PREPARATION OF LOW GLUTEN MUFFIN FROM COMPOSITE (WHEAT AND BUCK WHEAT) FLOUR BY USING (BUTTER AND AVOCADO) FAT

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

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Approval Letter

This ***dissertation*** entitled ***Preparation of Low Gluten Muffin From Composite (Wheat And Buck Wheat) Flour By Using (Butter And Avocado) Fat*** presented by **Bhagawan Thapa** has been accepted as the partial fulfillment of the requirement for the **B. Tech.** degree in **Food Technology**.

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Abstract

This work was carried out to develop low-gluten muffins by substituting wheat with varying ratios of buckwheat while maintaining a consistent blend of avocado and butter and evaluating its sensory and physicochemical properties. Raw material (wheat flour, buckwheat flour, butter, sugar, and egg) and avocado (Hass variety) were collected from the local market of Dharan. Proximate analysis of wheat flour, buckwheat flour, and avocado puree was carried out. The experimental design involved the formulation of samples denoted as A, B, C, D, E, F, and G, wherein a composite flour mixture was employed. Mixed design (simple lattice design) was used to formulate the recipe. This mixture comprised various ratios of wheat to buckwheat flour, specifically 100:0, 75:25, 66:33, 50:50, 33:66, 25:75, and 0:100, respectively. These ratios were meticulously combined with sugar (60%), egg (57%), and baking powder (1.42%) per 100 parts of the flour mixture. The incorporation levels of the flour ranged systematically from 0 to 100 parts. The superior product obtained through sensory evaluation and acceptability period was estimated in ambient conditions.

Moisture content, crude protein, crude fat, crude fibre, total ash, and carbohydrate content of wheat flour was found to be 11.53%, 10.18%, 1.13%, 0.45%, 0.46%, and 87.74% respectively, and 12.8%, 13.59%, 3.05%, 1.48%, 0.96%, and 79.95% respectively for buckwheat flour. Through sensory evaluation, the muffin incorporated 50 parts wheat flour and 50 parts buckwheat flour (sample D) was superior in comparison to all other muffin formulations. Statistical analysis ($p \leq 0.05$) showed that the substitution of buckwheat flour significantly improved all the physicochemical attributes (total ash content, fibre, crude protein, crude fat) except carbohydrate compared to muffin without buckwheat flour whereas significantly decreased the specific loaf volume of muffin. The free fatty acid as oleic acid and peroxide value of sample D at day 0 was found to be 1.635 mg KOH/g oil and 1.366 Meq O2/kg fat respectively. Sample D was fit for consumption for day 4 and day 10 respectively.

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List of Abbreviations

Abbreviation	**Full form**
ANOVA	Analysis of variance
AV	Acid value
PV	Peroxide value
Wb	Wet basis
Db	Dry basis
LSD	Least significant difference
SD	Standard deviation
AACC	American Association of Cereal Chemists
TPA	Texture Profile Analysis
AOAC	Association of Official Analytical Chemists
MUFA	monounsaturated fatty acids
SFA	saturated fatty acids
PUFA	polyunsaturated fatty acids
NCGS	Non-celiac gluten sensitivity
SDF	Soluble dietary fiber

Part I

Introduction

1.1 General Introduction

Muffins are sweet, high-calorie baked products that are highly appreciated by consumers due to their good taste and soft texture (Martínez-Cervera *et al.*, 2012). Muffins can be categorized as a quick bread product made with baking powder as a leavening agent (Arifin *et al.*, 2019). The principal ingredients of muffins include flour, sugar, fat, and egg. Each ingredient plays an important role in the structure, appearance, and eating quality of the final product. Butter is commonly used as a fat ingredient in muffin formulation. It contributes to the desirable mouthfeel, and unique texture in muffins as well as provides unique aroma, and flavor extension (Brown, 2018).

Traditionally, a muffin batter recipe is mainly composed of wheat flour (WF), sucrose, vegetable oil, egg, and milk (Sanz *et al.*, 2009). Flour contains carbohydrates as well as the proteins glutenin and gliadin, which work jointly to hold other ingredients together and provide structure to the finished baked product (Baniya, 2022). Starch gelatinizes when it is hydrated and heated, breaking hydrogen bonds and causing starch granules to expand, giving the batter a more solid structure (McWilliams, 2006).

Gluten is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin (Biesiekierski, 2017). Celiac disease, also known as gluten-sensitive enteropathy and nontropical sprue, is a prevalent autoimmune disorder that is triggered by the ingestion of wheat gluten and related proteins of rye and barley in genetically susceptible individuals (Briani *et al.*, 2008). The classical presentation of Celiac disease consists mainly of gastrointestinal symptoms associated with malabsorption including diarrhea, steatorrhea, weight loss, or failure to thrive. Other extra-intestinal symptoms include iron deficiency, recurrent abdominal pain, aphthous stomatitis, chronic fatigue, short stature, and reduced bone density (Kelly *et al.*, 2015).

Buckwheat is a gluten-free pseudocereal that belongs to the Polygonaceae family (Sanchez *et al.*, 2011). The most widely grown buckwheat species include common buckwheat (Fagopyrum esculentum) and tartary buckwheat (*F. tataricum*), it is a pseudocereal but its grains belong to cereals because of their similar use and chemical composition (Z.-L. Zhang *et al.*, 2012). Among a variety of buckwheat species, nine have agricultural and nutritional value (Krkošková and Mrázová, 2005). Buckwheat grains are an important source of microelements, such as Zn, Cu, Mn, and Se (Stibilj *et al.*, 2004), and macroelements: K, Na, Ca, and Mg (Wei et al. 2003). With 80% unsaturated fatty acids more than 40% are constituted by polyunsaturated fatty acid (PUFA) (Krkošková and Mrázová, 2005). The significant contents of rutin, catechins, and other polyphenols as well as their potential antioxidant activity are also of significance to the dietary value (Oomah and Mazza, 1996; Watanabe, 1998). Moreover, buckwheat grains are a rich source of TDF (total dietary fibre), and soluble dietary fibre (SDF), and are applied in the prevention of obesity and diabetes (Brennan, 2005).

Fat is also an important component in muffins. Fat is used as a tenderizer and lubricant on the oil side and with the solid fat it also provides structure. Fat also contributes to the creaminess, appearance, palatability, texture, and lubricity of foods and increases the feeling of satiety during meals (Romanchick-Cerpovicz *et al.*, 2002). It was used as a moisture barrier. Without the fat to prevent the loss or gain of moisture the baked product may either dry out or be soggy (Bennion and Bamford, 1997). Fat contributes to most of the calories in an avocado. A 1000-kJ portion of avocado contains about 25 g of fat, most of which are healthier monounsaturated fatty acids (MUFA). Most lipids found in avocados are polar lipids (glycolipids and phospholipids), which play a fundamental role in various cellular processes such as the functioning of the cell membranes as second messengers These lipids are also used to make emulsions of water and lipids.

Increasing sensitivity to wheat gluten, an increase in the number of cases of celiac disease, and obesity the serious public health problems all around the world. Celiac disease (CD) is a life-long autoimmune disease in the small intestine that affects genetically susceptible individuals worldwide. CD is one of the most common genetic diseases that result from both environmental (gluten) and genetic (HLA and non-HLA genes) factors (Gujral *et al.*, 2012). CD is a serious genetic autoimmune disease that damages the villi of the small intestine and interferes with the absorption of nutrients from food (National Foundation for Celiac Awareness, 2011). Greater awareness of celiac disease throughout the world has led to growing demand for gluten-free products such as cookies, bread, pasta, and cakes (Gallagher *et al.*, 2003). According to a study done at the Centre for Celiac Research at the University of Maryland, individuals who took 50 mg of gluten per day experienced villous atrophy after just 90 days, who consumed either 10 mg or no gluten showed no discernible alterations.

Therefore, several gluten-free bakery products containing buckwheat have been developed, such as gluten-free bread (Torbica *et al.*, 2010; Wronkowska *et al.*, 2010), biscuits (Schober *et al.*, 2003), spaghetti (Verardo *et al.*, 2011) and crackers (Sedej *et al.*, 2011).

1.2 Statement of Problem

Non-communicable diseases (NCDs), such as obesity and type 2 diabetes, are related to an unbalanced diet (Lustig et al., 2012; Malik et al., 2006; Palmer et al., 2008). Therefore, consumers have become aware of the issue and changed their food habits by reducing sugar and fat consumption while increasing their dietary fiber intake (Bianchi et al., 2023). On the other hand, developing novel and healthier food products is a real challenge for bakers and food companies. Due to the rising incidence of gluten-related illnesses and the growing desire for better food options, the consumption of gluten-free and low-gluten goods has significantly increased. The current study intends to respond to this trend by addressing the following major issues related to making low-gluten muffins with composite flour (wheat and buckwheat) and fats from both conventional (butter) and non-conventional (avocado) sources. Halliday and Noble (1946) reported that both trans and saturated fats should be avoided for a healthier lifestyle. Researchers have been experimenting with a variety of ingredients in order to develop adequate fat replacers to meet the high customer demand for healthy products, taking into account both fat quality and fat importance in bakery products. Avocado lipids are made up of 71% monounsaturated fatty acids (MUFA), 13% polyunsaturated fatty acids (PUFA), and 16% saturated fatty acids (SFA)

1.3 Objectives

1.3.1 General Objectives

The general objective of this dissertation work is preparation of low gluten muffins from composite (wheat and buck wheat) flour by using (butter and avocado) fat.

1.3.2 Specific objectives

1. To carry out the chemical analysis of raw material i.e. wheat flour, buckwheat flour, and avocado puree.
2. To prepare the buckwheat flour and wheat muffin at their different proportions.
3. To analyze the physical, chemical and sensory properties of prepared muffins.
4. To estimate the acceptability period of the muffin.
5. To perform cost evaluation of the product.

1.4 Significance of study

Non-celiac gluten sensitivity (NCGS) is an emerging syndrome, affecting up to 6% of the general population in Western countries, although the exact prevalence is as yet unknown (Sapone *et al.*, 2012). NCGS consists of a combination of intestinal and/or systemic symptoms, similar to those of celiac disease (CD) and irritable bowel syndrome (IBS) (Volta *et al.*, 2015). The most frequent intestinal symptoms are bloating and abdominal pain, alternating bowel habits, and nausea. Additional extra-intestinal manifestations include exhaustion, lack of wellbeing, headaches, anxiety, “foggy mind”, arm/leg numbness, muscle or joint pain and depression (Eswaran *et al.*, 2013a). In recent years, an increase of the diagnosed cases of celiac and non-typical celiac diseases, or allergic reaction/ intolerances to gluten consumed in food products, caused a growing interest in gluten-free products. A diet based on gluten-free products is characterized by a low content of some nutritional components such as proteins and mineral components, as well as non-nutritional but physiologically important components like dietary fibre (Wronkowska and Soral-Smietana, 2008).

This study will focus on the scope of preparation of low gluten muffin from composite flour. Once the study is completed, it will be beneficial to the health of the consumer. Furthermore, the improved bakery product can be produced.

1.5 Limitations of the study

1. Instrumental textural analysis was not carried out due to lack of rheometer. Since, Texture Profile Analysis (TPA) was performed on muffins at a 25°C. The samples (20 mm × 20 mm × 20 mm) were measured by a two-bite compression test using rheometer.

Part II

Literature review

2.1 Muffins

Muffins are individual-sized baked quick bread products characterized by their distinctive round shape, leavened primarily through the use of baking powder or baking soda rather than yeast. Muffins are often characterized by a rounded shape, either domed or flat on top, depending on the recipe and baking conditions. The exterior may have a slightly golden-brown color, adding to the visual appeal. English muffins originating in London were made from yeast dough, in contrast to the quick bread muffins served in early America (Cross, 2004). Consumption of baked products constitutes an important part of a daily breakfast considering that people are continually grabbing meals on the go (Baniya, 2022). Among baked products, muffins rank third in breakfast products and attract a broad range of consumers (Rosales-Soto *et al.*, 2012). The principal ingredients of muffins include flour, sugar, fat, and egg. Each ingredient plays an important role in the structure, appearance, and eating quality of the final product (Arifin *et al.*, 2019).

2.2 Chemical composition of muffins

Chemical composition of muffin is shown in Table 2.1

Table 2.1 Chemical composition of muffin

Constituent	Value
Moisture, %	20.33
Fat, %	14.37
Protein, %	17.6
Carbohydrate, %	44.28
Total dietary fibre, %	2.22
Ash, %	1.21

Source: Rahman *et al.* (2015)

2.3 Ingredients and their role in muffin making

2.3.1 Flour

Flour is the primary ingredient in baked products. Flour represents 30–40% of the total batter weight in most cake muffins. Flour contains starch and the proteins glutenin and gliadin, which hold other ingredients together to provide structure to the final baked product. Hydration and heat promote gelatinization of starch, a process that breaks hydrogen bonds, resulting in swelling of the starch granule, which gives the batter a more rigid structure (McWilliams, 2006).

2.3.1.1 Requirements of flour characteristic

The flour should be free flowing, dry to touch, creamy in color and free from any visible bran particles. It should also have a characteristic taste and should be free from musty flavor and rancid taste. The characteristics as required in flour is shown in Table 2.2.

