EFFECT OF AVOCADO PUREE AS FAT REPLACER IN THE COMPOSITE FLOUR (WHEAT AND OATS) MUFFIN

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2022

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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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July, 2022

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Approval Letter

This *dissertation* entitled **Effect of Avocado Puree as Fat Replacer in the Composite Flour (Wheat and Oats) Muffin** presented by **Barsha Baniya** has been accepted as the partial fulfilment of the requirement for the **B. Tech. degree** in **Food Technology.**

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Acknowledgements

I would like to express my heartfelt gratitude to my respected guide Prof. Mr. Pashupati Mishra for his kind support, excellent guidance, encouragement and constructive recommendations throughout the work.

I am really thankful to Asst. Prof. Navin Gautam (Head of Department, Department of Food Technology) and Assoc. Prof. Dr. Dil Kumar Limbu (Campus Chief, Central Campus of Technology) and Prof. Basanta Kumar Rai (Head of Department, Central Department of Food Technology) for providing opportunity and making available all the facilities to carry out the best in this dissertation work.

I also express my sincere thanks to my teachers, staff of library and laboratory for their direct and indirect co-operation and suggestions without whom this work could not have been possible.

I cannot stay from thanking all my classmates for their help in preparing me for this through all those experiences and memories which will never be forgotten in my whole life. Special thanks must be bestowed to my senior Nabindra Shrestha, Sanjog Kharel, Shiva Kumar Shrestha, Archana Shrestha, my friends Bhawana Poudel, Sunita Karki, Sangita Rijal and all my beloved juniors for their support during the completion of this work. I would like to thank all those whose perceptions, observation and inputs have helped me directly or indirectly.

No words in this world can ever express the love, encouragement, inspiration and moral support given by my parents and family members during my tough days.

Date of submission:

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Abstract

This work was carried out to prepare avocado puree incorporated composite flour (wheat and oats flour) muffin and evaluate its sensory and physicochemical properties. Raw material (oats flour, butter, sugar and egg) and avocado (Hass variety) were collected from the local market of Dharan and Dhankuta respectively. Proximate analysis of oats flour, wheat flour and avocado puree (de-pitting and homogenized) were carried out. The puree was incorporated at the level of 0 - 65 parts with 65 - 0 parts butter and named as sample A, B, C, D and E respectively. All the ingredients were mixed with sugar 60%, egg 57%, baking powder 1.42% per 100 parts of flour mixture (75% oats flour and 25% wheat flour) respectively. The superior product obtained through sensory evaluation and acceptability period was estimated in ambient and refrigerated condition.

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 8.4%, 13.34%, 9.38%, 1.48%, 1.53%, and 74.27% respectively for oats flour and 72.64%, 3.826%, 78.74%, 11.69%, 2.607% and 3.167% respectively for avocado puree. Through sensory evaluation, muffin incorporated with 32.5 parts puree and 32.5 parts butter (sample C) was superior in comparison to all other muffin formulation. Statistical analysis ($p \leq 0.05$) showed that substitution of avocado puree significantly improved all the physicochemical attributes (total ash content, fibre, crude protein) except carbohydrate and crude fat compared to composite flour muffin without puree whereas significantly decrease the specific loaf volume of muffin. The free fatty acid as oleic acid and peroxide value of sample C at day 0 was found to be 1.435 mg KOH/g oil and 1.561 Meq O_2/kg fat respectively. The sample C was fit for consumption for day 4 and day10 respectively. No colony of yeast and mold and coliform were found till those days.

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List of Abbreviations

Abbreviation	Full form
ANOVA	Analysis of variance
AV	Acid value
PV	Peroxide value
TPC	Total plate count
Wb	Wet basis
Db	Dry basis
LSD	Least significant difference
SD	Standard deviation

Part I

Introduction

1.1 General introduction

Muffins are a sweet, high-calorie baked food that customers admire for their exquisite flavor and soft texture (Martinez-Cervera *et al.*, 2012). Muffins are quick bread as “quick-acting” chemical leavening agents are used instead of yeast, “longer-acting” biological leavening agent (Hui *et al.*, 2007). The principal ingredients of muffins are flour, sugar, fat, and egg which play an important role in the structure, appearance, and eating quality of the final product (Karaoğlu and Kotancilar, 2009; Martinez-Cervera *et al.*, 2012).

