1.410±0.11

Source: Ifesan et al. (2015)

Like many other plant seeds, avocado seeds contain a variety of substances that could be regarded as anti-nutritional components. Plants use these substances as part of their natural defenses against predators and to protect their seeds. While some of these substances may

have potential health benefits in moderation, overconsumption could have negative effects. An anti-nutritional constituents of avocado seed flour as shown in Table.

Table 2.8 Anti-nutritional constituents of avocado seed flour

Parameter	Value (mg/100g)
Tannin	6.98±0.04
Total oxalate	14.98±0.03
Phytic acid	3.18±0.16

Source: Ifesan et al. (2015)

2.5.3 Preparation of avocado seed flour

Avocado seeds were served as a raw material for this study. The seeds were washed and the outer covering of the seeds were manually removed while washing. The washed seeds were chopped and air dried.

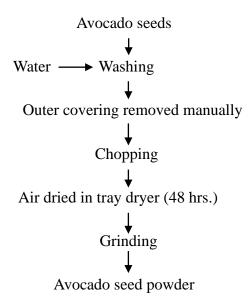


Fig. 2.2 Preparation of avocado seed powder

Source:- Mahawan et al. (2015)

2.5.4 Benefits of avocado seed

- Avocado seed flour is rich in fiber, protein, healthy fats, and can potentially contribute to the development of healthier and more sustainable baked goods.
 Avocado seeds to prepare a beverage as an antioxidant tea and the use of seeds as a culture medium (R. G. Araújo *et al.*, 2018).
- ii. Avocado seed is underutilized and represents a large portion of the fruit, thus its use can be an alternative to reduce the production cost of edible oil (Duarte *et al.*, 2016).
- iii. Avocado seed has high in tannins which give bitter taste and red color. Presence of tannins in the body can improve the appetite and reduce respiratory problem and circulatory disorder like lowering blood pressure and reduce of cholesterol in blood. Avocado seed contains a lower concentration of magnesium and calcium, required for blood clotting, muscle contraction and strong teeth and bone (Doyle *et al.*, 2006).
- iv. Avocado seed possess a high concentration of potassium and phosphorous, maintains a steady heartbeat and fluid balance in the body and strong bones and synthesis of DNA and phospholipids respectively (Doyle *et al.*, 2006).

2.6 Preparation of muffin

Dry and damp materials were sorted first. The dry ingredients were wheat flour, baking powder, and sugar. The wet ingredients included an egg, water, and butter. Before adding the milk and oil, the egg was beaten for two minutes. In a separate bowl, all of the dry ingredients were thoroughly mixed. The dry and wet ingredients were then combined to form a mixed muffin batter as given by Rahman *et al.* (2015) as shown in Fig 2.7.

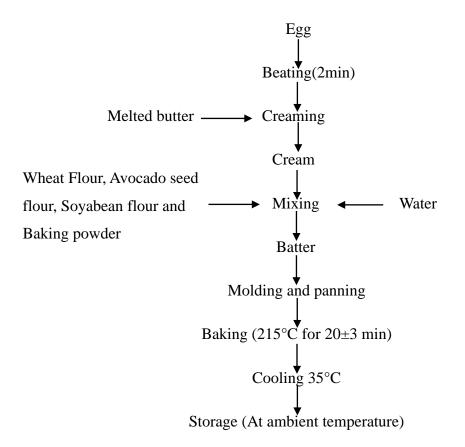


Fig. 2.3 Flow chart for the Preparation of muffins

Source: Rahman et al. (2015)

2.7 Baking profile

Baking is the most crucial step in muffin production because if it is not done correctly, the product loses its eating quality. During baking, the food is cooked, flavor and color are developed, and the raw dough is converted into an edible snack known as a muffin. The primary purpose of baking is to remove excess moisture from the dough by gently heating it (Bloksma, 1990). Every baking process depends on heat transfer from a hot source to the finished product. Heat is transported largely through three processes during baking:

conduction, convection, and radiation. During baking, radiation transmits the majority of the heat to the dough pieces, with convection transmitting only a minor fraction of the heat until the air velocity in the tunnel surpasses 5 feet per second, at which point convection transmits a bigger portion of the heat. Aside from these three modes of heat transfer, high frequency heating is used, which has a quicker moisture removal rate (Smith, 1972).

Every oven used till date consists of four basic parts.

- 1. A heat sources
- 2. A base (sole or hearth), capable of being heated, on which the dough piece is placed.
- 3. A cover over the base, making up a chamber in which to retain the heat.
- 4. A closable opening through which the dough piece can be put into and taken from the baking chamber.

During baking the dough undergoes gradual changes physically as well as chemically.

Physical changes include:

- 1. Formation of a film crust on the dough
- 2. Melting of the fat in the dough
- 3. Gas release and volume expansion
- 4. Conversion of water into steam
- 5. Escape of carbon dioxide, other gases and steam

Chemical changes include:

- 1. Gas formation
- 2. Starch gelatinization
- 3. Protein changes
- 4. Caramelization of sugar
- 5. Dextrinization

The effect of temperature in the baking oven on fresh dough is seen in Table 2.9.

Temperature (°F)	Changes occurred
90-100	Skin formation on the top crust (as a result of surface moisture evaporation).
90-120	CO ₂ evolution within crumb (lower CO ₂ solubility).
90-150	CO_2 Gas expansion (CO_2 and steam) generates a volume increase.
90-210	Starch gelatinization (muffin structure).
125-210	Irreversible protein coagulation.
170-250	The dextrinization process (surface gloss).
370-400	Skin formation on the top crust (as a result of surface moisture evaporation).

Table 2.9 Temperature related changes in muffin during baking

Source: Mukhopadhyay (1990)

During baking, more steam is required in the oven than is created by the moisture in the dough and the combustion of the fuel. Steam delivered into the baking chamber at the moment of dough entry or very early in the oven's passage aids in the formation of a shiny crust, the avoidance of fractured crusts, increased volume, and, to some extent, agitation of the oven atmosphere. The utilization of rapid moving fans recirculating air at 2000 cubic feet per minute can obviate the need for steam injection. The oven dampers are crucial for relieving the strong positive pressure created by high heat evaporation; similarly, if high moisture cookies or biscuits are desired, the dampers in the last zone must be closed (Smith, 1972).

Part III

Materials and methods

3.1 Raw materials

3.1.1 Avocado

Avocado (*Persea americana*) was harvested from the single farm at the coordinates 26.9835° N, 87.3215° E in Pakhribas, Dhankuta, Nepal.

3.1.2 Wheat flour

'Fortune' wheat flour was brought from local market of Dharan.

3.1.3 Soybean

Soybeans (*Glycine max*) were brought from local market in Dharan

3.1.4 Butter

The standard Amul butter was used.

3.1.5 Sugar

Sugar in the form of powdered sugar was used and obtained from the Dharan market.

3.1.6 Baking powder

'Weikfied baking powder double action,' made and packed by Weikfied food Pvt. Ltd., Pune, India, was purchased at a Dharan supermarket.

3.1.7 Egg

Eggs were brought from local market in Dharan.

3.1.8 Water

Potable water was provided in Dharan's Central Campus of Technology.