Table 2.2 Requirements of flour characteristic

Characteristics	Requirements
Moisture content	13.0% max
Gluten content on dry basis	7.5% max
Total ash on dry basis	0.5% max
Acid insoluble ash on dry basis	0.5% max
Protein (Nx7.5) on dry basis	9.00%
Alcohol acidity as H2SO4 in 90% alcohol	0.10%
Water absorption	5%
Sedimentation	22%
Granularity	To satisfy the taste

Source: Arora (1980b)

2.3.2 Shortening

Shortening contributes to the eating qualities of tenderness, flavor, texture, and a characteristic mouth feel. Fat keeps the crumb and crust soft and helps retain moisture, and thus contributes to keeping qualities or shelf-life. Fat enhances the flavor of baked products because flavor components dissolve in fat (McWilliams, 2006). The main action of the fat or shortening during mixing is to avoid the gluten forming proteins to come in contact with water by insulating the gluten forming protein molecules due to its hydrophobic nature. Hence, less tough dough with desired amount of gluten formation can be obtained. Thus, shortened baked products possess less hard, crispier nature and can easily melt in mouth. The fat(butter) should possess reasonable shelf life on its own without the addition of antioxidants. The acid value and peroxide value of the extracted fat should not exceed 0.5 mg KOH/g oil and 10 MeqO2/kg fat respectively (Mukhopadhyay, 1990). The acid value and peroxide value of the extracted fat (butter) should not exceed 6 mg KOH/g oil and 10 MeqO2/kg fat respectively

However, bakery products are often not consumed and referred to by health conscious and obese people owing to the high fat content. The addition of the functional ingredients to bakery products has risen in popularity due to the ability to reduce risk of chronic diseases beyond basic nutritional function (Eswaran *et al.*, 2013).

2.3.3 Fat replacer

Fat replacers are substances used in food manufacturing to mimic the texture and mouthfeel of fats while reducing the overall fat content in a product. These substances are employed to create low-fat or reduced-fat versions of various food items without sacrificing taste and texture.

There are three types of fat replacers, according to *Leveille and Finley* (1997): fat mimetics, low-calorie fats, and fat substitutes.

Fat mimetics have the same size and mouth feel as fats; however, they give fewer calories to the body. Starch, cellulose, pectin, protein, and dextrins are common fat-mimicking substances. Fat mimics reduce calories not just because they have a lower caloric density than fats, but also because they include a lot of water, which substitutes some of the fat.

Low-calorie fats are genuine fats with a structure that assures they offer the body few calories. For example, salatrim has both short and long fatty acids. The short ones contain less calories, while the long ones are poorly absorbed, providing only approximately five calories per gram.

Fat Substitutes are the compounds that are functionally closest to fats. They are heat stable, which is not the case with all fat substitutes. Because of their chemical composition, these compounds supply fewer calories than normal fats. Olestra is an example of a fat replacement. Olestra is made up of sucrose sugar and from six to eight fatty acids. Because humans are unable to digest and absorb olestra due to the way the fatty acids are bonded to the sucrose, it provides no calories. In 2022 Barsha Baniya determined the effect of avocado puree as fat replacer in the composite flour (wheat and oats) muffin (Baniya, 2022).

2.3.4 Sweeting agent

Another important ingredient in muffins is sugar. Sugar is a main ingredient of muffins and other baked products, so removal or reduction of sucrose negatively affects product appearance, texture, and mouthfeel (Gao *et al.*, 2017). Therefore, the replacement of sucrose content by artificial or natural sweeteners in the production of bakery products represents a challenge for the food industry (Di Monaco *et al.*, 2018; Luo *et al.*, 2019; Sahin *et al.*, 2019). Intense or non-caloric sweeteners (sucralose, saccharin, cyclamate, stevia, etc.) have great sweetening power; however, they do not contribute to the formation of the body of the bakery product (Struck *et al.*, 2014). On the other hand, energy sweeteners (monosaccharides, disaccharides, polyalcohols, etc.) can give rise to bakery products with stable structure, but they tend to lack flavor. Nevertheless, some natural agents may combine the best qualities of both groups of sweeteners: good sweetening power and a stable structure of the final bakery product; this group of sweeteners includes agave syrup (AS) (Liang and Were, 2018; Rothschild *et al.*, 2015; Struck *et al.*, 2014; Zamora-Gasga *et al.*, 2014).

2.3.5 Leavening agent

Leavening agents are substances that are used in baking to make dough or batter rise, resulting in a lighter and softer texture in the finished product. There are two main types of leavening agents: chemical and biological. Chemical leaveners include ammonium and sodium bicarbonate, whereas biological leaveners include yeasts. Similarly, mechanical leavening can be accomplished by mechanically agitating the dough matrix to include air. The combination of two or more chemicals can also result in the formation and incorporation of gas; for example, the reaction between ammonia bicarbonates and sodium with acidulants (Baniya, 2022). Among leavening agents, baking powders are widely used in muffin preparation. Commercial formulations are generally a mixture of inorganic compounds (baking soda and acid salts) that react in presence of moisture and heat to release CO_2 (Carullo *et al.*, 2020). Baking powders differ from one another in relation to diverse acidic constituents. Most of the common acid components are phosphate salts, showing the drawback of negatively affecting the flavor of the final product (Beeren *et al.*, 2019).

2.3.6 Whole egg

Beaten egg white, like fat, helps to retain gas bubbles, while egg alone acts as a binder (Bhaduri, 2013).

2.3.7 Water

A certain amount of water is also needed for the formation of gluten and gelatinization of starch, which plays an important role in forming the muffin frame work (Rismaya *et al.*, 2022). The matrix network structure is crucial in trapping air or gas during baking, which in turn determines the expansion volume (Rathnayake *et al.*, 2018). Dissolved minerals and organic matters present in water can affect the flavor, color and physical attributes of the finished baked (Smith, 1972).

The water used in the baking product should be potable and odorless if required, although no significant effect has been noticed due to the hardness, but demineralization is recommended if the mineral content is too higher which might cause an adverse in product color (Arora, 1980). The moisture content between 15-30% is acceptable (Sani *et al.*, 2014).

2.4 The muffin method of mixing

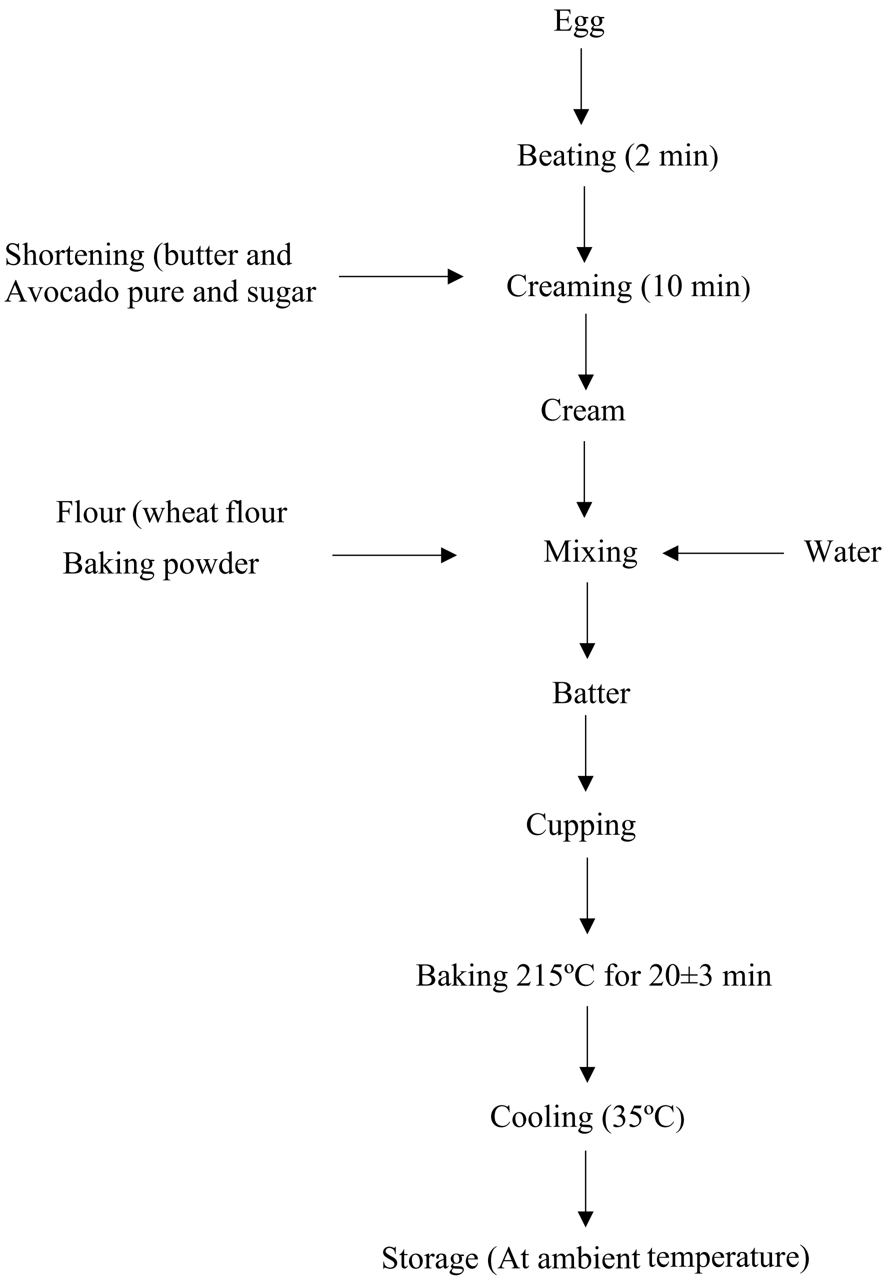
The muffin method is a mixing technique commonly used in baking muffins and other quick breads. It is designed to produce a tender and moist texture in the finished product. The key to the muffin method is to mix the wet and dry ingredients separately and then combine them with minimal mixing.

2.5 Objective of mixing

The primary objective in mixing is to achieve a homogenous mixture; generally, this means, attaining a nearly uniform distribution of the ingredient. A distinction may be drawn between batch and continuous process. Overall, the concentration of the ingredient should uniformly distributed in the output stream, should not vary with time and the processing of each part of the mixture should be same (Ashokan *et al.*, 2013).

2.6 Preparation of muffin

First ingredients were divided into dry and wet ingredients. The dry ingredients included wheat flour, buckwheat flour, baking powder and sugar. The wet ingredients were egg, water and butter. The egg was beaten for 2 min, butter and grinded sugar was creamed for 10 min separately. In a separate bowl, all dry ingredients along with beaten egg and creamed butter 11 and sugar were mixed, to obtain muffin batter. The batter was filled in paper muffin cup. The muffins were baked at 215°C in oven for 20±3 min (Khouryieh *et al.*, 2005).



Source: Lamsal (2018)

Figure 2.1 Flow chart of wheat muffin

2.7 Baking Profile

Baking is a decisive stage in the production of bakery products for most of the quality attributes of the final products depend on it (Markus Schirmer *et al.*, 2011). It is a complex process that combines heat and mass transfer between the environment and the product, in which both are responsible of transforming the mixture or initial dough into the final product (Purlis, 2011).

The baking process itself is a decisive factor in producing high-quality baked goods. Baking is considered a simultaneous heat and mass transfer process, characterized by a rapid increase of the core temperature and the development of a dry surface crust. Also, the increase of the internal temperature is associated to several chemical reactions and physical changes, responsible for both the transformation of the cake batter into crumb and the product volume expansion. In consequence, baking process conditions—oven temperature, baking time, and oven humidity—strongly influence the development of all quality attributes (Asselman *et al.*, 2007).

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

Temperature in the baking oven has different effect on the raw dough, which is shown in Table 2.3

Table 2.1 Temperature related changes in muffin during baking

Temperature °C	Changes occurred
32.22-37.78	Top crust skin formation (Evaporation of surface moisture)
32.22-48.89	Evolution of CO2 within crumb (Less solubility of CO2)
32.22-65.56	Increase in volume due to CO2
32.22-98.89	Gas Expansion (CO2 and steam)
51.67-98.89	Starch gelatinization (Muffin structure)
76.67-121.11	Coagulation of protein (Irreversible)
187.78-204.44	Dextrinization (surface gloss)

Source: Mukhopadhyay (1990)

More steam is required in the oven during baking than that produced by the moisture in the dough and the combustion of the fuel. Steam introduced into the baking chamber, either at the time the dough pieces are placed in the oven or at a point very early in their journey through the oven, aids in the formation of a shiny crust, the prevention of cracked crusts, increased volume, and to some extent agitation of the oven atmosphere. The use of fast-moving fans recirculating air at speeds of 2000 cu ft. per min can eliminate the necessity for steam injection. The oven dampers are important for releasing the strong positive pressure caused by high heat evaporation within the oven; similarly, if high moisture cookies or biscuits are wanted, the dampers in the last zone must be closed (Smith, 1972).