The major ingredient in muffins is flour (Bhaduri, 2013). 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. 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, 2001).

Gluten is a key protein component of wheat flour that is thought to be the cause of celiac disease. Life-long gluten-free diet has been considered as the only effective treatment for celiac disease (Bhaduri, 2013). Celiac disease has an ever-increasing number of internal and external consequences. Internal consequences include the persistence of a flat intestinal mucosa, which is often followed by a decrease in enzyme activity and as a result, a vitamin and mineral deficiency, which can lead to a range of inadequacies. External signs include dermatitis, herpetiformis (pale skin), and dry skin (Hui *et al.*, 2007).

The oat (*Avena sativa*) belongs to the poaceae family. Oats are a small cereal crop in terms of the volume of grain produced each year or the area cultivated for production. Oats are a nutrient-dense cereal that also contains a considerable quantity of soluble fibre and minerals. Vitamin E (tocotrienols), phytic acid, phenolic acid, protein, lipids (unsaturated fatty acids), and minerals are abundant in oats (W. S. Ahmad *et al.*, 2014).

Fat is one of the main ingredients in bakery product like muffin. Fat gives a softer texture and helps to prevent the CO_2 bubbles from escaping from the mixture too soon, it also produces emulsifying properties and holds considerable amounts of liquid to increase and extend cake softness and interrupts the protein particles to break gluten continuity to tenderize the crumb (Bennion and Bamford, 1973).

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. Previous research has identified a number of natural fat-replacement substances, including mung bean paste, pawpaw fruit puree, apple sauce, and okra gum (Adair *et al.*, 2001; Hayek and Ibrahim, 2013; Hu and Lai, 2017; Wiese and Duffrin, 2003)

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; Pieterse *et al.*, 2005). Avocados have a calorie density of 1.7 calories per gram, with 114 calories, 4.6 grams of fibre, 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). Avocado lipids are made up of 71% monounsaturated fatty acids (MUFA), 13% polyunsaturated fatty acids (PUFA), and 16% saturated fatty acids (SFA) (USDA, 2011).

1.2 Statement of problem

Nutrition and health awareness, concern lead consumers to choose reduced- or low-fat/-calorie foods. In the baking industry, low-fat and low-calorie food products have been investigated and created on a regular basis to meet consumer demand for healthier food options (Sandrou and Arvanitoyannis, 2010). Previous research has shown that avocado puree can be used as a fat substitute in oatmeal cookies and butter cake, with up to 50% avocado substitution for butter (Marina *et al.*, 2016; Wekwete and Navder, 2008). However, the potential of avocado puree as a fat replacer in oat muffin has not been tested. In view of the preceding, this study was conducted to develop fat-reduced oat muffin while determining the nutritional characteristics of oat muffin incorporating avocado puree. To identify the optimal level of avocado substitution in muffin recipes, the sensory quality and acceptability of muffin was also tested.

1.3 Objectives

1.3.1 General objectives

The general objective of this dissertation work is to study the effect of avocado puree as fat replacer in the composite flour (wheat and oats) muffin.

1.3.2 Specific objectives

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

1.4 Significance of study

Obesity is linked to a slew of health issues (USA, 2013). Obesity was 42.4% among adults in 2017–2018, with no significant variations between men and women across all individuals or by age group. Year after year, the rate of obesity is rising(Hales *et al.*, 2020). Similarly, the wheat protein (gluten) causes the celiac disease (Hui *et al.*, 2007). Consumers are concern about the gluten-free and low calorie diet (Sandrou and Arvanitoyannis, 2010). This study will focus on the scope of avocado puree as the fat replacer in oats muffin. 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 Limitation of the study