3.1.9 Equipment and chemicals

The necessary apparatus and chemicals were obtained from the Central Campus of Technology laboratory, Dharan.

3.2 Methods

3.2.1 Design of experiment

Design expert 13 was used to create the recipe. Mixed design (simple lattice design) was used to formulate the recipe. The independent variable for the experiment is concentration of avocado seed flour (0-10) and soyabean flour (0-20) used to prepare muffin.

3.2.1.1 Formulation of recipe

The recipe formulation for the avocado seed and soybean flour as substitutes muffin were carried out as per in table 3.2. The given is on different basis:

Ingredients	Wheat flour	Avocado seed flour	Soyabean flour
A	93.26	0	6.74
В	88.31	0	11.69
С	100	0	0
D	83.36	0.73	15.91
Е	78.38	1.62	20
F	78.38	1.62	20
G	96.12	3.88	0
Н	96.12	3.88	0
Ι	88.5	4.68	6.82
J	83.06	5.67	11.27
Κ	83.06	5.67	11.27
L	75.33	7.15	17.52
М	87	10	3
Ν	87	10	3
0	78.17	10	11.83
Р	70	10	20

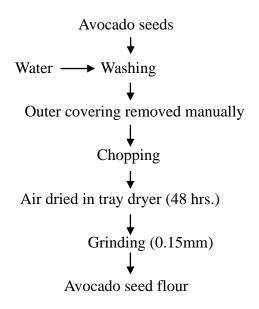
Table 3.1 Recipe formulation for muffin

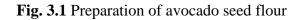
All the ingredients were mixed with sugar 60%, egg 57%, butter 68%, baking powder 2.8% per 100 parts of flour mixture.

Name	Goal	Range
Specific loaf volume	To be in range	1.5-2.5
Protein content	To be maximize	To be determined
Potassium content	To be maximize	To be determined
Ash content	To be minimize	To be determined

Table 3.2 Different response for experimental goal of muffin

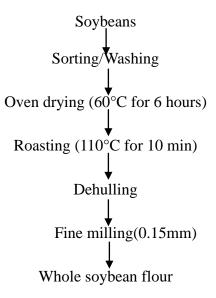
3.2.1.2 Preparation of avocado seed flour

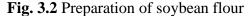




Source: Mahawan et al. (2015)

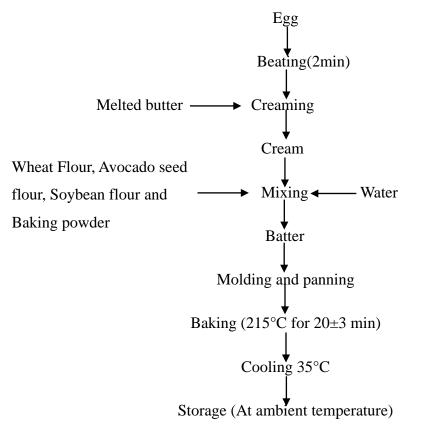
3.2.1.3 Preparation of soybean flour

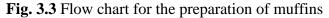




Source: Agume et al. (2017)







Source: Rahman et al. (2015)

For the muffins, the egg was beaten for 2 minutes and the shortening and sugar were creamed for 10 minutes. Water, composite flour, and baking powder were mixed along with these ingredients to make a batter. The batter was formed and panned and cooked at 215° C for 20 ± 3 minutes to make muffins (Rahman *et al.*, 2015). Here is the modification is in the flour. Different proportion of wheat flour, soyabean flour and avocado seed flour are used for the preparation of muffin as suggested by Design Expert 13.

3.2.1.4 Muffin making process

3.2.1.4.1 Mixing

The fundamental goal of mixing is to achieve a homogeneous mixture, which generally involves achieving a roughly uniform distribution of the elements. A contrast can be made between batch and continuous processes. Overall, the concentration of the ingredient should be equally distributed in the output stream, should not vary with time, and the processing of each section of the combination should be the same (Ashokan *et al.*, 2013).

3.2.1.4.2 Baking

After mixing, the batter should be poured in a mold and cooked as soon as possible. Muffin batters that will not be prepared immediately should be carefully wrapped in plastic wrap and refrigerated until ready to bake in the oven. Carbon dioxide is produced once the baking powder enters the solution. If the mixture is allowed to stand for an extended period of time, some of the carbon dioxide will escape, resulting in a baked product with a typically coarse cell structure (Trimbo and Miller, 1966).

3.2.1.4.3 Cooling

Before packing, the muffin is allowed to cool to room temperature after being removed from the oven. Moisture travels from the inside to the crust and to the atmosphere during cooling; if the moisture content of the crust rises significantly during chilling, the texture of the crust becomes leathery and harsh, and the pleasant crispness of freshly cooked muffin is lost. Excessive cold drying causes weight loss and poor crumb properties. The goal of cooling is to reduce the temperature while minimizing moisture loss (Yu and Kies, 1993). When the muffin cools, the sugar in it gives it strength and stiffness.

3.2.2 Analytical procedure

3.2.2.1 Moisture content

The moisture content of raw materials and finished products was determined using a hot air oven as described by (Ranganna, 1986)

Moisture Content =
$$\frac{W_{initial} - W_{final}}{W_{initial}} \times 100\%$$

Where, $W_{initial}$ = Weight of sample before drying

 W_{final} = Weight of sample after drying

3.2.2.2 Crude protein

The crude protein (N 6.25) content of raw material and product was determined using the micro kjeldahl technique (Ranganna, 1986).

Protein content (%, wb) =
$$\frac{(\text{sample titer-blank titer}) \times \text{N of HCl} \times 14 \times 100 \times 100}{\text{Aliquot (ml)} \times \text{wt. of sample (g)} \times 1000}$$

3.2.2.3 Crude fat

The crude fat content of the samples was measured using the Soxhlet equipment using petroleum ether as the solvent (Ranganna, 1986).

Crude fat =
$$\frac{W_2 - W_1}{W} \times 100\%$$

Where, W_1 = weight of beaker

 W_2 = weight of oil extracted + beaker

W = weight of sample

3.2.2.4 Crude fiber

The crude fiber was determined using a chemical technique in which the sample was treated with boiling dilute sulphuric acid, boiling sodium hydroxide, and then alcohol under standardized conditions (Ranganna, 1986).

Crude fiber (%, wb) = $\frac{(\text{Residue-Ash})g \times (100\text{-F})}{\text{sample (g)}}$

3.2.2.5 Total ash

By using the dry ashing approach as described in Ranganna (1986).

Ash content =
$$\frac{W_3 - W_1}{W_2 - W_1} \times 100\%$$

Where, W_1 = weight of empty crucible

 W_2 = weight of sample + crucible before ashing

W₃= weight of sample + crucible after ashing

3.2.2.6 Potassium content

Solid and ash samples were prepared using the ASTM C114-09 standard procedure (Junsomboon and Jakmunee, 2011). The material was precisely weighed to 1.0000 g and placed in a 150 mL beaker. Then, 20 mL of water and 5 mL of strong hydrochloric acid were added sequentially, followed by 50 mL of water. The suspension was digested for around 15 minutes on a hot plate before being filtered through a Whatman No. 40 filter paper into a 100 mL volumetric flask. Finally, water was added to the mark to create a ready-to-analyze solution.