2.8 Buck Wheat

Buckwheat is a traditional crop in Asia and Central and Eastern Europe (Wijngaard and Arendt, 2006). Buckwheat (Fagopyrum esculentum Möench) is an annual crop, it is a pseudocereal but its grains belong to cereals because of their similar use and chemical composition (Campbell, 1997). Common buckwheat (Fagopyrum esculentum Moench) is the most commonly grown species, while two other species of buckwheat (*F. tataricum* Gaertner and *F. emarginatum*) have been cultivated on a small scale (Marshall and Pomeranz, 1982; Mazza and Oomah, 2005). Buckwheat is the only pseudocereal that contains rutin, hence it is a beneficial source of this flavonoid. Other phenolic compounds and flavones such as hyperin, quercitrin, and quercetin have been detected and isolated from immature buckwheat seeds (Koyama *et al.*, 2013).

2.8.1 Chemical and Nutritional composition of Buck Wheat

Buckwheat grains contain a variety of nutrients, the main compounds being: proteins, polysaccharides, dietary fibre, lipids, rutin, polyphenols, micro- and macroelements (Kim *et al.*, 2004). The total content of components depends on the variety or environmental factors (Bárta *et al.*, 2004).

In literature, the protein content of buckwheat grains has been reported to range from 12% to 18.9% as shown in the table below.

Table 2.2 Content of proteins in buckwheat grains

S.N.	N (% d.m.) x 6.5
A	12.0 - 13.0
B	12.11
C	13.30 - 15.55
D	8.51 - 18.87
E	12.02

Source: A: Steadman *et al.* (2001)

B: Si-quan and Zhang (2001)

C: Yi-min Wei *et al.* (2003)

D: Krkošková and Mrázová (2005)

E: Stempińska and Soral-Śmietana (2006)

Buckwheat proteins are rich in arginine and lysine, the primary amino acids limiting the content of proteins in cereals, whereas the contents of methionine and threonine in buckwheat proteins are low as shown in the table below.

Table 2.3 Amino acids of buckwheat grains

Amino acids	A (% w/w)	B (% w/w)	C (% w/w)
Lysine	6.17	4.9	5.68
Histidine	2.44	1.4	2.52
Arginine	8.85	5.4	11.16
Glutamic acid	15.37	9.7	19.38
Aspartic acid	9.1	5.2	9.54
Threonine	4.04	1.9	3.5
Serine	4.89	2.4	4.61
Proline	4.57	2.6	7.93
Glycine	6.23	4.2	5.66
Alanine	4.83	3	3.89
Valine	4.97	3.4	4.26
Isoleucine	3.41	2.6	3.12
Leucine	6.12	2.8	5.94
Methionine	0.99	1.6	2.3
Tyrosine	1.94	1.5	3.03
Phenylalanine	4.42	2	4.3
Tryptophane	2.14	1.5	2

Source: A: Soral-Śmietana *et al.* (1984)
B: Yi-min Wei *et al.* (2003)
C: Tomotake *et al.* (2006)

Buckwheat is rich in potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). P, K, and Mg are most concentrated in bran, particularly in the bran from which the hulls were removed before milling the grains (Christa & Soral-Śmietana, 2008). Buckwheat may be an important nutritional source of such microelements as iron (Fe), manganese (Mn), and zinc

(Zn) (Y-M Wei *et al.*, 1995). The different minerals found in the buck wheat is given in the table below.

Table 2.4 Minerals in Buck Wheat

Elements	Values
P (%)	0.1
K (%)	0.15
Ca (%)	0.001
Mg (ppm)	0.09
Cu (ppm)	8.1
Fe (ppm)	67
Mn (ppm)	5
Zn (ppm)	34

Source: De Francischi *et al.* (1994)

2.8.2 History of Buck Wheat cultivation

China was the place where the cultivation of buckwheat commenced (Radics and Mikóházi, 2010). Buckwheat diffused to the Himalayan regions and Tibet from southern China. Buckwheat probably diffused to Japan through northern China and the Korean peninsula. As for the European emergence of buckwheat, it diffused first to northern China than it reached Europe and Pakistan via the Silk Road (Murai and Ohnishi, 1996). Although buckwheat became popular in Europe in the Middle Ages, it may already had been introduced into Europe in very ancient times (first or second century or earlier) on the basis of archaeological evidences (Ohnishi, 1993).

2.8.3 Buck Wheat as a functional food

Buck wheat is recognized as a good source of nutritionally valuable protein, lipid, dietary fiber, and minerals, and in combination with other health-promoting components, such as phenolic compounds and sterols, it has received increasing attention as a potential functional food (Krkošková and Mrázová, 2005). It has been described that the consumption of Buck wheat and buck wheat-enriched products is related to a wide range of biological and healthy activities: hypocholesterolemic, hypoglucemic, anticancer, and anti-inflammatory (Gimenez-Bastida and Zielinski, 2015).

2.8.4 Buck Wheat flour

Although other parts of the buckwheat plant can be used for human consumption and animal feed, buckwheat is now mainly grown for the production of seeds (Belton and Taylor, 2002). Buckwheat seeds are usually processed into flour. Buckwheat seeds are dehulled before milling or the flour is sieved afterward. The milling of buckwheat grains provided an average flour yield of 56.5% (Wijngaard and Arendt, 2006)

2.9 Wheat

Wheat is botanically named as Triticum vulgare. Wheat is counted among the 'big three' cereal crops, with over 600 million tonnes being harvested annually (Shewry, 2009). Wheat flour for muffin making is obtained from the endosperm in the form of particle size enough to pass through a flour sieve usually 100 mesh per linear inch (Kent-Jones and Amos, 1967). Apart from its major constituent starch, wheat flour also contains many other types of substances of which the gluten, the non-starch polysaccharides as well as the lipids are the most important in terms of their impact on the processability of the raw material and in terms of the quality of the final products (Goesaert *et al.*, 2005). The gluten forming proteins (glutenin and gliadin) constitute about 75-80% of the total flour proteins (Mukhopadhyay, 1990).

Gluten is elastic, cohesive and rubbery and holds together the various ingredients of the dough. Thus gluten is the necessary framework, forming the sustaining wall of the whole structure of baked products (Bohn, 1957). The gluten matrix and its resulting functions are essential to determining the dough quality of bread and other baked products such as pasta, cakes, pastries, and biscuits. Gluten is heat stable and has the capacity to act as a binding and extending agent and is commonly used as an additive in processed foods for improved texture, flavor, and moisture retention (Biesiekierski, 2017).

Wheat flour used for making muffin should be the product obtained by milling cleaned hard or soft wheat or a combination of both types. Flour strength is usually defined by the percentage of protein present in the flour. Weak flour is casually accepted as flour with low percentage of protein. Usually, this protein is inferred to be gluten, which when the flour is made into a dough with water, will become very extensible under stress, yet when the stress is removed it will not fully return to its original dimensions. Further, the amount of stress required to facture the dough piece is less than that required under identical conditions when strong flour is used (Smith, 1972).

The flour should be free flowing, dry to touch, should be creamy in colour and free from any visible bran particles. It should also have a characteristic taste and should be free from musty flavour and rancid taste.

In 2018 Lamsal reported respective proximate values of moisture content, crude protein, crude fat, crude fibre, total ash, gluten content and carbohydrate were 11.56 (wet basis), 9.17, 1.07, 0.45, 0.44, 9.1, 88.89% respectively (Lamsal, 2018). Similarly Sarwar in 2010 found that of 11.50 (wet basis), 11.3, 0.90, 0.30, 0.60, 8.9 and 86.9% respectively (Sarwar, 2010) and Khanal in 1997 found that of 11.97 (wet basis), 10.32, 1.02, 0.56, 0.83, 9.2 and 87.27% respectively (Khanal, 1997).

Table 2.5 Chemical composition of wheat

Parameter %	A	B	C
Crude protein	10.32	11.3	9.17
Crude fat	1.02	0.9	1.07
Crude fiber	0.56	0.3	0.45
Total ash content	0.83	0.6	0.44
Gluten	9.2	8.9	9.1
Carbohydrate	87.27	86.9	88.89

Source A = Khanal (1997)

B = Sarwar (2010)

C = Lamsal (2018)

2.10 Avocado

The avocado tree (Persea americana Mill.) belongs to the family Lauraceae and is one of the few commercially significant members of the genus Persea (E. Yahia and Woolf, 2011). The name “avocado” also refers to the fruit of the tree, which is characterized by an oval or pear-shape, with a rough or leathery skin, and a large seed; it is sometimes known as the avocado pear or alligator pear. It is a highly caloric fruit rich in vitamins, minerals, folates, potassium, and fibre, with a unique lipid composition (Slater *et al.*, 1975). Avocado is an energetic fruit with high nutritional value and is considered a major tropical fruit, since it is rich in protein and contains fat soluble vitamins lacking in other fruits, including Vitamins A and B, and median levels of vitamins D and E (Duarte *et al.*, 2016).

Scientific Classification

Kingdom	Plantae
Subkingdom	Viridiplantae
Super division	Embryophyta
Division	Tracheophyta
Sub-division	Spermatophyta
Class	Magnoliopsida
Order	Laurales
Family	Lauraceae-laurels
Genus	Persea Mill.- bay
Species	Persea Americana Mill.- avovado

Source: Shrestha (2022)

The avocado fruit can be round, pear shaped, or oblong, and the skin of the fruit may vary in texture and colour. The skin may be pliable to woody, smooth to rough, and green-yellow, reddish-purple, purple, or black in colour (E. Yahia and Woolf, 2011). After harvest, the fruit completes maturation, with major changes in metabolism and higher respiratory rate, and thus high production of ethylene, being highly perishable under environmental conditions leading to the production of high amounts of waste. In this sense, the avocado pulp processing can contribute to its best use, either as a food product or for oil extraction (Kluge *et al.*, 2002; Rocha, 2010).

Avocado is a fruit which had a caloric density of 1.7 kcal per gram and a half unit (~70 g) is composed by 114 kcal, 4.6 g of fibers, 345 mg of potassium, 19.5 mg of magnesium, 1.3 mg of vitamin E and 57 mg of phytosterols (Weschenfelder *et al.*, 2015). Avocado oil is predominantly monounsaturated, a property which is thought to confer distinct health benefits (Gupta *et al.*, 2018). Avocados also contain significant amounts of other beneficial healthy compounds including tocopherols (vitamin E), plant pigments, sterols, fibre, and folate as shown in table below.

Table 2.6 Avocado fruit composition

Component	Quantity
Water (%)	74.4
Lipids (%)	20.6
Proteins (%)	1.8
Fibre (%)	1.4
Ash (%)	1.2
Glucose	0.3
Fructose	0.1
Sucrose	0.1
Malic acid	0.32
Citric acid	0.05
Oxalic acid	0.03
Ascorbic acid	11
Thiamine	0.07
Riboflavin	0.12
Nicotinic acid	1.9
Vitamin B6	0.62
Folic acid	0.04
Biotin	0.006
α-carotene	0.29

β-carotene	0.03
Criptoxanthin	0.16
Potassium	480
Phosphorus	27
Calcium	14
Magnesium	23
Sodium	2
Iron	0.7
Zinc	0.5

Source: E. M. Yahia (2001)

2.11 Butter

Butter is one of the oldest forms of preserving fat components of milk. Its manufacture dates back to some of the earliest historical records (David Hettinga, 2005). Butter is a dairy product made exclusively through the churning process of the pasteurized cream, which has been separated from milk (generally cow's milk), the excess water (buttermilk) being removed. The butter flavor is given by the diacetyl, other substances such as butyric, propionic and formic acid, acetaldehyde acetoin having a smaller contribution (Jain *et al.*, 2020). Butter is a water-in-oil (W/O) emulsion, generally recognized as having a minimum of 80 g milk fat 100 g^{-1} and a maximum of 16 g moisture 100 g^{-1}. It is probably one of the oldest milk products, being produced by the concentration of milk fat following the destabilisation of the oil-in-water (O/W) milk or cream emulsion (Wilbey, 2009). Butter is derived from milk and contains high-quality nutrients, including carbohydrates, proteins, fats, and micronutrients, in easily absorbed forms (ÖZTÜRK YILMAZ and Altinci, 2018). In addition to its high-fat content, butter has substantial amounts of vitamin A (retinol equivalent) 653.0 µg/100 g, vitamin E (tocopherol equivalent) 2.2 mg/100 g, between 183 and 248 mg/100 g cholesterol, and minor amounts of calcium, phosphorus, vitamin K 60 µg/100 g, vitamin D 1.2 µg/100 g and also a low protein content. Additionally, it is a well-known fact that butter’s color is given by the presence of carotene (lycopene), vitamin A, and other fat-soluble pigments (Queirós *et al.*, 2016).

Part III

Material and methods

3.1 Raw materials

3.1.1 Buckwheat Flour

The buckwheat flour named ‘Fortune’ was brought from local market of Dharan, Sunsari.

3.1.2 Wheat flour

The wheat flour named ‘Fortune’ was brought from local market of Dharan, Sunsari.

3.1.3 Butter

Standard ‘Amul Pasteurized butter’ was used made from fresh cream.

3.1.4 Sugar

Sugar in the form of pulverized sugar was used and brought from the market of Dharan.