1. Instrumental textural analysis was not carried out.

Part II

Literature review

2.1 Muffins

A muffin is defined as the type of bread that is baked in small portions. Many forms are somewhat like small cakes or cup-cakes in shape, although they usually are not as sweet as cupcakes and generally lack frosting. Muffins are produced similar to low-ratio cakes, but the batter is placed in small cup-shaped mould before baking. English muffins originating in London were made from yeast dough, in contrast to the quick-bread muffins served in early America (Hui *et al.*, 2007). Consumption of baked products constitutes an important part of a daily breakfast considering that people are continually grabbing meals on the go. Among baked products, muffins rank third in breakfast products and attract a broad range of consumers (Rosales-Soto *et al.*, 2012). Most are produced from refined soft wheat flour, although whole wheat muffins and batters supplemented with blueberries, raspberries, dates, nuts, chocolate chips, raisins, carrots, and other products are gaining popularity (Serna-Saldivar, 2012).

A high quality muffin described by Halliday and Noble (1946) is as follows: To be good, muffins should be very light, so light in fact that when one picks them up one is surprised that anything of their size should weigh so little. The outside should be baked to a golden-brown shade; should be symmetrical in shape, with no tendency to form peaks or knobs at the top; and should have a somewhat pebbled, rather than a smooth and even surface. The inside should show round holes of fairly uniform size but should have none of the long, narrow ones sometimes called “tunnels”.

2.2 Chemical composition of muffins

Chemical composition of muffin is shown in Table 2.1

Table 2.1 Chemical composition of muffin

Constituents	Values
Moisture, %	20.33
Fat, %	14.37
Protein, %	17.60
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

In order to make muffins, the endosperm of *Triticum vulgare* is milled into flour with particles large enough to pass through a flour sieve typically 100 mesh per linear inch (Kent and Amos, 1983). Besides carbohydrates, flour contains glutenin and gliadin, two proteins that work together to keep other ingredients together and provide structure to the final baked dish After being hydrated and heated, starch expands, breaking hydrogen bonds and giving the batter additional structure (McWilliams, 2001).

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% min
Total ash on dry basis	0.5% max
Acid insoluble ash on dry basis	0.5% max
Protein (N×7.5) on dry basis	9.0%
Alcohol acidity as H_2SO_4 in 90% alcohol	0.1%
Water absorption	5%
Sedimentation	22%
Granularity	To satisfy the taste

Source: Arora (1980)

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 flavour components dissolve in fat (McWilliams, 2001). 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 MeqO$_2$/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 MeqO$_2$/kg fat respectively (DFTQC, 2018).

However, bakery products are often not consumed and refered 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

The concept "fat replacer" refers to a wide range of products that replace some or all of the fat in foods, with the purpose of preserving as much of the food's sensory aspects as possible while lowering fat and calorie content.

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 with little 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.

Carbohydrate, protein, and fat are all used as fat substitutes. Fat mimetics are carbohydrate and protein-based fat substitutes; low-calorie fats or fat substitutes are fat-based fat substitutes. Several fat substitutes are frequently combined in one product by manufacturers (Jones and Roller, 1996).

Khalil (1998) investigated the physical and sensory features of cakes made with carbohydrate-based fat substitutes. Using carbohydrate-based fat replacers enhanced the batter's specific gravity significantly ($p \leq 0.05$), especially at the 25% and 50% replacement levels. Wekwete and Navder (2008) investigated the effectiveness of two alternative fat replacers, avocado pure and Oatrim, in partially replacing (50%) butter fat. Samkaria (2018) also performed a thesis on apple pomace replacing fat in which the fat content decreased, increasing the crude fibre, moisture content and ash content. The sensory aspect is also affected by the replacement of the fat, in which the appearance and color lowers after the full fat replacement. It was also pronounced in the cake with the cantaloupe as fat replacer (Hussien, 2016)

2.3.4 Sweeting agent

Another important ingredient in muffins is sugar. Sugar cane and sugar beet are the most common sources of sugar used in muffins. Sugarcane has a sucrose content of 16-22 %, while sugar beet has a sucrose content of 8-9 %. When manufacturing muffins, different types of sugar are used, such as crystalline, crushed, liquid, brown, or soft sugar, depending on the product. Pulverised sugar is the most common type of sugar used in muffin baking. It's possible that this is due to its easily soluble nature, which fools the tongue into thinking it is sweet. The crystalline size has an impact on muffin sweetness, shortness, and spreadability (Whitely, 1971).