3.2.2.7 Carbohydrate

The total carbohydrate content was measured using the difference method according to Ranganna (1986).

Carbohydrate = 100-(protein + fat + ash + crude fiber)

3.2.2.8 Volume

The muffin loaf volume was determined using the mustard seed displacement method, which involved placing the muffin in a beaker of known volume. A known volume box will be filled with seed, and the weight of seed necessary to simply fill the box will be recorded. The seed is poured back into the box after the sample has been injected. The volume of the product is equal to the volume of the seed displaced (Committee, 2000).

3.2.2.9 Weight of muffin

The weight of the muffin was determined using a weighing balance accessible at the Central Campus of Technology in Dharan (Committee, 2000).

3.2.2.10 Specific loaf volume

The volume of the muffin loaf was calculated by dividing the volume by the weight (Committee, 2000).

Specific loaf volume = $\frac{\text{weight of muffin}}{\text{volume of muffin}}$

3.2.3 Sensory analysis

Semi-trained panelists comprised of Central Campus of Technology faculty and students will conduct the sensory analysis for overall quality. Texture, appearance, color, aroma, taste, and overall acceptance are the sensory evaluation parameters. Sensory evaluation was carried out using the 9-point Hedonic Scale.

3.2.4 Data analysis

The collected data was statistically evaluated using Genstat Discovery Edition 12 for Analysis of Variance (ANOVA) at a 5% threshold of significance. The proximate analysis and sensory evaluation data were subjected to one and two way ANOVA respectively.

Part IV

Results and discussion

This work was carried out to evaluate the impact of avocado seed and soybean flour as wheat flour substitutes in composite flour muffins. As a nutritious breakfast food, muffins are popular. Initially, the primary raw materials were subjected to proximate analysis.

4.1 The proximate composition of wheat, avocado seed and soybean flour.

The proximate composition of wheat flour, avocado seed flour and soybean flour were determined. The results are presented in Table 4.1

Parameters	Wheat	Avocado seed	Soybean flour
Moisture (wb)	11.21±0.12	14.51±0.55	10.45±0.15
Protein (db)	10.28±0.11	7.74±0.93	35.93±2.22
Fat (db)	1.14±0.43	0.71±0.12	19.68±1.43
Crude fiber (db)	0.36±0.10	4.93±0.22	4.33±0.31
Ash content (db)	0.47±0.07	2.81±0.13	5.82±0.43
Carbohydrate (db)	87.45±2.69	68.9±3.05	34.18±1.60
Potassium (mg/100g)	10.6±0.11	40.18±0.1	1756±35

Table 4.1 The proximate composition of wheat, avocado seed and soybean flour

*Values are the means of triplicates, and figures in parenthesis represent the standard deviations of the triplicates.

4.1.1 Chemical composition of wheat flour

The moisture content, protein, fat, crude fiber, ash and carbohydrate of wheat flour were found to be 11.21, 10.28, 1.14, 0.36, 0.47, 87.45%. According to (DFTQC, 2017), proximate values were 13.3, 11, 0.97, 0.3, 0.83, 85.27%. There is an inequality between the standard value and the calculated value. Varieties, climatic circumstances, soil type, maturity, fertility, and other factors may contribute to differences in proximate composition.

4.1.2 Chemical composition of avocado seed flour

The moisture content, protein, fat, crude fiber, ash content, potassium content and carbohydrate of avocado seed flour were found to be 14.51, 7.74, 0.71, 4.93, 2.81, 4.18, 68.9%. According to Ejiofor *et al.* (2018), proximate values and potassium content were 15.10, 15.55, 17.90, 2.26, 7.1, 49.03% and 4.160mg/g respectively. There is an inequality between the standard value and the calculated value. The proximate composition could be due to variances in cultivar, growth, location, and seed processing.

4.1.3 Chemical composition of soybean flour

The moisture content, protein, fat, crude fiber, ash content, and carbohydrate of soybean flour were found to be 10.45, 35.93, 19.68, 4.33, 5.82, 34.18%. According to Ojha *et al.* (2014) proximate values were 10.33, 37.72, 20.53, 4.56, 5.75 and 31.41 respectively. There is an inequality between the standard value and the calculated value. Varieties, climatic circumstances, soil type, maturity, fertility, and other factors may contribute to differences in proximate composition.

4.2 Effect of avocado seed and soybean flour on response factor of the muffins

Observed values of responses (specific loaf volume, protein content, potassium content and ash content) were shown in Appendix C.

4.2.1 Effect on specific loaf volume

The specific loaf volume varied from 1.89 to 2.39 cm³/g. In Appendix C.1 and C.2, the quadratic model was best fitted to describe the effect of avocado seed, soybean flour and wheat flour on a specific loaf volume of muffins. The model F value was found significant (p<0.0001). The best-fitted quadratic model for the effect of variables on specific loaf volume was given best described by coded equation 4.1

Specific loaf volume = -0.4419A+2.05B+2.21C+3.02AB+4.62AC-0.1034BC....4.1

Where A, B and C are the coded values of avocado seed flour, soybean flour and wheat flour. A, B, C, AB, AC and BC are model terms.

In the quadratic equation 4.1, avocado seed (A) had a significant (P<0.05) negative effect and soybean flour (B) and wheat flour (C) had a positive effect on specific loaf

volume at a 95% level of confidence. The interaction terms avocado seed and soybean flour (AB), avocado seed and wheat flour (AC) had a significant positive effect on specific loaf volume. Also, the interaction term avocado seed and wheat flour (BC) had a significant (P<0.05) negative effect on specific loaf volume. The combination of 3.88 parts avocado seed flour, 0 parts soybean flour, and 96.12 parts wheat flour (samples G and H) were peak-specific loaf volume.

With the combined effect of avocado seed, soybean flour and wheat flour, the specific volume of muffin increased in (Fig:4.1). This graph shows the interaction between avocado seed flour, soybean and wheat flour.

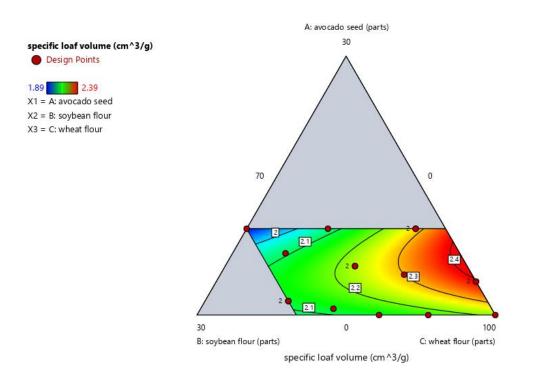


Fig. 4.1 Contour plot of a specific volume of muffin as a function of the avocado seed, soybean flour and wheat flour.

From fig. 4.1 it is clear that increase in avocado seed and a slightly decrease in the specific loaf volume of muffin. Increase in soybean flour and wheat flour increase in specific volume. Also, the increase in the combine effect of avocado seed and soybean flour, avocado seed and wheat flour with an increase in specific loaf volume. And increase the combined effect of soybean and wheat flour decrease in specific loaf volume.