3.1.5 Baking powder

Baking powder named ‘Ajanta’ was brought from the local market of Dharan.

3.1.6 Egg

The eggs were brought from local market of Dharan.

3.1.7 Avocado

Avocado (Persea americana) was collected from Pakhribas, Dhankuta, Nepal with the co-ordinates of 26.9835° N, 87.3215° E.

3.1.8 Apparatus and chemicals required

Apparatus and chemicals required were utilized from Central Campus of Technology laboratory. The apparatus and chemical used are in Appendix E.

3.2 Method of Experiment

3.2.1 Methodology

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 buckwheat flour used to prepare muffins. In an experimental study, an independent variable is one that is varied or manipulated in order to examine its effect. Wheat and buckwheat flour are independent variable. A dependent variable is the variable that changes as a result of the independent variable manipulation. Sugar, butter, avocado puree, baking powder, egg and water are dependent variable.

3.2.2 Formulation of recipe

The recipe formulation for the buckwheat flour incorporated muffin was carried out as given in Table 3.1. The amount given is on a part basis.

Table 3.1 Recipe formulation for muffin

Ingredients	A	B	C	D	E	F	G
Wheat Flour	100	75	66.66	50	33.33	25	0
Buckwheat	0	25	33.33	50	66.66	75	100
Sugar	60	60	60	60	60	60	60
Butter	32.5	32.5	32.5	32.5	32.5	32.5	32.5
Avocado Pure	32.5	32.5	32.5	32.5	32.5	32.5	32.5
Baking Powder	1.42	1.42	1.42	1.42	1.42	1.42	1.42
Egg	57	57	57	57	57	57	57
Water	31	31	31	31	31	31	31

Baniya (2022)

For the preparation of muffin egg was beaten for 2 min, creaming of shortening and sugar for 10 min. Along with these ingredients water, fats (50% butter and 50% avocado fat) and baking powder was added and mixed to form a batter. The batter was moulded, panned and baked at 215ºC in oven for 20±3 min to form muffins (Lamsal, 2018).

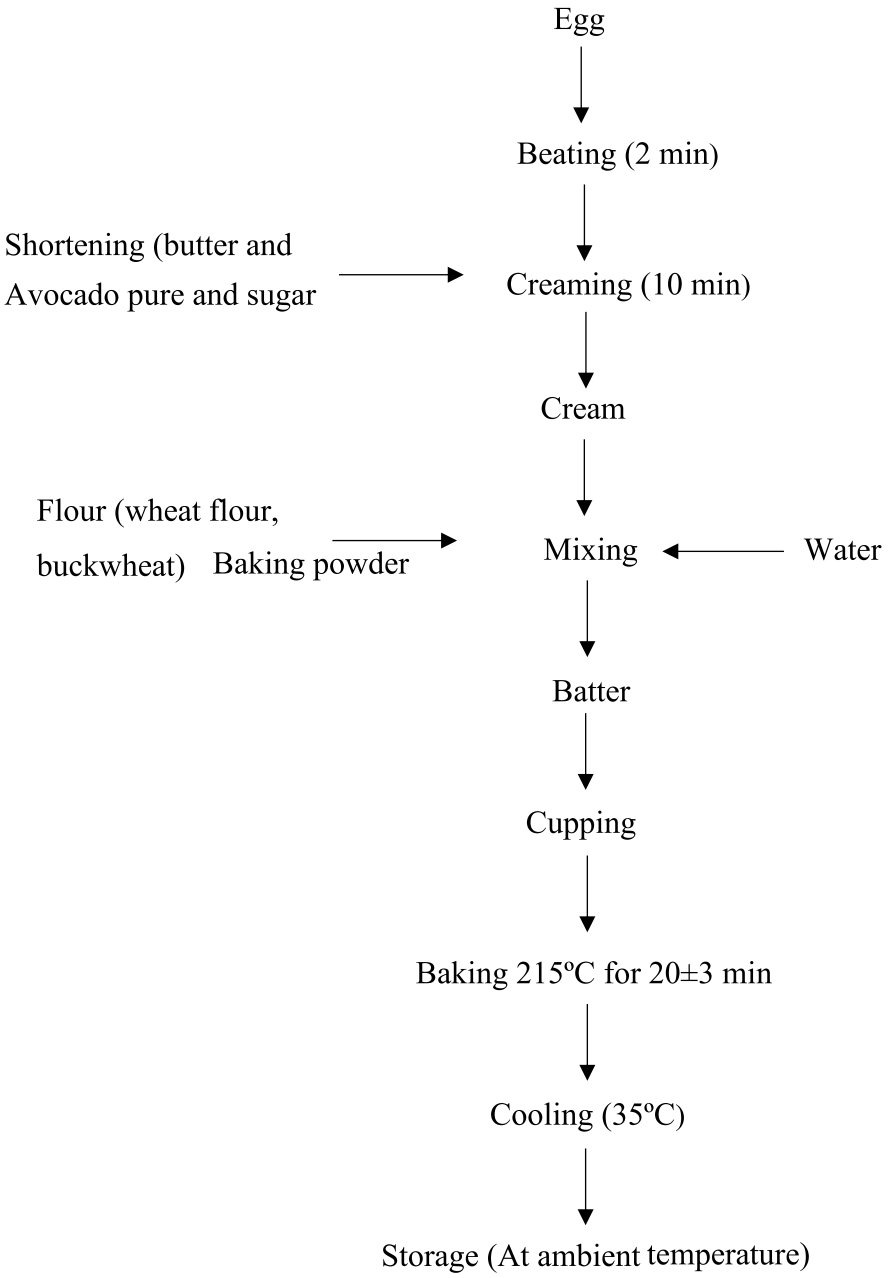


Figure 3.1 Flow chart of Buckwheat flour incorporated muffin.

Source: Lamsal (2018)

3.3 Analysis of raw material and product

3.3.1 Physical parameter analysis

3.3.1.1 Color and surface

Color and surface were determined by visual inspection method. The Wheat flour and wheat flour were spread on separate tray and color and surface was diligently examined. Similarly, the color of the muffin samples was analysed.

The appearance property, i.e. color of the muffin, is affected by the interaction of different factors. The natural pigments of the wheat flour and buckwheat flour such as phenolics, anthocyanins, tannins, carotenoids, and xanthophylls (Serna-Saldivar, 2012). The chlorophyll content present in the avocado puree also gives the color in the muffin (Ashton *et al.*, 2006). The processing factors affecting color are Maillard, browning, and caramelization reaction in which reducing sugar and protein plays the vital role (Serna-Saldivar, 2012).

3.3.1.2 Specific loaf volume of the muffin

First volume and weight of the muffin was determined. Volume was determined by rapeseed displacement method as mentioned in (AACC, 2016) for muffin and weight by physical balance. In this case, usually rape or canola seeds or pearled barley, take the place of a liquid. The process is quite straightforward. A box of known volume was filled with seed and the weight of seed required to just fill the box is noted. The sample was introduced, and the seed poured back into the box. The volume of seed displaced is equal to the volume of the product. The more seed that is displaced the larger the product volume (Stauffer, 2001). Different factor affect the muffin volume include the carbon dioxide production, thermal change of the structure due to protein denaturation and starch gelatinization (M Schirmer *et al.*, 2012).

Specific loaf volume = (Volume of the muffin)/ (Weight of the muffin)

3.3.1.3 Weight loss (WL)

According to (Ureta *et al.*, 2014) weight loss, WL, was calculated as the percentage difference between initial and final product weight (wet basis), Wi and Wf, respectively,

$$\text{WL}(\%) = \frac{W_i - W_f}{W_i} \times 100$$

3.3.1.4 Crust and crumb ratio

Crust/crumb ratio was calculated according to (Le-Bail *et al.*, 2011): the samples were removed from the oven and cooled for a few minutes. The crust was separated from the crumb using a scalpel, considering the crust as the dried and brown surface located at the upper zone of the muffin (Jusoh *et al.*, 2009). Crust to crumb ratio was expressed as the mass ratio on wet basis and dry basis.

3.3.1.5 Cell uniformity and size

Cell structure can be evaluated by making a vertical cut in the muffin to form two equal halves and then making an ink print or photo copy (Noble, 1946). A desirable muffin should have a uniform cell structure without tunnels (Noble, 1946).

3.3.2 Physicochemical analysis

3.3.2.1 Moisture content

Moisture content of the sample was determined by heating in an oven at 100 ± 5°C to get constant weight constant weight

$$\text{Moisture content } \% = \frac{\text{Initial weight - final weight}}{\text{Initial weight}} \times 100\%$$

3.3.2.2 Crude fat

Crude fat content of the samples was determined by solvent extraction method using Soxhlet apparatus and solvent petroleum ether as per AOAC (2005).

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

Where, W1 = weight of beaker

W2 = weight of oil extracted + beaker

W = weight of sample

3.3.2.3 Crude protein

Crude protein content of the samples was determined indirectly by measuring total nitrogen content by micro Kjeldahl method as per AOAC (2005).

$$\text{Protein content} = \frac{(\text{sample blank}) \times \text{N of HCL} \times 14 \times 100 \times 100}{\text{Aliquot (ml)} \times \text{wt of sample (g)} \times 1000}$$

3.3.2.4 Crude fibre

Crude fibre content of the samples was determined by acid-base hydrolysis given by AOAC (2005).

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

3.3.2.5 Total ash

Total ash content of the samples was determined by dry ashing given by AOAC (2005) using muffle furnace.

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

Where, W1 = weight of empty crucible

W2 = weight sample + crucible before ashing

W3 = weight of sample + crucible after ashing

3.3.2.6 Carbohydrate

The carbohydrate content of the sample was determined by difference method as by (AOAC, 2005).

Carbohydrate (%) = 100 - (protein + fat + ash + crude fibre).

3.3.2.7 Calorific value

The determination of calorific value was performed by indirect calorimetry. calorific value (kcal/100g) = (% Fat × 9) + (% Protein × 4) + (% Carbohydrate× 3.75). The energy conversion factors applied were 9 kcal/g for fats, 4 kcal/g for protein, and 3.75 kcal/g for carbohydrate, as reported in Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences (1972) (Finglas, 2015). The percentage reductions of fat and caloric value were calculated by using formula; [(V1 – V2) / V1] × 100, where V1 is the value of control sample (Sample A) and V2 is the value of the test sample (Sample C) (Othman *et al.*, 2018).

3.3.3 Sensory analysis

The sensory analysis for overall quality will be carried out by semi-trained panelists, which consisted of faculties and students of Central Campus of Technology. The parameters for sensory evaluation are texture, appearance, color, aroma, taste and overall acceptability. Sensory evaluation was performed according to the 9- Point Hedonic Scale by 10 sensory panelists.

3.3.4 Statistical analysis

The obtained data was analysed statistically by Genstat (12th edition) developed by VSN International Limited for Analysis of Variance (ANOVA) at 5% level of significance and Microsoft excel 2016.

3.3.5 Acceptability period of muffin

The acceptability period of the product was determined by acid value, peroxide value of the extracted fat and moisture content of the muffin.

Part IV

Results and discussion

This work was carried out for the preparation of low gluten muffin formulation with different proportions of composite (wheat and buckwheat) flour with butter and avocado fats. As muffin is, a product widely favoured and consumed by the general population as a healthy breakfast food. At first, the major raw materials were subjected for proximate analysis.

4.1 Proximate composition of wheat flour and buckwheat flour

The proximate composition of wheat flour and Buckwheat flour was determined. Determined results are presented in Table 4.1.

Table 4.1 Proximate composition of wheat flour and Buckwheat flour (dry basis)

Parameters %	Wheat flour	Buckwheat flour
Moisture (wb)	11.53±0.377	12.8±0.098
Crude protein (db)	10.18±0.055	13.59±0.020
Crude fat (db)	1.13±0.021	3.05±0.01
Crude fibre (db)	0.45±0.008	0.963±0.015
Total ash (db)	0.46±0.021	2.43±0.010
Carbohydrate (db)	87.74±0.023	79.958±0.022

Values are the means of triplicates and figures in the parenthesis are standard deviation.

4.1.1 Chemical composition of wheat flour

Proximate analysis of the wheat flour for various parameters like moisture content (%), crude protein (%), crude fat (%), crude fibre (%), ash (%), gluten (%) and carbohydrate (%) (in dry basis except moisture content) were found to be 11.53%, 10.18%, 1.13%, 0.45%, 0.46%, and (Lamsal, 2018) found that of 11.56 (wet basis), 9.17, 1.07, 0.45, 0.44, 9.1, 88.89% respectively. The moisture content of wheat flour(11.53% wet basis) was lower than that suggested by Arora (1980a) i.e. 13% max, but no significant difference between the value obtained by (Sarwar, 2010). The crude protein content in wheat flour 10.18% was lower than that obtained by (Khanal, 1997; Sarwar, 2010)but higher than that obtained by (Lamsal, 2018). The lower concentration of the protein in the wheat flour might be due to the loss of nitrogenous material during the digestion of sample. This might have reduced the final protein content. The crude fibre in the wheat flour was found to be 0.45% which was no significant difference to the value obtained by Lamsal (2018). The degree of milling, extraction rate and amount of bran content in wheat flour and variety of the wheat might be the reason for variation in crude fibre. According to (Arora, 1980a), the maximum limit of total ash is 0.5% as the obtained value was 0.46%. which was no significant difference to the value obtained by than obtained by (Lamsal, 2018). The difference in proximate composition may be due to factors like varieties, climatic conditions, soil type, maturity, fertility and others.