2.3.5 Leavening agent

Leavening is a type of gassing agent that causes the dough to spring back or puff up, giving the final product a porous, open texture. 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. Baking powder, the most frequent and important leavening agent, should have the following characteristics. (W. H. Smith, 1972).

1. Maximum gas strength-greatest volume of gas for least weight of the product.
2. Proper balance of ingredients to prevent any impairment of the taste or appearance of the biscuit.

3. Innocuous ingredients and residues.
4. Optimum velocity of reaction to be susceptible to control.
5. Keeping quality under diverse and extreme conditions to remain unimpaired over reasonable periods of time.
6. Minimum cost of production, economical in use.

The chemical reaction during use of chemical leaveners and acidulants is as given in this section.

$$NH_4HCO_3 \xrightarrow{\text{Heat}} NH_3 + CO_2 + H_2O$$

Ammonium bicarbonate → Ammonia + Carbon dioxide + Water

$$(NH_4)_2CO_3 \xrightarrow{\text{Heat}} 2NH_3 + CO_2 + H_2O$$

Ammonium carbonate → Ammonia + Carbon dioxide + Water

The chemical equations for the reaction of soda and acidulants are as follows:

$$NaHCO_3 + HX \longrightarrow NaX + CO_2 + H_2O$$

Sodium bicarbonate + Acid → NaX + Carbon dioxide + Water

$$NaHCO_3 + C_4H_5O_6K \longrightarrow C_4H_4O_6NaK + CO_2 + H_2O$$

Cream of tartar → Sod. Pot. Tartrate

Both sodium and ammonium bicarbonate react with acidic ingredients if any, in the dough. Use of an excess ammonium bicarbonate makes the dough more alkaline and thereby may affect the protein structure of the dough. Use of an excess sodium bicarbonate will affect the crumb and crust color often with an accompanying unpleasant or soapy taste, unless any acidic material is used to neutralize the residual sodium carbonate (Bohn, 1956).

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

Water is one of the most important ingredients during muffin making. Quality of water used has a great effect on the product. Dissolved minerals and organic matters present in water can affect the flavor, color and physical attributes of the finished baked product (W. H. 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). With the storage time the muffin moisture content decreased strongly, between first and second week there was 50% reduction in the moisture content after 12 weeks of storage in dark at room temperature (Grillo *et al.*, 2014). The moisture content between 15-30% is acceptable (Sain *et al.*, 2014).

2.4 The muffin method of mixing

The muffin method is a technique whereby two mixes are created; a mix of wet ingredients (eggs, soft or liquid fat, milk and sugar) and a mix of dry ingredients (flour, leavening and flavorings like cocoa powder). Once they are prepared, and the oven is preheated, the two are combined and stirred together very briefly before the finished batter is panned and baked (Miller, 1971).

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, oats 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 and sugar were mixed, to obtain muffin batter. The batter was filled in paper muffin cup. The muffins were baked at 215ºC in oven for 20±3 min (Khoueyieh *et al.*, 2005). Lamsal (2018) made modification on the flour composition with the oats flour of 75 parts and 25 parts of wheat flour for the preparation of muffin.