Avocado seeds are firm and dense, they can prevent muffin batter from expanding during baking, resulting in a lower total volume. Furthermore, the high oil content of avocado seeds may change the texture and moisture absorption of the muffins, perhaps resulting in a heavier and more compact crumb structure, influencing the specific loaf volume. Furthermore, avocado seeds may provide bitterness, impacting the taste and overall appeal of the muffins. Soybean flour, on the other hand, can influence the particular loaf volume due to its high protein and fat content. While it may add structure to the muffins, the lack of gluten may cause reduced flexibility, resulting in denser and less voluminous muffins than those produced with wheat flour (Demirkesen *et al.*, 2023).

4.2.2 Effect on protein content

The protein content varied from 10.28 to 16.2%. Table C.3 and C.4 a linear model was best fitted to describe the effect of avocado seed, soybean flour and wheat flour on the protein of muffins. The model F value was found significant (p<0.0001). The best-fitted linear model for the effect of variables on protein content was given best described by coded equation 4.2

Protein content = 10.52A+17.12B+10.88C.....4.2

Where A, B and C are the coded values of avocado seed flour, soybean flour and wheat flour. A, B and C are model terms.

In the linear equation 4.2, protein content had a significant (P<0.05) positive effect of avocado seed (A), soybean flour (B) and wheat flour (C) at a 95% level of confidence.

With the combined effect of avocado seed, soybean flour and wheat flour, the protein content of the muffin increased and reached maximum level in (Fig:4.2). This graph shows the interaction between avocado seed flour, soybean and wheat flour.

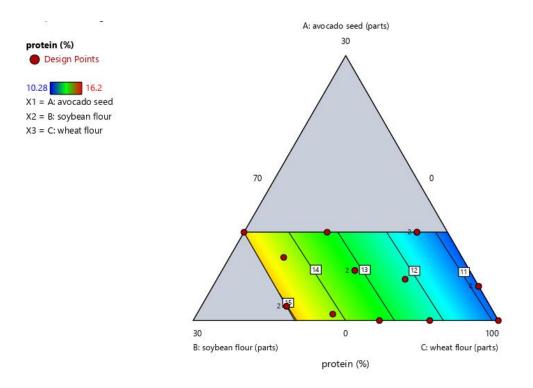


Fig. 4.2 Contour plot of the protein content of muffin as a function of the avocado seed, soybean flour and wheat flour.

From fig. 4.2 it is clear that an increase in avocado seed, soybean flour and wheat flour parts increase in protein content of the muffin.

Avocado seed into muffins may contribute to a minor increase in protein content, the biggest impact on protein levels will come from utilizing soybean flour, which contains roughly 30- 35% protein. It was found that increasing the amount of soybean and avocado flour. While avocado seeds contain protein, their content is minimal when compared to soybean flour. To increase the protein content of muffins, replacing a portion of ordinary flour with soybean flour is a more successful technique (Pauter *et al.*, 2018).

4.2.3 Effect on potassium content

The potassium content varied from 10.36 to 44.39mg/g. Table C.5 and C.6 a linear model was best fitted to describe the effect of the avocado seed, soybean flour and wheat flour on the potassium of muffins. The model F value was found significant (p<0.0001). The best-fitted linear model for the effect of variables on potassium content was best described by coded equation 4.3.

Potassium content = +7.85A+61.41B+10.61C.....4.3

Where A, B and C are the coded values of avocado seed flour, soybean flour and wheat flour. A, B and C are model terms.

In the linear equation 4.3, potassium content had a significant (P<0.05) positive effect of avocado seed (A), soybean flour (B) and wheat flour (C) at a 95% level of confidence.

With the combined effect of avocado seed, soybean flour and wheat flour, the potassium content of muffin increased and reached maximum level (Fig:4.3). This graph shows the interaction between avocado seed flour, soybean and wheat flour.

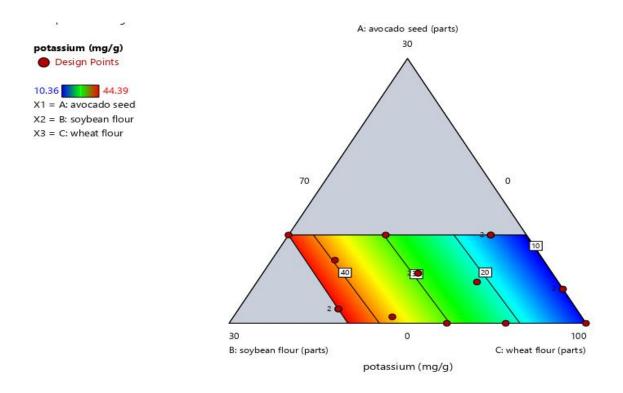


Fig. 4.3 Contour plot of potassium content of muffin as a function of avocado seed, soybean flour and wheat flour.

From fig. 4.3 it is clear that an increase in avocado seed, soybean flour and wheat flour parts increase in potassium content of the muffin. The addition of avocado seed and soybean flour to muffins may have a minor influence on potassium content. It was found that increasing the amount of soybean and avocado flour. While avocado seeds may contain potassium, the amount is minimal in comparison to the flesh of the fruit, therefore

their contribution may not have a substantial impact on the overall potassium level of the muffins. Soybean flour, on the other hand, is quite high in potassium, which means that including it in the muffin recipe can significantly increase the potassium level. However, the overall effect will be determined by the amount of soybean flour utilized (Topkaya and Isik, 2019).

The muffins with avocado seed and soybean flour had better nutritional profiles than the control muffins made simply of wheat flour. The composite muffins had higher protein and potassium content, which contributed to their higher nutritional value. This research shows that avocado seed and soybean flour can be successfully blended into composite flour muffins as wheat flour alternatives. The muffins improved in nutritional content as the quantity of alternative flours increased. The improved nutritional profile, especially the higher protein and fiber content, may appeal to health-conscious consumers.

An effect of avocado seed and soybean flour on the protein content and potassium content of the muffins with up to lower substitution was well-accepted, implying that these formulations might be launched into the market as a healthier and more nutritious option. At greater substitution levels, however, the different aromas of avocado seed and soybean flour may have reduced overall acceptance. More studies might be done to increase the acceptability of muffins with higher replacement levels by improving the formulation or integrating flavor-masking methods.

4.2.4 Effect on ash content

The ash content varied from 1.69 to 2.11%. Table C.7 and C.8 a special cubic model was best fitted to describe the effect of the avocado seed, soybean flour and wheat flour on the ash content of muffins. The model F value was found significant (p<0.0001). The best-fitted special cubic model for the effect of variables on ash content was best described by coded equation 4.4

Ash content = +2.61A+2.54B+1.69C-2.15AB-0.3081AC-0.7660BC+2.74ABC......4.4

Where A, B and C are the coded values of avocado seed flour, soybean flour and wheat flour. A, B, C, AB, AC, BC and ABC are model terms.

In the special cubic equation 4.4, ash content had significant (P<0.05) positive effect of avocado seed (A), soybean flour (B) and wheat flour (C) at 95% level of confidence. The interaction term avocado seed and soybean flour (AB), soybean flour and wheat flour (BC), avocado seed and wheat flour (AC) had significant negative effect on specific loaf volume. Also, interaction term avocado seed, soybean flour and wheat flour (ABC) had significant (P<0.05) positive effect on ash content.