4.1.2 Chemical composition of Buckwheat flour

Proximate analysis of the oats flour for various parameters like moisture content (%), crude protein (%), crude fat (%), crude fibre (%), ash (%),and carbohydrate (%) (in dry basis except moisture content) were found to be 12.8%, 13.59%, 3.05%, 1.48%, 0.96%, and 79.95% respectively as given in Table 4.1.

The moisture content is found no significant difference than reported by (Poudel, 2012), analyzed moisture content for common buckwheat (11.81%) and tartary buckwheat (11.14%). The moisture content of unmalted common buckwheat has found to be 11.4% as reported by (Devrajan *et al.*, 2017), whereas the moisture content of unmalted tartary buckwheat was in the range 10.2-11.5% as reported by (Thakur *et al.*, 2017). The variation in moisture content may be due to genetic and environmental factors.

The ash content of common buckwheat before malting was 2.43 % which has no significant difference to the research that was conducted by (Qin *et al.*, 2010) and 1.4 %-2.5% by (Dogra and Awasthi, 2015). The ash content of common buckwheat before malting was 2.43 % similar to the research that was conducted by (Qin *et al.*, 2010) and 1.4 %-2.5% by (Dogra and Awasthi, 2015). The fat content of common buckwheat was reported to 3.06% in (G. Zhang *et al.*, 2015) and 1.6%- 2.9% by (Dogra and Awasthi, 2015) and 3.16% in (Sindhu and Khatkar, 2016). It has been reported that the decrease in fat content might be due to increase activity of lipase enzyme (Devrajan *et al.*, 2017). Crude fiber is found in the range of 0.77%-0.96% that has been reported by (Khan *et al.*, 2013). Similarly the content of very low crude fiber is also found in (Baljeet *et al.*, 2010).

The ash content of common buckwheat before malting was 2.43 % similar to the research that was conducted by (Qin *et al.*, 2010) and 1.4 %-2.5% by (Dogra and Awasthi, 2015). The carbohydrate content of buckwheat was found to be 79.95% which is similar to the result as reported in (Khan *et al.*, 2013).

4.2 Effect of buckwheat flour on the physical parameters of muffins

The effect of a buckwheat flour on the physical parameters of muffins are given below:

4.2.1 Volume of the muffins

The change in the volume of muffins with the incorporation of buckwheat flour is shown in Fig. 4.1

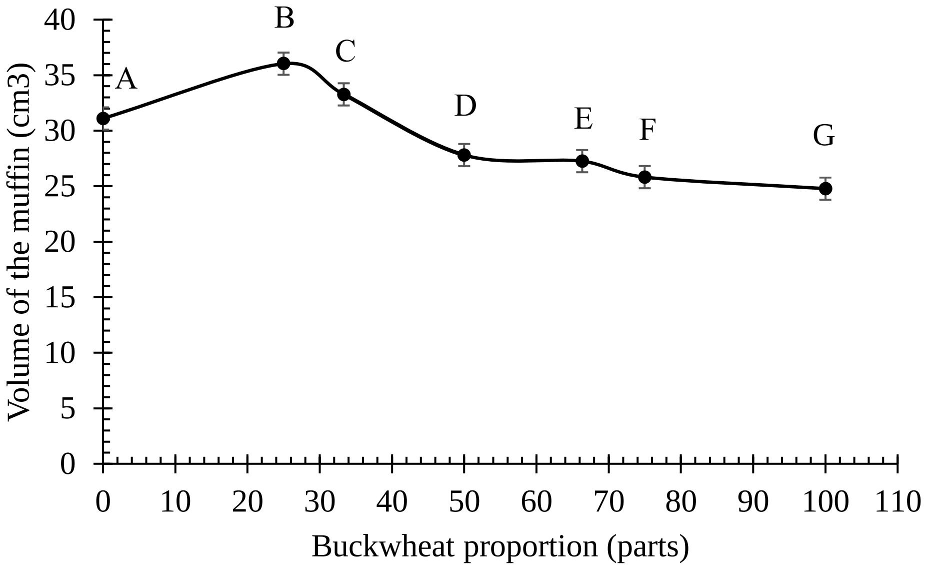


Figure 4.1 Effect of Buckwheat on the volume of the muffins

From Fig. 4.1 it is seen that the incorporation of the buckwheat to certain level increased the volume and then the volume gradually decreased. With respect to the control (sample A) the volume of Sample B and Sample C increased significantly, it may be due to the adequate moisture that trapped in the muffin and made the muffin rise in volume during the time of baking. Other factors affecting the muffin volume include the carbon dioxide production, thermal change of the structure due to protein denaturation and starch gelatinization (M Schirmer *et al.*, 2012).

The volume of the muffin increases due to the expansion of water vapor during the holding and baking process (Berglund and Hertsgaard, 1986). Increasing the amount of buckwheat flour means an increase in dietary fiber and decrease in gluten. The decrease in the volume of Sample D, E, F and G might be due to the dilution of gluten and disruption of gluten network and due to dilution of baking powder. The sample D and Sample E were observed unleavened which might be due to the lack of entrapment of air into the batter. Similar type of trend was seen in research where significantly decrease in volume of muffin was seen as the amount of buckwheat flour increased (Lee and Bae, 2015).

4.2.2 Specific loaf volume

The incorporation of the avocado puree to a certain level increased the specific loaf volume and then the specific loaf volume gradually decreased. The LSD shows that formulations A, B, C, D, E, F and G are significantly different among themselves at 5% level of significant which is clear from the figure below.

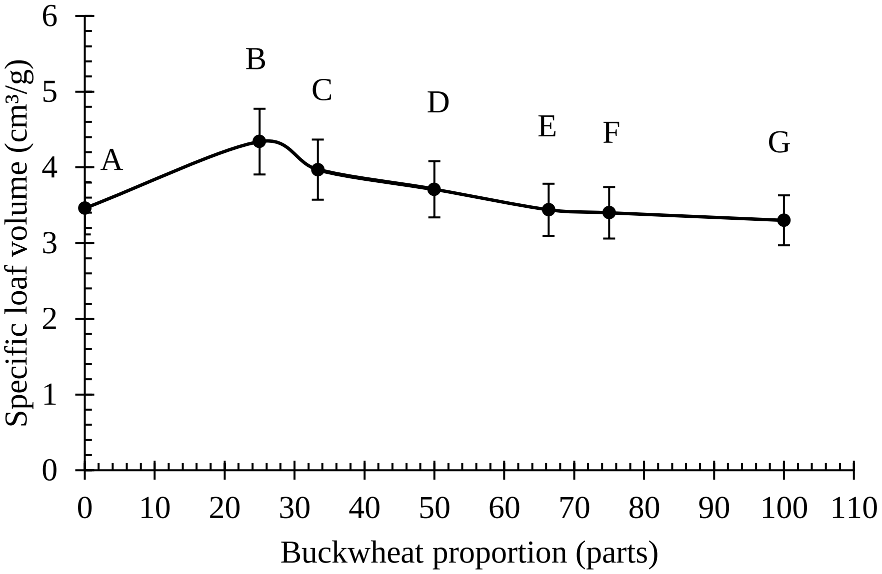


Figure 4.2 Effect of Buckwheat on the specific loaf volume of the muffins

The gradual decreased might be due to the effect of emulsifiers, as it helps the incorporation of air bubbles during mixing (Seyhun *et al.*, 2005). Increasing buckwheat flour content in cake formulation might make incorporation of air in cake batter more difficult due to higher fibre content, resulting in decreasing specific volume (Yıldız *et al.*, 2018). Since sample B has the maximum specific loaf volume than the other samples, it is regarded as the best sample. Lamsal (2018) also find similar trend with the oat flour incorporated muffin.

4.2.3 Cell uniformity and size

The cell size and uniformity of sample A and sample D was observed. The muffin with a uniform cell structure without tunnels is desirable (Halliday, 1946**).** The porosity depends on the number and size of pores (Ureta *et al.*, 2014). Sample A has a large air space except that there is an almost uniform cell structure which is as shown in Fig. 4.4.

Fig. 4.3 Photograph and binary image of sample A muffin cross-sectional area

Similarly, sample D has more homogeneous pores with significant amount of larger air space, due to which the volume of sample A muffin is higher than sample D muffin. In both samples A and D there was no formation of tunnels.

Fig. 4.4 Photograph and binary image of sample D muffin cross-sectional area

4.3 Sensory analysis

Statistical analysis of sensory scores obtained from 11 semi-trained panelists using 9-point hedonic rating scale (9= like extremely, 1= dislike extremely) for low gluten muffin from composite (wheat and buck wheat) flour by using (butter and avocado) fat. Panelists are those who have tasted muffins. The ANOVA and LSD table for sensory evaluation are presented in Appendix B. Here A (100 parts wheat and 0-part buckwheat), B (75 parts wheat and 25-part buckwheat), C (66.66 parts wheat and 33.33 parts buckwheat), D (50 parts wheat and 50 parts buckwheat), E (33.33 parts wheat and 66.66 parts buckwheat), F (25 wheat and 75 parts buckwheat), G (0 part wheat and 100 parts buckwheat).

4.3.1 Appearance

The mean sensory score for appearance were found to be 5.700, 5.800, 6.500, 8.200, 7.100, 6.800 and 6.800 for the muffin formulation A, B, C, D, E, F and G respectively. Statistical analysis showed that partial substitution of wheat flour with buckwheat flour had significant effect ($p < 0.05$) on the appearance of the different muffin formulations at 5% level of significance. The sample A and B were not significantly different to each other but slightly different to C and F, which is shown graphically in Fig 4.4 There was no significance difference between samples C, E, F and G. Sample D got the highest score whereas sample B got the lowest score among the samples. Bars with different alphabets indicates significant difference ($p < 0.05$).

(Parida *et al.*, 2018) also shows similar trend while substituting wheat flour with buckwheat flour in muffin formation.

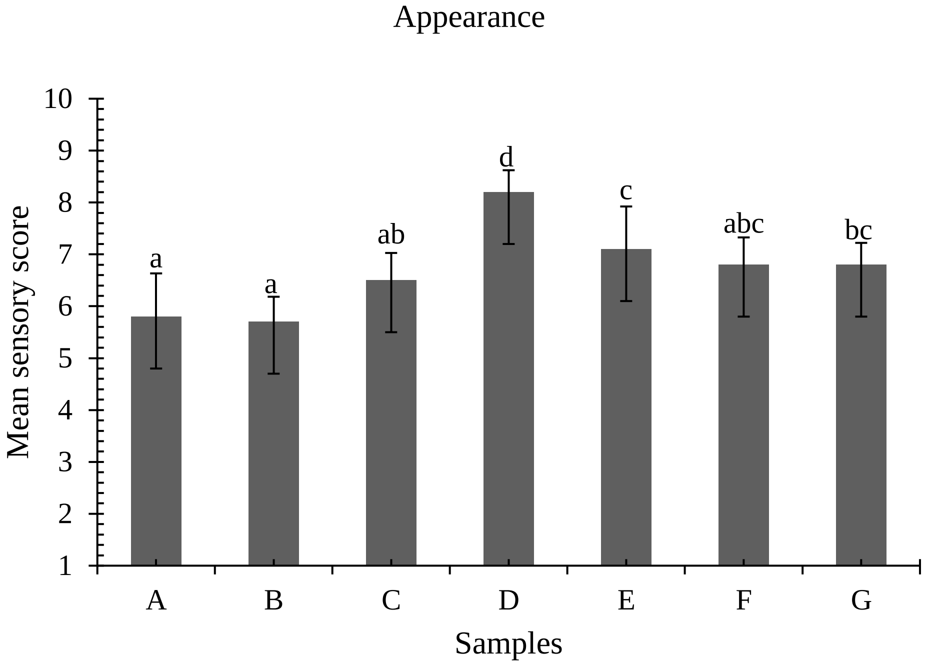


Figure 4.5 Mean sensory score for the appearance of muffins of different formulation

Based on the statistical analysis of the sensory data, the appearance of sample D was found to be the best among the seven different samples.

4.3.2 Aroma

The mean sensory score for aroma were found to be 5.900, 6.100, 6.100, 8.400, 6.900, 6.500 and 7.700 for the muffin formulation A, B, C, D, E, F and G respectively. Statistical analysis showed that partial substitution wheat with buckwheat had significant effect ($p < 0.05$) on the aroma of the different muffin formulations at 5% level of significance. There was slightly different in between the samples D, E and G whereas there was no significant difference between the samples A, B, C and F shown in fig 4.5. Sample D had the highest score while sample A recorded the lowest score.