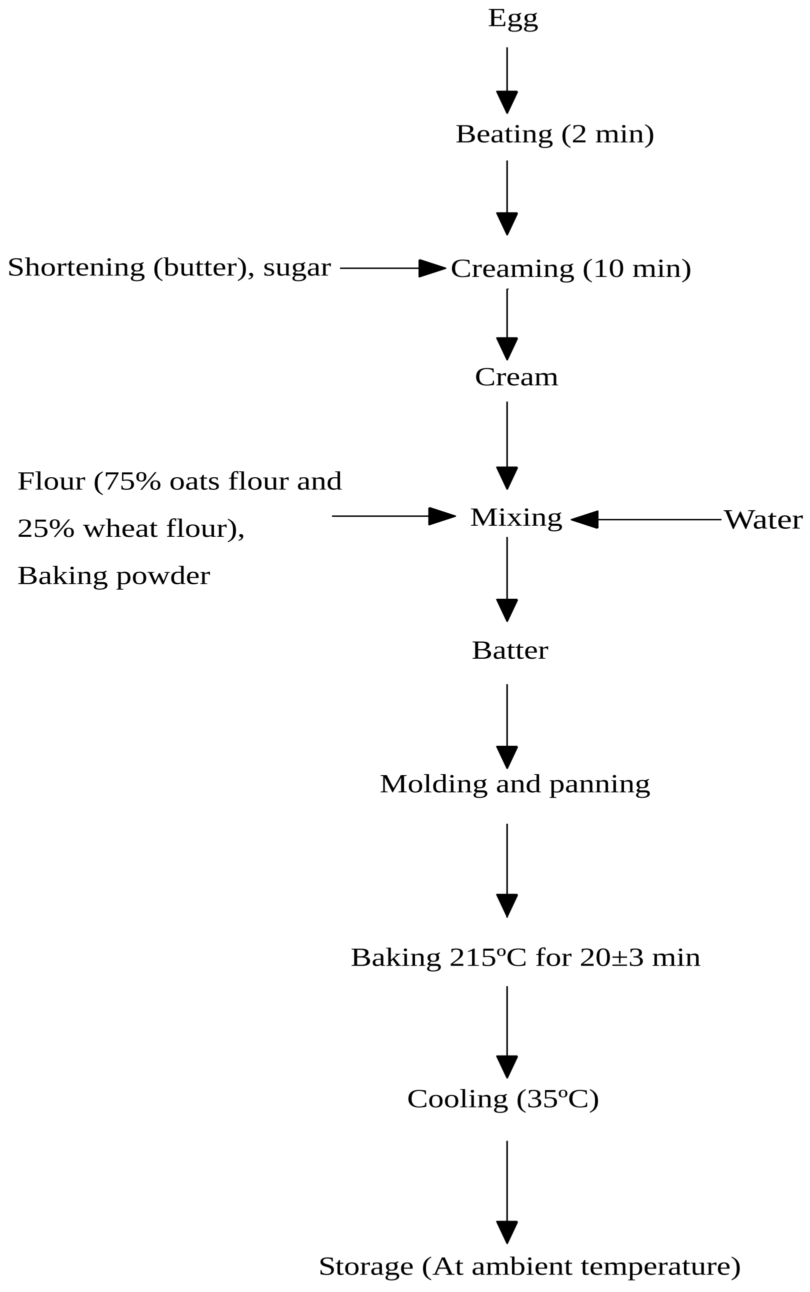

Fig. 2.1 Flow chart of oats muffin

Source: Lamsal (2018)

2.7 Baking profile

Baking is the most important phase in muffin production since the product loses its eating quality if it isn't done properly. The product is cooked, the flavor and color are produced, and the uncooked dough is transformed into an edible snack known as a muffin during baking. Baking's main goal is to eliminate extra moisture from the dough by gradually heating it (Bloksma, 1990). Every baking process is reliant on heat transfer from a hot source to the cooked result. During baking, heat is transferred primarily through three methods: conduction, convection, and radiation. During baking, radiation provides the majority of heat transmission to the dough pieces, with convection providing only a small portion of the heat transfer until the air velocity in the tunnel exceeds 5 feet per second, after which convection provides a larger portion of the heat transfer. Aside from these three heat transmission modalities, high frequency heating is also used, which has a faster moisture removal rate (W. H. Smith, 1972).

Every oven used till date consists of four basic parts.