With the combined effect of the avocado seed, soybean flour and wheat flour, the ash content of the muffin increased and reached maximum level in (Fig:4.4). This graph shows the interaction between avocado seed flour, soybean and wheat flour.

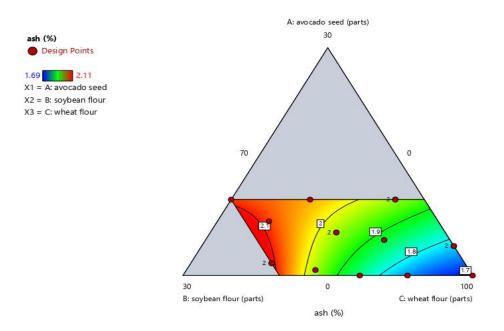


Fig. 4.4 Contour plot of ash content of muffin as a function of avocado seed, soybean flour and wheat flour.

From fig. 4.4 it is clear that an increase in avocado seed, soybean flour and wheat flour parts increases in ash content of the muffin. The increase in combined effect of avocado seed and soybean flour, avocado seed and wheat flour and soybean flour and wheat flour parts decrease in ash content. Also, with an increase in avocado seed, soybean flour and wheat flour parts, ash content increases.

The combination of avocado seed and soybean flour might affect the ash content of a muffin. The ash content is the inorganic mineral content that remains after all organic material has been burnt off during analysis. Avocado seeds and soybean flour both include

minerals that can contribute to the ash level of the muffin recipe when used together. The amount of avocado seed and soybean flour used in the muffin recipe will have an impact on the ash content. In general, the larger the quantity of these elements in the muffin batter, the higher the ash level of the final baked product (Pauter *et al.*, 2018).

4.3 Avocado seed and soybean flour impact physical characteristics of muffin.

The physical analysis is carried out to determine the effect of avocado seed and soybean flour on the muffin structure. Volume, weight and specific loaf volume are all considered. For each physical parameter, 16 values are taken and a table is displayed.

Sample	Volume in cc	Weight in g	Specific loaf volume
			in cc
А	65.83	32.33	2.15
В	66.53	30.33	2.13
С	65.26	28.16	2.22
D	67.83	31.4	2.13
E	68.06	32.4	2.12
F	68.06	32.23	2.12
G	66	28.06	2.39
Н	66.13	27.93	2.39
Ι	68.8	29.6	2.3
J	70	30.93	2.23
Κ	70.06	30.86	2.23
L	69.33	34.06	2.09
М	70.13	30.46	2.28
Ν	70.13	30.46	2.28
0	70.46	35.2	2.07
Р	70.33	36.26	1.89

Table 4.2Effect of avocado seed and soyabean flour on the volume, weight and specificloaf volume of muffin.

From Table 4.2, it is seen that the combination of avocado seed and soybean flour can have varied impacts on the volume of muffins, depending on the proportions used and the recipe. Avocado seeds are generally not suggested for baking due to their hardness, density,

and probable bitterness. Even when pounded into a powder, the high oil content may have an adverse effect on the texture and moisture balance of the muffin. Soybean flour, a gluten-free option, on the other hand, can boost the nutritional profile of muffins due to its high protein and fat content. However, adding too much soybean flour may result in dense and heavy muffins since it lacks the flexibility of gluten seen in regular wheat flour. Traditional muffin recipes that use wheat flour are more likely to produce the proper volume, texture, and flavor.

The addition of avocado seed and soybean flour to muffin recipes might affect the weight of the muffins in different ways. Avocado seeds are not a typical baking ingredient due to their hardness, density, and potential bitterness, and their inclusion may add weight to the muffins without significantly improving flavor or texture. Furthermore, the avocado seeds high oil content may contribute to the muffin's increased weight. Soybean flour, on the other hand, is a protein and fat-rich alternative to typical wheat flour that can affect muffin weight because it is denser than certain other flours. The combined effect of utilizing avocado seed and soybean flour will be determined by the exact amounts and other ingredients in the recipe. While soybean flour can be used in moderation to increase the nutritious content of muffins, avocado seed is generally avoided because it can result in thicker and heavier muffins. For best results, start with small test batches and modify ingredient ratios to reach the desired muffin weight and texture.

The effect of avocado seed and soybean flour on the specific loaf volume of muffins might be significant and primarily unfavorable. Because avocado seeds are firm and dense, they can prevent muffin batter from expanding during baking, resulting in a lower total volume. Furthermore, the high oil content of avocado seeds may change the texture and moisture absorption of the muffins, perhaps resulting in a heavier and more compact crumb structure, influencing the specific loaf volume. Furthermore, avocado seeds may provide bitterness, impacting the taste and overall appeal of the muffins. Soybean flour, on the other hand, can influence the particular loaf volume due to its high protein and fat content. While it may add structure to the muffins, the lack of gluten may cause reduced flexibility, resulting in denser and less voluminous muffins than those produced with wheat flour. To get good results in a specified loaf volume, avoid using avocado seeds and carefully mix soybean flour, establishing the proper proportion with other components to retain a suitable muffin texture and size. In the above table samples G and H were found to be more specific

loaf volume (2.39) as compared to other samples. Samples G and H were the best formulations (96.12 parts wheat flour, 3.88 parts avocado seed flour, and 0 parts soybean flour) due to the specific loaf volume of muffin.

4.4 Avocado seed and soybean flour impact sensory characteristics.

A sensory evaluation of muffins made with different levels of avocado seed flour and soybean flour incorporation according to recipes was performed. The hedonic rating system was used to analyze the color, smell, look, texture, taste, and general acceptability of the samples (1 = detest severely, 9 = like exceedingly) (Ranganna, 1986). The samples were shown to ten panelists. They were instructed to evaluate their sensory judgments about a variety of muffin parameters. It was proposed that the panelists create score sheets based on their perceptions.

4.4.1 Appearance

The mean sensory scores of maximum and minimum appearances were found to be 7.556, 7.556 and 5.444 for the muffin formulations E, F and M respectively. Statistical analysis showed that the partial substitution of flour with avocado seed flour and soybean flour had a significance effect (p < 0.05) on the appearance of different muffin formulations. Sample A, I, J, K, L, and O were not significantly different from each other while samples D and E were not significantly different to each other but from M, N and P. Also, samples B, D, E, F, G and H were not significantly different from M, N and P which is shown in graphically Fig: 4.5.

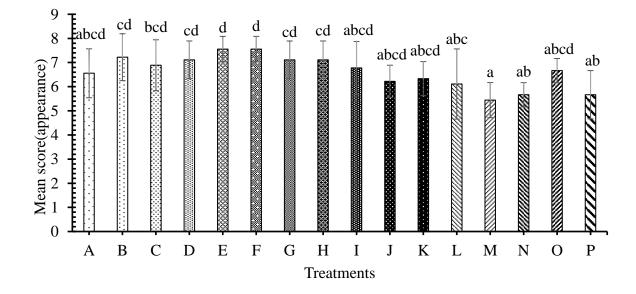


Fig. 4.5 Characteristics of product with respect to appearance. The alphabet above the bar graph shows a significant difference.