Furthermore, (Ma *et al.*, 2013) showed that, according to panelists, common buckwheat is a better material than tartary buckwheat for gluten-free noodle production. (Chopra *et al.*, 2014) described the reduction of flavor score of wheat cookies with 75% buckwheat addition to high flavonoids concentration, which are probably responsible for creating the bitter taste of buckwheat flour.

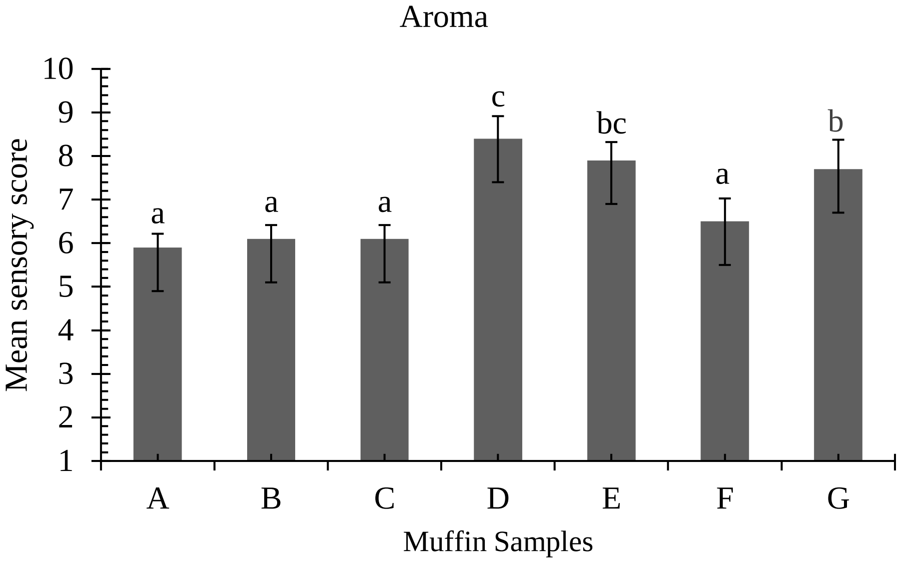


Figure 4.6 Mean sensory score for aroma of muffins of different formulation s

Based on the statistical analysis of sensory data, the aroma of sample D, E, F and G was no significant difference.

4.3.3 Color

The mean sensory score for color were found to be 6.000, 6.100, 6.400, 8.600, 7.600, 7.400 and 8.200 for the muffin formulation A, B, C, D, E, F and G respectively. Statistical analysis showed that partial substitution of wheat with buckwheat had significant effect ($p < 0.05$) on the color of the different muffin formulations at 5% level of significance. There is no significant difference between the samples A, B and C whereas there is there is significant difference between the samples A and D. The samples E and F have no significant difference whereas there is slightly difference between them, and sample G shown as in fig 4.6. The mean sensory score for sample D is the highest and sample A was found lowest.

A similar result was observed in muffin with Buckwheat added as a flour replacer, in which full flour replacement lowered the color score as compared to control (sample A) (Parida *et al.*, 2018). Similar observation was observed in the Buckwheat replacer in (Lee and Bae, 2015).

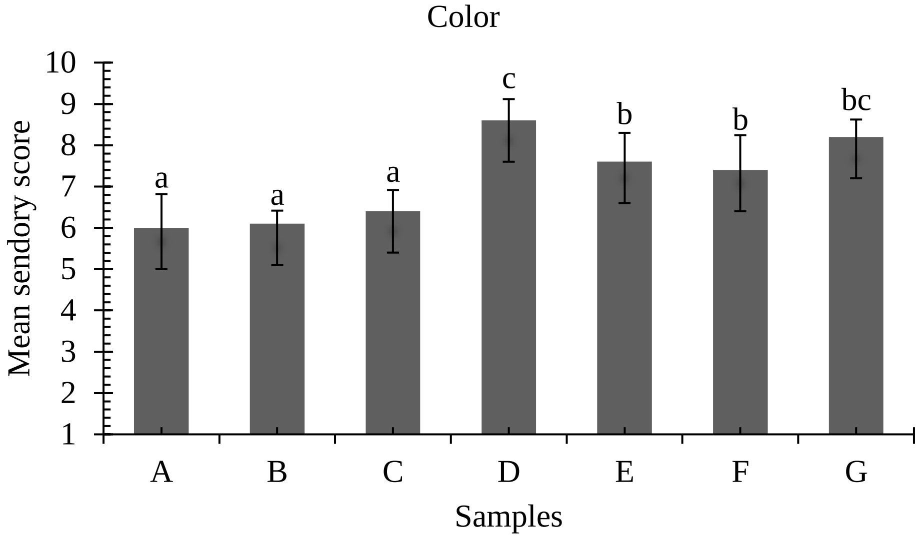


Figure 4.7 Mean sensory score for color of muffins of different formulations

Based on statistical analysis of the sensory data, the color of sample D, E, F and G was no significant difference

4.3.4 Texture

The mean sensory score for texture were found to be 5.700, 5.700, 6.100, 8.600, 7.300, 6.700 and 6.700 for the muffin formulation A, B, C, D, E, F and G respectively. Statistical analysis showed that partial substitution of wheat with buckwheat had significant effect ($p < 0.05$) on texture of the different muffin formulations at 5% level of significance. The samples A, B and C show no significant difference between them whereas samples E, F and G also show no significant difference between themselves. Sample D shows a significant difference with respect to other samples shown in fig 4.7. The mean sensory score for sample D is the highest and sample A and B was found to be the lowest.

The lowering trend of texture scores of cookies with the increase in supplementation. In the present study the decrease in breakability scores is due to the increased hardness of chapattis because of high protein content in buckwheat flour except gluten. In a study (Atuonwu, 2010) reported the decrease in texture of cookies when supplemented with pumpkin seed flour.

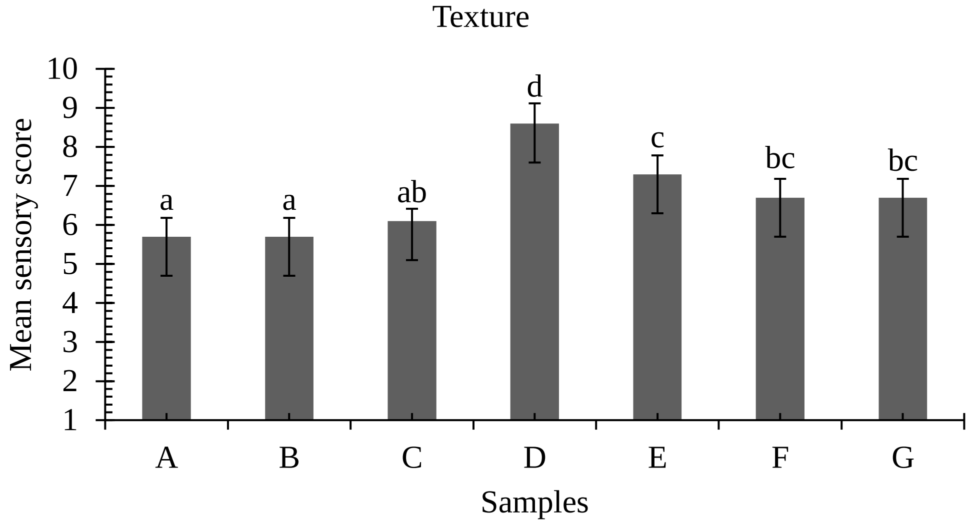


Figure 4.8 Mean sensory score for texture of muffins of different formulations

Based on statistical analysis of the sensory data, the texture of sample D was found to be best among seven samples.

4.3.5 Taste

The mean sensory score for texture were found to be 6.100, 6.100, 6.400, 8.600, 7.400, 6.800 and 6.300 for the muffin formulation A, B, C, D, E, F and G respectively. Statistical analysis showed that partial substitution of wheat with buckwheat had significant effect ($p < 0.05$) on taste of the different muffin formulations at 5% level of significance. Samples A, B, C, E, F and G do not show significant difference between them. D sample shows significant difference as compared to other samples shown in fig 4.8. The mean sensory score for sample D is the highest and sample A and B was found to be the lowest.

The lowering trend of taste scores of cookies with the increase in supplementation level of rice bran was studied by (Younas *et al.*, 2011). Similar observation for the pattern of taste while substituting wheat flour with buckwheat flour in muffin preparation was seen in (Bae and Jung, 2013)

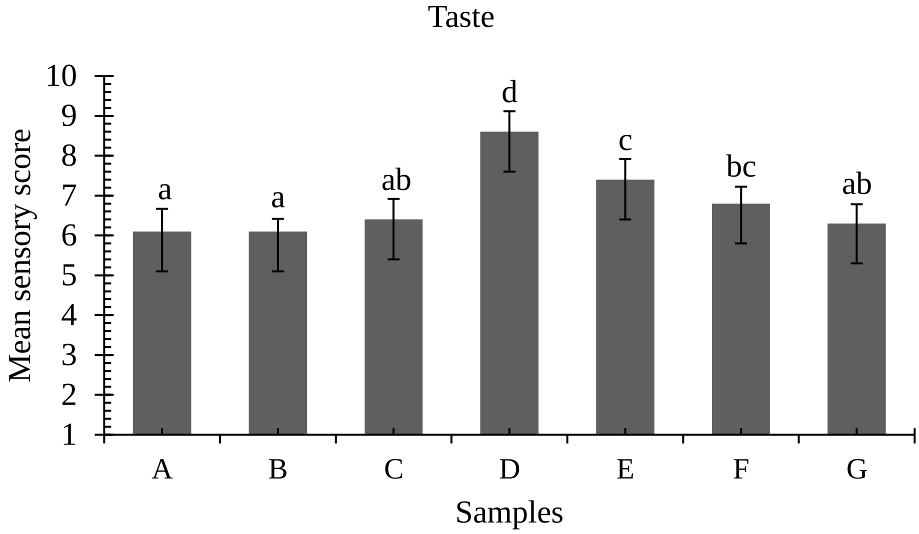


Figure 4.9 Mean sensory score for taste of muffins of different formulation

Based on statistical analysis of the sensory data, the taste of sample D was found to be best among seven samples.

4.4.6 Overall acceptability

The overall score for different muffin samples were obtained as 6.100, 6.100, 6.400, 8.600, 7.400, 6.800, 7.000 for the muffin formulation sample A, B, C, D, E, F and G respectively. Statistical analysis showed that partial substitution of wheat with buckwheat had significant effect ($p < 0.05$) on overall of the different muffin formulations at 5% level of significance. The sample D was significantly different to other samples, which is shown graphically in Fig. 4.9. The sample A, B, C, E, F and G do not have significant difference among themselves. Sample D got the highest score than remaining samples. Samples A and B were the least accepted by panelists. C and F scored slightly higher than A and B while E and G scored slightly higher than C and F and are not significantly different.

Similar observation of overall acceptability was seen in (Effect of Buckwheat (*Fagopyrum esculentum*) Powder on the Physicochemical and Sensory Properties of Emulsion-type Sausage). Similar in the study of (Bano *et al.*, 2014) same pattern was observed. Also same pattern was seen in the lowering trend of overall acceptability scores of cookies with the increase in supplementation level of rice bran was observed by (Younas *et al.*, 2011). In another similar study (Oluwamukomi *et al.*, 2005)studied the decrease in overall acceptability of biscuits when supplemented with soy flour. (Olatidoye and Sobowale, 2011) studied the similar pattern in on supplementation of full fat soybean flour with cassava flour

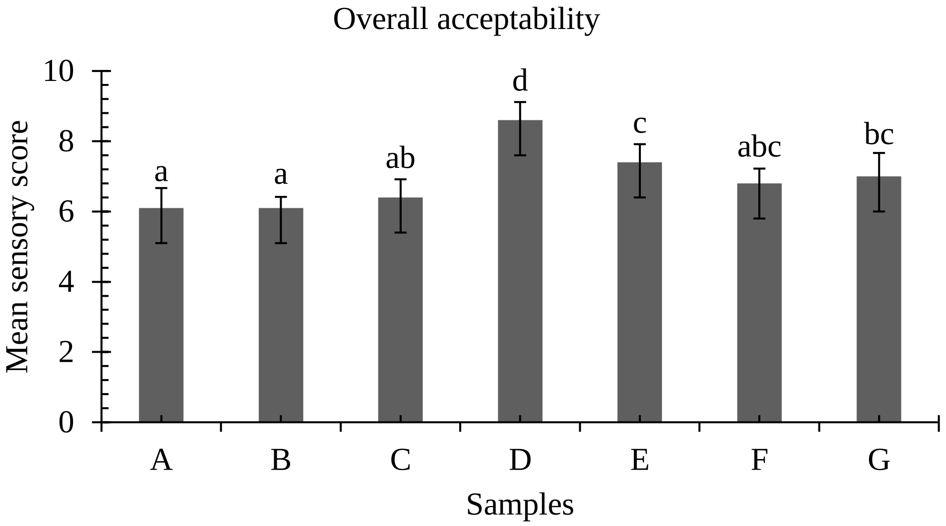


Figure 4.10 Mean sensory score for overall acceptability of muffins of different formulation

The appearance, color, aroma, taste and texture of the sample D was very much liked. With respect to the control, i.e. sample A, sample D got a high score in terms of overall acceptability as shown in Fig. 4.9. Therefore, overall acceptability of the muffin up to 50 parts (in 100 parts) substitution of wheat by buckwheat sample D was found to be significantly superior based on the sensory characteristics of muffins.