1. A heat source
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

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

Table 2.3 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 CO_2 within crumb (Less solubility of CO_2).
32.22-65.56	Increase in volume due to CO_2
32.22-98.89	Gas expansion (CO_2 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 (W. H. Smith, 1972).

2.8 Oats

Cereal grains are used to feed a substantial portion of the world's population. Wheat, rice, and maize are the most often consumed grains. These grains are either consumed whole or fractionated. Oats are still a prominent cereal crop in developing countries, and the most widely grown species is *Avena sativa*, sometime known as common covered white oats. Oats grow best in cool, moist climates because they require more moisture to generate a given unit of dry matter than all other cereals besides rice (Prasad *et al.*, 2015). Oats are mostly grown throughout North America and Europe, primarily in Russia, Canada, and the United States of America. It is mostly used for animal feed and, to a lesser extent, as human food. Because of the growing use and interest in oats as a human health food, their use as animal feed has steadily reduced (A. Ahmad *et al.*, 2010).

2.8.1 Chemical composition and nutritive value of oats

The biochemical composition of the oats grain is as shown in Table 2.4.

Table 2.4 Biochemical composition of oats grain (Value ± SD)

Parameters	Husked oats	Naked oats
Protein content, %	10.58±0.67	15.71±1.10
Fat content, %	5.15±0.19	9.66±1.87
Starch, %	48.08±0.29	31.55±3.72
Total dietary fibre, %	17.63±1.52	22.97±1.89
β-glucan, %	3.15±0.19	3.29±0.26

Source: Zhao *et al.* (2014)

Oats are rich in carbohydrate. Besides, protein is the second vital constitute of the oats. Significant amount of fat is also present in the oats. Apart from this it is rich in minerals and vitamins. Table 2.5 gives a general idea about the chemical constitutes of oats.

Table 2.5 Proximate constituents of oats

Constitutents	%
Moisture	10.7
Protein	13.6
Fat	7.6
Carbohydrate	62.8
Minerals	1.8

Source: DFTQC (2012)

2.8.2 Nutritional components of oats

2.8.2.1 Oats starch

About 60% of the oat grain is made up of starch. It is primarily found in endosperm. There is a significant difference in physicochemical properties between oats starch and other cereal starches. Distinct cultivars of oats have different physicochemical properties as well. The amount of interaction between and among starch chains within the amorphous and crystalline portions of native granules, as well as the chain length of the amylose and amylopectin fractions of oats starch, are likely to be the cause of these variances. Oats starch has unusual characteristics, such as tiny granule size, a well-developed granule surface, and a high lipid content (Berski and Ptaszek, 2011).

2.8.2.2 Oats protein

Oats are regarded as a low-cost protein source with high nutritional value. Oats have a distinct protein profile and a high protein level of 11–15 %. Albumins (water-soluble), globulins (salt water-soluble), prolamins (soluble in dilute alcohol solution), and glutelins (soluble in dilute alcohol solution) are the four forms of cereal proteins (soluble in acids or bases). In comparison to other cereal grains, oat protein differs not only in structural features but also in protein fraction distribution. Other cereals, such as wheat and barley, have a distinct protein matrix that the oat lacks. The store protein in wheat and certain other cereals is insoluble in salt solutions, although a considerable fraction of salt water soluble globulins in oats also belong to the endosperm storage proteins (C. Klose *et al.*, 2009).

The amount of prolamins (15%) in oats is lower than the amount of globulins (80%) in total oats protein. Prolamins (avenins) are oat protein fractions with a low molecular weight. These prolamins can be dissolved in 50–70% ethyl alcohol or 40% 2-propyl alcohol. In comparison to the other protein fractions, prolamins have a high glutamine and proline content and a low lysine content (Robert and Nozzolillo, 1985). The storage function of avenins, a kind of prolamin, is similar to that of other cereal prolamins. Because they are difficult to completely solubilize and are reliant on the extraction solvent and solvent concentration, glutelin levels have been reported to range from 5 to 66 % of total protein. Water soluble albumin makes up the majority of the total metabolically active proteins in oats. Albumins make up roughly 1–12% of the total protein in oats. Albumin and globulin have more lysine than other proteins. As a result, oats have a higher lysine level than other cereals, but a lower glutamic acid and prolamin concentration (Lapvetelainen and Aro, 1994).