4.4.2 Color

The mean sensory scores of maximum and minimum colors were found to be 7.222 and 5.444 for the muffin formulations E and M respectively. Statistical analysis showed that the partial substitution of flour with avocado seed flour and soybean flour had a significance effect (p < 0.05) on the color of different muffin formulations. The sample N was not significantly different with other but from B, D, F, G, H and O which is shown in graphically Fig: 4.6.

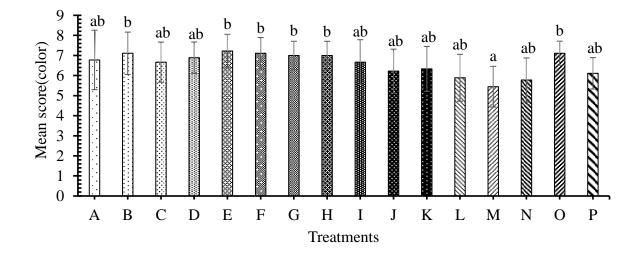


Fig. 4.6 Characteristics of product with respect to color. The alphabet above the bar graph shows a significant difference.

4.4.3 Taste

The mean sensory score of maximum taste was found to be 6.778 for the muffin formulations F, H and I respectively. Also, the mean sensory score of minimum taste was found to be 5.889 for the muffin formulation A, M, and P respectively. Statistical analysis showed that the partial substitution of flour with avocado seed flour and soybean flour had a significance effect (p < 0.05) on the taste of different muffin formulations. The samples A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P were not significantly different from each other which is shown in Fig: 4.7.

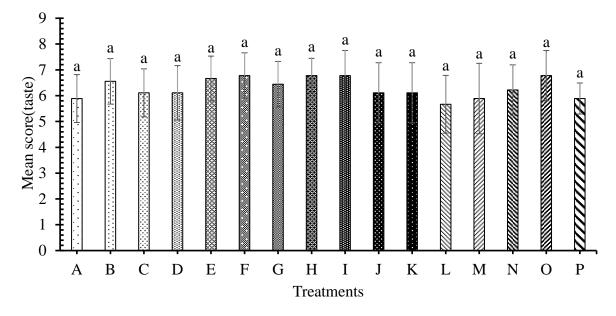


Fig. 4.7 Characteristics of product with respect to taste. The alphabet above the bar graph shows a significant difference.

4.4.4 Aroma

The mean sensory score of maximum aroma was found to be 6.667 for the muffin formulations H and I. Also, the mean sensory score of minimum aroma was found to be 5.889 for the muffin formulation M and O respectively. Statistical analysis showed that the partial substitution of flour with avocado seed flour and soybean flour had a significance effect (p < 0.05) on the aroma of different muffin formulations. The samples A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P were not significantly different from each other which is shown in Fig: 4.8.

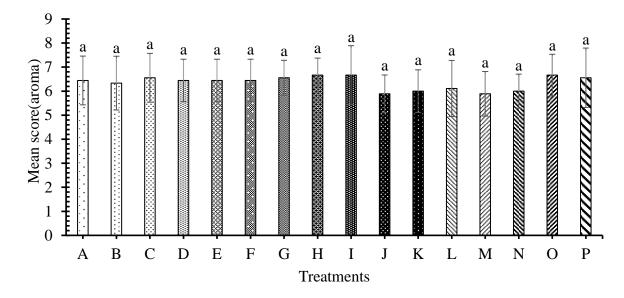


Fig. 4.8 Characteristics of product with respect to aroma. The alphabet above the bar graph shows a significant difference.

4.4.5 Texture

The mean sensory scores of maximum and minimum textures were found to be 7.111 and 5.556 for the muffin formulations F and M respectively. Statistical analysis showed that the partial substitution of flour with avocado seed flour and soyabean flour had a significance effect (p < 0.05) on the aroma of different muffin formulations. The samples F and M were significantly different from each other, but not another sample which is shown in Fig: 4.9.

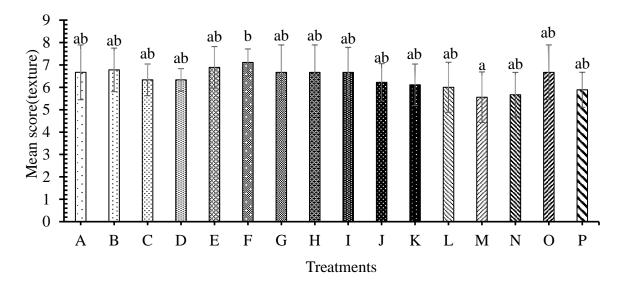


Fig. 4.9 Characteristics of product with respect to texture. The alphabet above the bar graph shows a significant difference.

4.4.6 Overall acceptability

The mean sensory scores of maximum and minimum overall acceptability were found to be 7.222 and 5.667 for the muffin formulations F and M respectively. Statistical analysis showed that the partial substitution of flour with avocado seed flour and soybean flour had a significance effect (p < 0.05) on the aroma of different muffin formulations. The samples A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P were not significantly different from each other which is shown in Fig: 4.10.

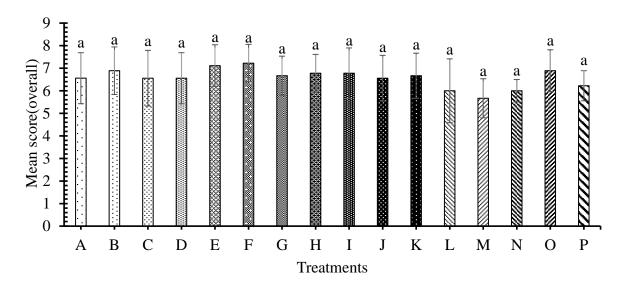


Fig. 4.10 Characteristics of product with respect to overall acceptability. The alphabet above the bar graph shows a significant difference.

4.5 Cost evaluation

The cost of prepared muffins was computed by adding the costs of all raw materials used and excluding the costs of packaging, labor, processing, and profit margin. The cost calculation of the product (Rs/100g) is 34.40 given in Appendix F.

Part V

Conclusion and recommendations

5.1 Conclusions

On the basis of research work, the following conclusions have been drawn.

- The use of avocado seed flour may result in decreased muffin volume, increased weight, and decreased specific loaf volume, but soybean flour may have a less obvious impact on these metrics or possibly boost specific loaf volume slightly due to its protein content.
- 2. The addition of avocado seed flour and soybean flour to muffins boosts protein content while moderating potassium and ash content, resulting in higher nutritional value.
- 3. The sensory quality of muffins prepared with soybean flour and avocado seed did not significantly change.
- 4. The superior way of formulation was discovered to be 3.88 parts avocado seed flour, 0 parts soybean flour, and 96.12 parts wheat flour based on the effect of avocado seed and soybean flour on the specific loaf volume of the muffin (2.39).
- 5. The cost of avocado seed flour and soybean flour replacement muffins was discovered.

5.2 Recommendations

The experiment can be extended using the recommendations that follow:

- 1. Composite flour muffins can be prepared with a combination of avocado seed flour, soybean flour and wheat flour for better nutrients.
- 2. The shelf life of avocado seed flour and soybean incorporated muffin can be studied.