Here we aim to use the maximum portion of avocado puree than the butter as the shortening for the preparation of muffin. Design expert 13 was used to create the recipe. Mixed design was used to formulate the recipe. It is concerned with maximum use of avocado puree with the high sensory score and desirability. 37.3357 parts avocado puree and 27.6643 parts butter can be used having the sensory quality such as appearance, color, aroma, texture, taste, overall acceptability as 7.19278, 7.0001, 6.89761, 6.82302, 7.10987 and 7.11218 respectively (corresponding value Appendix A). The muffin will have the desirability of 0.857. The avocado puree and butter portion with the sensory quality and desirability is as shown in Fig. 4.2

4.4 Proximate composition of products

Thus, from statistical sensory analysis, the substitution of wheat by 50 parts buckwheat had significant different with the control sample. So, sample D was found to be the best muffin sample containing 50 parts wheat and 50 parts buckwheat parts shortening required. The proximate composition of sample D and control muffin (sample A) were presented in Table 4.2.

Table 4.2 Proximate composition of product

Parameters	Sample A (Control)	Sample D (Best)
Moisture % (wet basis)	13.293±0.626	20.073±1.008
Crude Protein % (dry basis)	11.293±0.627	15.573±1.305
Crude Fat % (dry basis)	19.708±0.045	22.372±0.321
Crude Fiber % (dry basis)	1.157±0.225	7.268±0.215
Total ash % (dry basis)	2.460±0.066	3.679±0.073
Carbohydrate % (dry basis)	64.256±0.915	50.215±0.890
Crumb moisture % (wet basis)	17.275±0.275	26.273±0.623
Crust moisture % (wet basis)	7.956±0.705	11.353±0.612
Weight loss (%)	14.081±0.316	17.466±0.523
Crust/Crumb ratio	0.46±0.016	0.432±0.013
Caloric value	514.791±0.416	465±0.946
Caloric value reduction (%)	-	9.063

Values are the means of triplicates and figures in the parenthesis are standard deviation of the triplicates. Values in the column having different superscripts are significantly different at 5% level of significant.

The moisture content, crude protein, crude fat, crude fiber, ash and carbohydrate of sample A were found to be 13.293%, 11.293%, 19.708%, 1.157%, 2.460% and 64.256% respectively and that of sample D(best) were found to be 20.073%, 15.573%, 22.372%, 7.268%, 3.679% and 50.215% respectively. The LSD shows that these proximate values are significantly different from sample A. It is observed that there is a significant difference in the moisture content of sample A and sample D with the moisture content 13.293% and 20.073% respectively. Moisture content increased significantly ($p < 0.05$) in muffins incorporating buckwheat as compared to control. The high moisture content in Sample D can be explained by the high moisture content of buckwheat flour when used as a substitute for wheat flour. The results of current study are in line with findings of (Fessas *et al.*, 2008) and (Shalini and Laxmi, 2007) who studied higher moisture content (13.6%) in buckwheat flour than wheat flour which results higher moisture content in Sample D.

The crumb moisture content and crust moisture content of sample A were found to be 17.275% and 7.956% respectively and that of sample D were found to be 26.273% and 11.353% respectively. The weight loss and crust/crumb ratio of sample A were found to be 14.081%, 0.46 respectively and that for sample D were found to be 17.466% and 0.432 respectively. The higher moisture content makes it prone to microbial attack, but it also gives the characteristic firmness to the muffins.

Crude protein content in muffins showed a significant increment ($p > 0.05$) with the incorporation of Buckwheat flour, ranging from 11.293% to 15.573%. In another similar study (Si-quan and Zhang, 2001) and (Bonafaccia *et al.*, 2003) reported 11.28% and 11.4% crude protein content in buckwheat flour and (Paterson and Piggott, 2006), (Bilgiçli, 2009) and (Butt *et al.*, 2004) found similar results in whole wheat flour i.e., 10.58, 11.4 and 10.94% respectively.

The muffin made with 50 percent Buckwheat flour, or Sample D, was approved. There was a substantial increase in fat content from 19.708% to 22.372% at the 5% level of significance following the addition of buckwheat flour. The results regarding fat content are at par with the finding of (Bonafaccia *et al.*, 2003)and (Si-quan and Zhang, 2001) who reported 2.45% and 3.2% fat content in buckwheat flour and (Mepba *et al.*, 2007) reported 1.1% and 0.82% fat content in whole wheat flour.

The ash content of muffins increased in avocado incorporated muffin. The increase in ash content may be due to the high mineral content in the avocado puree i.e. potassium, phosphorus, calcium and iron (Watnick, 2009). The ash content ranged from 2.460% in wheat flour muffin (Sample A) to 3.679% in the Buckwheat flour incorporated wheat flour muffin (Sample D). In another study (Akhtar *et al.*, 2008) reported 1.95% ash content in buckwheat flour and 1.62% in whole wheat flour respectively.

It was observed that crude fiber content is also significantly increased in Buckwheat incorporated muffin which was due to higher crude fiber content in avocado puree than that of butter. The crude fiber ranged from 1.157% in Wheat flour muffin (Sample A) to 7.268% in the Buckwheat flour incorporated with wheat flour muffin (Sample D). Our results of present study for crude fiber in buckwheat flour are in agreement with findings of (Si-quan and Zhang, 2001) who reported 8.3% and (Khetarpaul and Goyal, 2009) evaluated 1.85% in whole wheat flour.

Carbohydrate content significantly decreased ($p < 0.05$) following the significant difference of buckwheat flour incorporation, from 64.256% (sample A) to 50.215% (Sample D). Buckwheat flour incorporation contributed to the increase in carbohydrate content in muffin formulation since other ingredients were kept at a constant amount. Decreased of the carbohydrate may be due to low amount of carbohydrate content in wheat flour in compared to buckwheat as suggested by (Wijngaard and Arendt, 2006).

The muffin incorporating buckwheat flour was accepted up to 50 parts buckwheat (in 100 parts of composite flour) incorporation i.e. sample D and 9.063% lower calorie content compared to the full wheat counterpart. The nutritional value also increased significantly or insignificantly.

4.5 Shelf-life evaluation of the muffin

The shelf life of the muffin was studied for 8 days with triplicate samples. The samples were stored in ambient temperature (25 ± 3 °C). The acid value, peroxide value of the extracted fat, and moisture content of the product was evaluated from the date of manufacture up to 8 days.

4.5.1 Change in acid value

In general, acid value is the indication of free fatty acid content in the product. The increment in the fatty acid of the product was found increased with storage time and also depends on storage condition. Here, at the ambient temperature the acid value of sample A was observed to be 1.01 at initial which reached 1.38, 1.707, 2.0311, 3.906 within 2, 4, 6, and 8 days respectively. Similarly, for sample D acid value was 1.635 at initial which reached 2.245, 3.561 and 5.387 within 2, 4 and 6 days respectively but the acid value was below the unacceptability level of 6 mg KOH/mg of oil as described by (DFTQC, 2018) till 8th and 6th day of analysis for sample A and sample D respectively. The change in the acid value of sample A and sample D is shown in Fig. 4.10. The acid value of sample D was greatly increased earlier than that of sample A. It might be due to the presence of high moisture compared to sample A.

The presence of lipase enzyme, which hydrolyses the fat present to the free fatty acid and glycerol (Oropeza, 2018). The increase in the fatty acid ultimately increases the acid value.

Change in pH value, following LAB metabolite build-up, affects enzyme activity in sour dough. Quick drop in pH leads to activation of LAB proteolytic enzymes and gluten degradation (Thiele *et al.*, 2002).

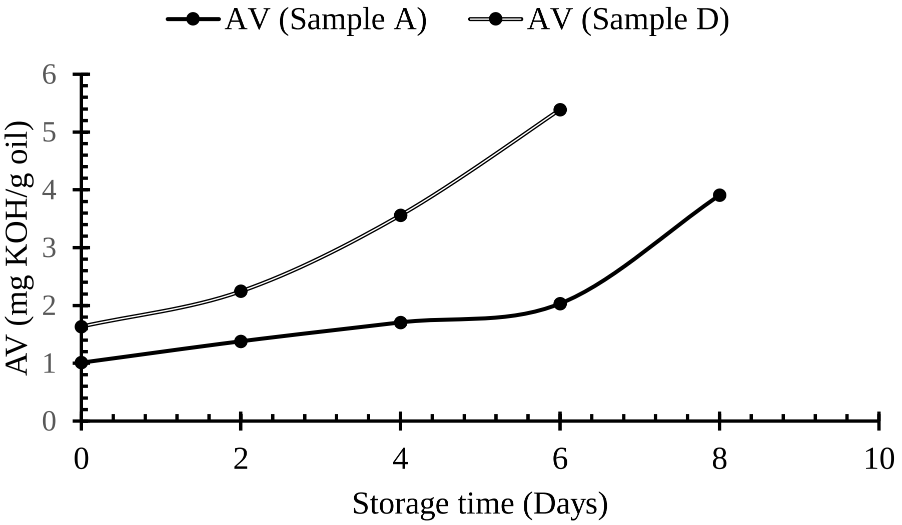


Figure 4.11 Change in acid value during storage at ambient temperature of sample A and sample D

4.5.2 Change in peroxide value

Peroxide value is a sensitive indicator of early stages of oxidative deterioration of fats and oils. Peroxide value provides a means of predicting the risk of the development of flavor rancidity. The peroxide value of sample A at ambient temperature was observed to be 2.405 at initial which reached 2.897, 3.201, 3.571 and 4.193 within 2, 4, 6, 8 days respectively. Similarly, for sample D peroxide value was 1.366 at initial which reached 2.099, 3.017 and 4.721 within 2, 4 , 6 days respectively but the PV obtained was far below the unacceptable level of maximum 10 MeqO2 /kg fat as described by (DFTQC, 2012; Mukhopadhyay, 1990) till the last date of analysis. The increase amount of unsaturated fatty acid is prone to rancidity. The change in peroxide value of sample A and sample D in ambient temperature is shown in Fig. 4.11.

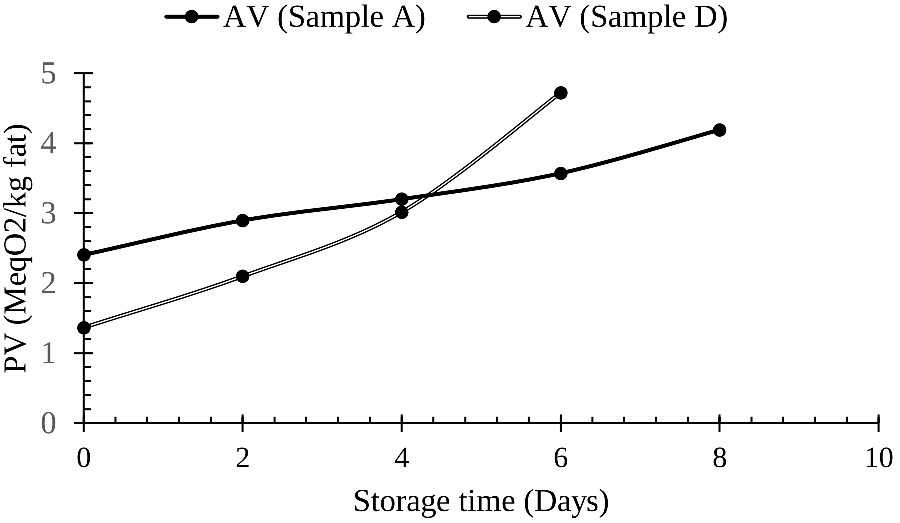


Figure 4.12 Change in peroxide value during storage at ambient temperature of sample A and sample D

4.5.4 Shelf life of the product

Sample A and sample D were kept in the ambient temperature and refrigerated temperature to observe the storage life of muffins. At ambient temperature sample A was fit for consumption for 6 days whereas sample D was fit to consume for 4 days respectively. Similar observation was seen in the research of (Lamsal, 2018).

4.6 Cost evaluation of products

The total cost associated with the control and best product was calculated and the cost of wheat muffin (sample A) and buckwheat flour incorporated with wheat muffin (sample D) was NRs. 27.857 and NRs. 31.28 per 100 g muffin respectively including overhead cost and profit of 10%. From the cost calculation given in appendix D at Table D.1 and D.2, it can be seen that the cost of the muffin is increased by 12.31% with the replacement of wheat by buckwheat flour.

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of the research, the following conclusions can be drawn. Since the work was done under controlled condition on a small scale, its generalization may warrant some reservations.

1. The moisture content, crude protein, crude fat, crude fiber, total ash content and carbohydrate of wheat flour, buckwheat flour and avocado puree were found to be at an acceptable level.

2. Different physical properties were analyzed such as volume, weight, specific loaf volume, weight loss, crust to crumb ratio of the muffin. There was slight increment of the volume, specific loaf volume and crust to crumb ratio and the weight sharply decreased.

3. The nutritional quality of the buckwheat incorporated muffin seemed to be enhanced in the case of fibre, fat content, ash content and protein content. The carbohydrate content of buckwheat incorporated wheat muffin is reduced to 50.215% and caloric valve reduction is 9.063%.

4. There was significant difference in the sensory quality of muffin up to 100 parts substitution of buckwheat flour.

5. The chemical analysis of the product shows acceptability of buckwheat incorporated muffin was up to four days at room temperature and ten days at refrigerated temperature without any artificial preservatives used.

6. The cost of buckwheat incorporated muffin is increased by 12.31%

7. The development of low-gluten muffins, achieved through strategic formulations incorporating varying ratios of wheat to buckwheat flour, represents a promising innovation with potential health benefits.

5.2 Recommendation

The experiment can be further continued with the following recommendations:

1. Entrepreneur can utilize buckwheat flour substituting the wheat up to 50 parts to prepare low gluten muffin without hampering consumer’s acceptance.

2. Texture of the prepared muffin can also be analyzed using texture meter.

Part VI

Summary

Muffins are a sweet, high calorie baked food that customers admire for their exquisite flavor and soft texture. The importance of fat in muffins is also highlighted, emphasizing its role in texture, moisture retention, and overall palatability. Increasing sensitivity to wheat gluten and increasing cases of celiac disease worldwide has created a need for gluten-free baked goods, leading to the development of products such as gluten-free breads, cookies, spaghetti, and crackers containing buckwheat flour.

For the preparation of low gluten muffin, Response surface methodology was used. Five different muffin formulation namely A, B, C, D, E, F and G with the buckwheat flour parts 0, 25, 33.33, 50, 66.66, 75 and 100 respectively. Sensory evaluation was carried out based on appearance, color, flavor, taste, texture and overall acceptability. The data obtained were statistically analyzed using two-way ANOVA (no blocking) at 5% level of significance. Sample D got the highest mean sensory score after sample E. The proximate analysis for moisture, crude protein, crude fat, crude fibre, total ash, gluten and carbohydrate were found to be 12.8%, 13.59%, 3.05%, 0.963%, 2.43%, null, 79.95% and 20.073%, 15.573%, 22.372%, 7.26%, 3.679% and 50.21% of buckwheat and Sample D (best) respectively. The loaf volume decreases with the increase in buckwheat proportion.

The AV, PV and moisture content of sample D at ambient temperature and refrigerated temperature at day 0 was found to be 1.01 mg KOH/g oil and 2.405 meq O2/kg fat and 13.17% respectively. At ambient temperature, AV, PV and moisture content of sample D reached 3.143 mg KOH/g oil, 3.571 meq O2/kg fat and 10.692% on day 6. Similarly, at refrigerated temperature, AV, PV and moisture content of sample D reached 5.335 mg KOH/g oil, 4.311 meq O2/kg fat and 9.67% on day 12. The cost of sample D was found to be NRs.31.28 per 100 g, which is 16.31% more than the cost of sample A.

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Appendices

Appendix A

Sensory Analysis Score Card

Name of the panelist: Date:

Name of the product: Preparation of low gluten muffin from composite (wheat and buckwheat) flour by using (butter and avocado) fat.

Dear panelist, you are provided with 5 samples of Avocado puree incorporated muffin on each proportion with variation on avocado puree content. Please test the following samples of muffin and check how much you prefer for each of the samples. Give the point for your degree of preference for each sample as shown below.

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

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

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

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

Parameters	Sample code						
	A	B	C	D	E	F	G
Appearance							
Color							
Aroma							
Texture							
Taste							
Overall acceptability							

Appendix B

ANOVA results of sensory analysis

Table B.1 ANOVA (no interaction) for appearance of buckwheat incorporated muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	42.8	7.1333	16.01	<.001
Panelist	10	3.8429	0.427	0.96	0.484
Residual	54	24.0571	0.4455		
Total	69	70.7			

Table B.2 ANOVA (no interaction) for color of buckwheat incorporated muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	64.4857	10.7476	27.34	<.001
Panelist	10	2.8714	0.319	0.81	0.608
Residual	54	21.2286	0.3931		
Total	69	88.5857			

Table B.3 ANOVA (no interaction) for aroma of buckwheat incorporated muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	52.6	8.7667	42.59	<.001
Panelist	10	1.4857	0.1651	0.8	0.616
Residual	54	11.1143	0.2058		
Total	69	65.2			

Table B.4 ANOVA (no interaction) for texture of buckwheat incorporated muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	63.2857	10.5476	44.8	<.001
Panelist	10	1.0857	0.1206	0.51	0.859
Residual	54	12.7143	0.2354		
Total	69	77.0857			

Table B.5 ANOVA (no interaction) for taste of buckwheat incorporated muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	49.8857	8.3143	34.61	<.001
Panelist	10	1.7286	0.1921	0.8	0.618
Residual	54	12.9714	0.2402		
Total	69	64.5857			

Table B.6 ANOVA (no interaction) for overall acceptability of buckwheat incorporated muffin

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	7	46.8857	7.8143	28.46	<.001
Panelist	10	1.7714	0.1968	0.72	0.691
Residual	54	14.8286	0.2746		
Total	69	63.4857			

Appendix C

Table C.1 t-test (two- sample assuming unequal variance) for moisture of the best sample (sample D) with control (sample A)

	Sample A	***Sample D***
Mean	13.293	20.07333333
Variance	0.391917	1.016133333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-9.896977492	
P(T<=t) one-tail	0.001096976	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.002193952	
t Critical two-tail	3.182446305	

Table C.2 t-test (two- sample assuming unequal variance) for protein of the best sample (sample D) with control (sample A)

	Variable 1	***Variable 2***
Mean	11.29333333	15.57333333
Variance	0.393633333	1.703033333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.119643271	
P(T<=t) one-tail	0.007212245	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.01442449	
t Critical two-tail	3.182446305	

Table C.3 t-test (two- sample assuming unequal variance) for crude fat of the best sample (sample D) with control (sample A)

	Sample A	***Sample D***
Mean	19.70833333	22.372
Variance	0.440324333	0.261889
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-5.505616637	
P(T<=t) one-tail	0.002654269	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.005308537	
t Critical two-tail	2.776445105	

Table C.4 t-test (two- sample assuming unequal variance) for crude fibre of the best sample (sample D) with control (sample A)

	Variable 1	***Variable 2***
Mean	1.157666667	7.268333333
Variance	0.020997333	0.072776333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	34.56278838	
P(T<=t) one-tail	2.66261E-05	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	5.32522E-05	
t Critical two-tail	3.182446305	

Table C.5 t-test (two- sample assuming unequal variance) for total ash of the best sample (sample D) with control (sample A)

	Sample A	***Sample D***
Mean	2.460333333	3.679333333
Variance	0.013290333	0.015721333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-12.39589799	
P(T<=t) one-tail	0.00012173	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.00024346	
t Critical two-tail	2.776445105	

Table C.6 t-test (two- sample assuming unequal variance) for carbohydrate of the best sample (sample D) with control (sample A)

	Variable 1	***Variable 2***
Mean	52.25	67.21
Variance	0.8377	0.7933
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-20.28921482	
P(T<=t) one-tail	1.74205E-05	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	3.48409E-05	
t Critical two-tail	2.776445105	

Table C.7 t-test (two- sample assuming unequal variance) for crumb moisture of the best sample (sample D) with control (sample A)

	Sample A	***Sample D***
Mean	17.275	26.27
Variance	0.99405	0.72
Observations	2	2
Hypothesized Mean Difference	0	
df	2	
t Stat	-9.716378454	
P(T<=t) one-tail	0.005213471	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.010426943	
t Critical two-tail	4.30265273	

Table C.8 t-test (two- sample assuming unequal variance) for crust moisture of the best sample (sample D) with control (sample A)

	Sample A	***Sample D***
Mean	7.956666667	11.35
Variance	0.076033333	0.3892
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-8.616913607	
P(T<=t) one-tail	0.001643312	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.003286624	
t Critical two-tail	3.182446305	

Table C.9 t-test (two- sample assuming unequal variance) for weight loss of the best sample (sample D) with control (sample A)

	Variable 1	***Variable 2***
Mean	14.081	17.466
Variance	0.099925	0.273747
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-9.59122374	
P(T<=t) one-tail	0.001202483	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.002404966	
t Critical two-tail	3.182446305	

Table C.10 t-test (two- sample assuming unequal variance) for crust/crumb ratio of the best sample (sample D) with control (sample A)

	Variable 1	***Variable 2***
Mean	0.460893698	0.432995442
Variance	0.000261273	0.000188053
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	2.279592086	
P(T<=t) one-tail	0.042412363	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.084824726	
t Critical two-tail	2.776445105	

Table C.11 t-test (two- sample assuming unequal variance) for caloric value of the best sample (sample D) with control (sample A)

	Sample A	*Sample D*
Mean	514.791	465
Variance	0.369453	0.502805333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	26.47243544	
P(T<=t) one-tail	6.05098E-06	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	1.2102E-05	
t Critical two-tail	2.776445105	

Appendix D

Table D.1 Cost calculation of the control (sample A)

Particulars	Cost (Rs/kg)	Weight in a lot (g)	Cost (Rs)
Wheat Flour	80	100	8
Buckwheat Flour	240	0	0
Sugar	90	65	5.85
Avocado puree	150	32.5	4.875
Butter	900	32.5	29.25
Baking Powder	300	57	17.1
Egg	125	1.42	0.1715
Raw materal cost			65.2525
Processing and labor cost (10% of raw material cost)			6.5255
Profit (10%)			7.17
Grand total cost			78.951
Average weight of muffin batter		283.42	
Number of muffins formed		24	
Per piece weight of muffin (g)		11.809	
Per piece cost muffin			3.289
Total cost of muffin (Rs/100g)			27.8565

Table D.2 Cost calculation for best sample (sample D)

Particulars	**Cost (Rs/kg)**	**Weight in a lot (g)**	**Cost (Rs)**
Wheat Flour	80	50	4
Buckwheat Flour	240	50	12
Sugar	90	65	5.85
Avocado puree	150	32.5	4.875
Butter	900	32.5	29.25
Baking Powder	300	57	17.1
Egg	125	1.42	0.1715
Raw materal cost			73.2555
Processing and labor cost			
(10% of raw material cost)			7.3252
Profit (10%)			8.05807
Grand total cost			88.63877
Average weight of muffin batter		283.42	
Number of muffins formed		24	
Per piece weight of muffin (g)		11.809	
Per piece cost muffin			3.69
Total cost of muffin (Rs/100g)			31.28

Appendix E

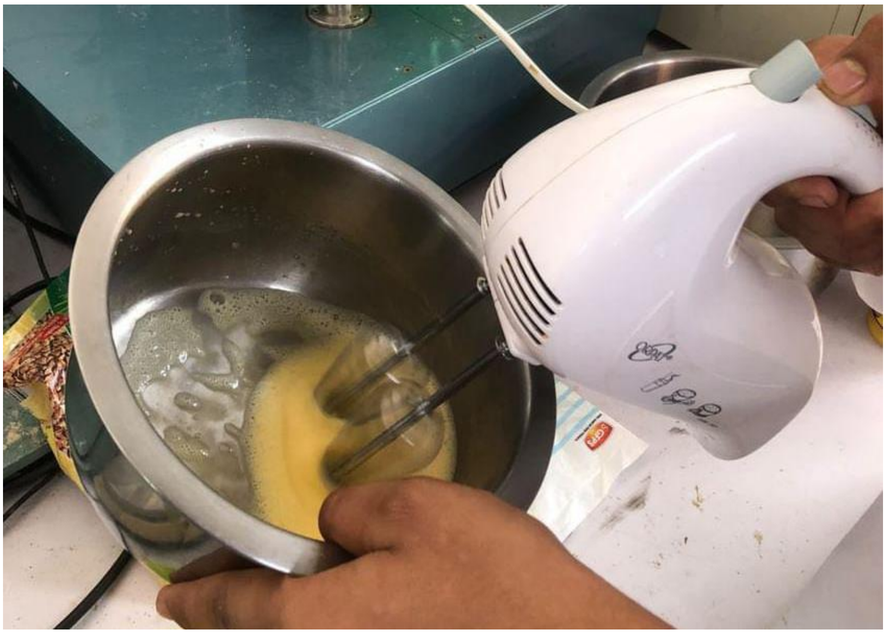
Apparatus

- Oven
- Electronic balance
- Measuring cylinder, beaker, pipette, Volumetric flask, conical flask, test-tube, funnel
- Soxhlet assembly
- Buchner filter assembly
- Hot air oven
- Muffle furnace
- Petriplate
- Dean and stark apparatus

Chemicals required

- Petroleum ether
- Acetone
- Sulfuric acid
- Sodium hydroxide
- Hydrochloric acid
- Boric acid
- Catalyst mixture
- MacConkey medium
- Plate count agar
- Alcohol
- Sodium thiosulphate
- Potassium dichromate
- Phenolphthalein

Colour Plates


P1: Creaming


P2: Panelist performing sensory


P3: Protein Determination

P4: cell uniformity of sample A(control)

P5: cell uniformity of sample D (best)