Part VI

Summary

Muffins are a sweet, high-calorie baked food that customers adore for their excellent flavor and delicate texture. Avocado (*Persea americana*) is a tropical fruit that is harvested once a year. However, it has not been commercialized in Nepal. Avocado seed contains more soluble fiber than oat powder and nearly every other food, and it contains 70% of the antioxidants present in an avocado whole. Avocado seed oil is also rich in antioxidants, lowers cholesterol, and aids in the prevention of disease. Soybean flour has substantial nutritional value as a flexible plant-based protein source. It is high in complete protein, which contains all of the essential amino acids needed for growth and development.

For the preparation and quality evaluation of muffin prepared from avocado seed and soybean flour. Response surface and methodology were used. 16 different muffin formulations with the avocado seed, soybean and wheat flour parts. The appearance, color, flavor, taste, texture, and general acceptance were all evaluated. The obtained data were statistically analyzed using two-way ANOVA (no blocking) at a 5% significant level. There is no significant difference from each other. The moisture content, protein, fat, crude fiber, ash and carbohydrate of wheat flour were found to be 11.21%, 10.28%, 1.14%, 0.36%, 0.47% and 87.45% respectively and 14.51%, 7.74%, 0.71%, 4.93%, 2.81%, 4.18% and 68.9% respectively for avocado seed flour and 10.45%, 35.93%, 19.68%, 4.33%, 5.82% and 34.18% respectively or soybean flour. Through analysis, the effect of avocado seed and soybean flour on the specific loaf volume of muffin (2.39) incorporated with 3.88 parts avocado seed flour, 0 parts soybean flour and 96.12 parts wheat flour sample were superior in comparison to all other muffin formulations.

Design Expert 13 was used to create the recipe. A mixed design was used to formulate the recipe. It is concerned with maximizing protein, potassium and minimizing ash content and desirability. 0.98151 parts avocado seed flour, 12.9255 parts soybean flour and 86.093 parts wheat flour can be used having the response quality such as 2.15674 specific loaf volume, 13.5535 protein content, 32.4103 potassium content and 1.89736 ash content respectively (corresponding value Appendix D). The muffin will have the desirability of 0.558.

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Appendices

Appendix A

Sensory Analysis Score Card

Specimen card for sensory evaluation by hedonic rating test

Sensory evaluation for avocado seed and soybean flour substitutes muffin

Name of panelist:

Date:

Dear panelist,

Please give your answer according to degree of preference.

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

Like extremely - 9	Like slightly - 6	Dislike moderately - 3
Like very much - 8	Neither like nor dislike - 5	Dislike very much - 2

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

Parameters	Appearance	Color	Test	Aroma	Texture	Overall acceptability
Sample						acceptacinty
А						
В						
С						
D						
Е						
F						
G						
Н						
Ι						
J						
K						
L						
М						
Ν						
0						
Р						

Any comment

Appendix B

ANOVA table of sensory analysis of avocado seed flour and soybean flour substitutes muffin.

Table B.1 ANOVA (Two-way no blocking) at 5% level of significance for appearance

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	L sd
Muffin_type	16	60.4706	3.7794	5.32	<.001	0.7862
Panelist	9	7.0588	0.8824	1.24	0.280	0.5721
Residual	128	90.9412	0.7105			
Total	152	158.4706				

Table B.2 ANOVA (Two-way no blocking) at 5% level of significance for color

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	L sd
Muffin_type	16	42.3268	2.6454	3.21	<.001	0.8466
Panelist	9	27.8954	3.4869	4.23	<.001	0.6160
Residual	128	105.4379	0.8237			
Total	152	175.6601				

Source of variation	d.f.	S.S.	m.s.	V.I.	F pr.	L sd
Muffin_type	16	19.7647	1.2353	1.38	0.162	0.8830
Panelist	9	19.2941	2.4118	2.69	0.009	0.6425
Residual	128	114.7059	0.8961			
Total	152	153.7647				

Table B.3 ANOVA (Two-way no blocking) at 5% level of significance for taste

Table B.4 ANOVA (Two-way no blocking) at 5% level of significance for aroma

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	L sd
Muffin_type	16	12.5490	0.7843	0.93	0.535	0.8555
Panelist	9	13.4248	1.6781	1.99	0.052	0.6225
Residual	128	107.6863	0.8413			
Total	152	133.6601				

Source of variation	d.f.	S.S.	m.s.	V.I.	F pr.	L sd
Muffin_type	16	27.3595	1.7100	2.08	0.013	0.8448
Panelist	9	27.8954	3.4869	4.25	<.001	0.6147
Residual	128	104.9935	0.8203			
Total	152	160.2484				

Table B.5 ANOVA (Two-way no blocking) at 5% level of significance for texture

Table B.6ANOVA (Two-way no blocking) at 5% level of significance for overall

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.	L sd
Muffin_type	16	23.6078	1.4755	1.56	0.088	0.9059
Panelist	9	15.0327	1.8791	1.99	0.052	0.6592
Residual	128	120.7451	0.9433			
Total	152	159.3856				

Appendix C

Source	Sequential p-value	Lack of fit p-value	Adjusted R ²	Predicted R ²	
Linear	0.0007		0.6207	0.4418	
Quadratic	< 0.0001		0.9990	0.9981	Suggested
Special cubic	0.3619		0.9990	0.9958	
Cubic	0.4997		0.9990	0.9760	
SP Quartic vs Quadratic	0.7043		0.9989	0.9897	
Quartic vs Cubic			1.0000		Aliased
Quartic vs Sp Quartic			1.0000		Aliased

Table C.1 Model summary statistics for specific loaf volume
--

 Table C.2 Analysis of variance (ANOVA) for a quadratic model of specific loaf volume.

Source	Sum o	of df	Mean	F-value	p-value	
	Squares		Square			
Model	0.2452	5	0.0490	3145.65	< 0.0001	Significant
Linear mixture	0.1647	2	0.0824	5282.33	< 0.0001	
AB	0.0158	1	0.0158	1015.87	< 0.0001	
AC	0.0484	1	0.0484	3105.10	< 0.0001	
BC	0.0004	1	0.0004	22.56	0.0008	
Residual	0.0002	1	0.0002			
		0				
Lack of Fit	0.0002	6	0.0002			not significant
Pure Error	0.0000	4	0.0000			
Cor Total	0.2454	1				
		5				

Source	Sequential	Lack of fit	Adjusted	Predicted	
	p-value	p-value	\mathbb{R}^2	\mathbb{R}^2	
Linear	0.0004	< 0.0001	0.6538	0.5565	Suggested
Quadratic	0.4638	< 0.0001	0.6477	0.4795	
Special cubic	0.9830	< 0.0001	0.6086	-0.1253	
Cubic	0.0010	< 0.0001	0.9542	0.1807	
Sp Quartic vs Quadratic	0.0991	< 0.0001	0.7834	-0.4427	Suggested
Quartic vs Cubic	< 0.0001		0.9996		Aliased
Quartic vs Sp Quartic	<0.0001		0.9996		Aliased

 Table C.3 Model summary statistics for protein

Table C.4 Analysis of variance (ANOVA) for the linear model of protein content

Source	Sum Squares	of	df	Mean Square	F-value	p-value	
Model	35.55		2	17.77	15.61	0.0004	significant
Linear mixture	35.55		2	17.77	15.61	0.0004	
Residual	15.24		13	1.17			
Lack of Fit	15.24		9	1.69	1314.93	0.413	not significant
Pure Error	0.0051		4	0.0013			
Cor Total	50.78		15				

Source	Sequential	Lack of fit	Adjusted	Predicted	
	p-value	p-value	\mathbf{R}^2	\mathbb{R}^2	
Linear	< 0.0001		0.9999	0.9999	Suggested
Quadratic	0.1531		0.9999	0.9999	
Special cubic	05153		0.9999	0.9998	
Cubic	0.1514		0.9999	0.9988	
Sp Quartic vs Quadratic	0.6953		0.9999	0.9993	
Quartic vs Cubic			1.0000		Aliased
Quartic vs Sp Quartic			1.0000		Aliased

Table C.5 Model summary statistics for potassium content

Table C.6 Analysis of variance (ANOVA) for linear model of potassium content

Sum of Squares	df	Mean	F-value	p-value	
		Square			
2353.72	2	1176.86	81712.50	< 0.0001	Significant
2353.72	2	1176.86	81712.50	< 0.0001	
0.1872	13	0.0144			
0.1872	9	0.0028			not significant
0.0000	4	0.0000			
2353.91	15				
	2353.72 2353.72 0.1872 0.1872 0.0000	2353.72 2 2353.72 2 0.1872 13 0.1872 9 0.0000 4	Square 2353.72 2 1176.86 2353.72 2 1176.86 0.1872 13 0.0144 0.1872 9 0.0028 0.0000 4 0.0000	Square Square 2353.72 2 1176.86 81712.50 2353.72 2 1176.86 81712.50 0.1872 13 0.0144	Square Image 2353.72 2 1176.86 81712.50 <0.0001

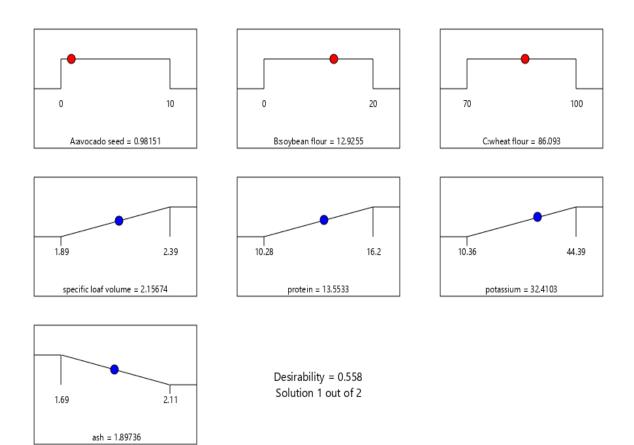
Sequential	Lack of fit	Adjusted	Predicted	
p-value	p-value	\mathbb{R}^2	\mathbb{R}^2	
0.0001		0.0627	0.0007	
<0.0001		0.8627	0.8007	
0.0035		0.9516	0.8969	
0.0120		0.9743	0.9624	Suggested
0.4543		0.9743	0.8438	
0.1013		0.9700	-0.0363	
		1.0000		Aliased
		1.0000		Aliased
	p-value <0.0001 0.0035 0.0120 0.4543	p-value p-value <0.0001 0.0035 0.0120 0.4543	<0.0001	p-value p-value R ² R ² <0.0001

Table C.7 Model summary statistics for ash content

 Table C.8 Analysis of variance (ANOVA) for special cubic model of ash content

Source	Sum of Squares	df	Mean	F-value	p-value	
			Square			
Model	0.2754	6	0.0455	95.79	< 0.0001	significant
Linear mixture	0.2465	2	0.1232	257.14	< 0.0001	
AB	0.0054	1	0.0054	11.21	0.0085	
AC	0.0002	1	0.0002	0.3140	0.5889	
BC	0.0097	1	0.0097	20.17	0.0015	
ABC	0.0407	1	0.0047	9.85	0.0120	
Residual	0.0043	9	0.0005			
Lack of Fit	0.0043	5	0.0009			not-significant
Pure Error	0.0000	4	0.0000			
Cor Total	0.2797	15				

Appendix D



Appendix E

Ingredients	Wheat flour	Avocad o seed flour	Soyabea n flour	Sp. loaf volume	Protein content	Potassium content	Ash content
А	93.26	0	6.74	2.15	12.21	21.65	1.75
В	88.31	0	11.69	2.13	12.42	30.42	1.84
С	100	0	0	2.22	10.28	10.65	1.69
D	83.36	0.73	15.91	2.13	12.42	37.57	1.98
Е	78.38	1.62	20	2.12	16.1	44.39	2.11
F	78.38	1.62	20	2.12	16.2	44.39	2.11
G	96.12	3.88	0	2.39	10.94	10.36	1.79
Н	96.12	3.88	0	2.39	10.93	10.36	1.79
Ι	88.5	4.68	6.82	2.3	14.83	21.73	1.83
J	83.06	5.67	11.27	2.23	13.25	29.21	1.99
K	83.06	5.67	11.27	2.23	13.26	29.21	1.99
1	75.33	7.15	17.52	2.09	13.34	39.61	2.06
Μ	87	10	3	1.89	14.99	43.49	2.09
Ν	87	10	3	2.28	11.25	14.75	1.96
0	78.17	10	11.83	2.28	11.24	14.75	1.96
Р	70	10	20	2.07	12.56	29.71	2.05

Table E.1 Recipe formulation of muffin for experimental design and resulted data

Appendix F

Ingredients	Rate	Amount	Cost (Rs)
Wheat flour	70/kg	100g	7
Avocado	300/kg	10	3
Soyabean	120/kg	20	2.4
Sugar	80/kg	60	4.8
Butter	850/kg	68	57.8
Egg	400	57	13.33
Baking powder	45/100g	2.8	1.26
Raw material cost			89.59
Processing and labour cost			8.959
(10% of raw material cost)			
Profit (10%)			10.802
Grand total cost			109.351
Average weight of muffin		317.8	
batter			
No of muffin formed		10	
Per piece weight of muffin		31.78	
Per price weight of muffin			10.935
Total cost of muffin (Rs/100g)			34.40

 Table F.1 Cost evaluation of muffin

Appendix G

Apparatus

- i. Oven
- ii. Electronic balance
- iii. Measuring cylinder, beaker, pipette, Volumetric flask, conical flask, test-tube, funnel
- iv. Soxhlet assembly
- v. Buchner filter assembly
- vi. Hot air oven
- vii. Muffle furnace
- viii. Petriplate
- ix. Dean and stark apparatus

Chemicals required

- i. Petroleum ether
- ii. Acetone
- iii. Sulfuric acid
- iv. Sodium hydroxide
- v. Hydrochloric acid
- vi. Boric acid
- vii. Catalyst mixture
- viii. Alcohol
 - ix. Sodium thiosulphate
 - x. Potassium dichromate
 - xi. Phenolphthalein

Color Plates



P1: Drying avocado seed

P2: Prepared samples



P3: Panelist performing sensory



P4: Prepared sample for chemical analysis