The consumption of gluten by a gluten intolerant individual causes Celiac disease. Gluten is a complex protein found in wheat and similar cereals such as barley and rye that is soluble in alcohol. Gluten causes an abnormal small intestine immune response characterized by villous atrophy and crypt hyperplasia in people who are genetically vulnerable (Fasano and Catassi, 2001), resulting in malabsorption of protein, lipids, carbohydrates, soluble vitamins, folate, and minerals, particularly iron and calcium. At the moment, the sole treatment option is to entirely eliminate gluten from the patient's diet. Oats have a more favorable and nutritionally useful protein fraction composition than other grains (Capouchova *et al.*, 2006). However, whether oats are safe for celiac sufferers has long been a point of contention. (Baker and Ream, 1976; Dicke *et al.*, 1953) urged complete oat exclusion, whereas (Baker and Ream, 1976; Dicke *et al.*, 1953; Janatinen *et al.*, 2006; Ripsin *et al.*, 1992) promoted oat inclusion in the celiac diet. The protein fractions albumins, globulins, prolamins (avenins), and glutelins determine whether oats can be used in a gluten-free diet.

Prolamins, along with glutelins, make up the reserve protein found in the grain endosperm, which accounts for 60–70% of cereal grain proteins. The prolamin components are more resistant to hydrolysis and thus more difficult to digest. In comparison to wheat (40–50 %), rye (30–50 %), and barley (35–45 %), oats have a low prolamin concentration

(10–15 % of total protein). According to Kumar and Farthing (1995) avenins (oats prolamins) may cause toxicity in celiac patients only if oats are taken in large numbers relative to rye and barley. The amount of prolamins in oats varies depending on the species, variety, and culture duration. However, oats are now acceptable ingredients under European Commission Regulation No. 41/2009, assuming the gluten concentration does not exceed 20 ppm (mg/kg) (Henkey, 2009).

2.8.2.3 Dietary fibre

Dietary fibres are an important component of a healthy diet. They are made up of a variety of plant-based compounds that are not digested by the human upper gastrointestinal tract. Polysaccharides such as cereal-glucan, arabinoxylans, and cellulose are among them. Dietary fibres are found in the grain's cell walls. The pericarp, seed coat, and outer layers all contribute significantly to the grain's insoluble dietary fibre content. Dietary fibre is defined as "the edible fraction of plant or similar carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine," according to the American Association of Cereal Chemists (AACC). Polysaccharides, oligosaccharides, lignin, and other plant compounds are included. Dietary fibres have a variety of physiological benefits, including laxation, blood cholesterol reduction, and blood glucose reduction (AACC, 2016).

2.8.2.4 Lipids

Oats have been demonstrated to provide a wide range of human health advantages, including reduced diabetes and obesity symptoms. The key component of oats responsible for these health advantages is -glucan, however other antioxidant chemicals and phenolic compounds in oats also have health benefits. Tocopherols, tocotrienols, phytic acid, flavanoids, and non-flavonoid phenoilc substances such as avenaantramides all contribute to oats' antioxidant activity (Tapola *et al.*, 2005).

2.8.3 Oats as a functional food

Oats have long been recognized as a healthy and nutritious cereal that is strong in soluble fibre and minerals. Regardless of how nutritionally dense a cereal is, it has physiological benefits such as lowering hyperglycemia, hyperinsulinemia, and hypercholesterolemia, among others. The main physiological effect of soluble fibre from oats is an increase in viscosity, which is caused by soluble fibre such as (1→3, 1→4) - β -D-glucan or β-glucan. Glycaemic, insulin, and cholesterol responses to diets have all been found to be influenced by β-glucan. Oats are a good source of functional elements including β-glucan, which have been shown to have health advantages in studies (W. S. Ahmad *et al.*, 2014).

Oats include a variety of dietary fibre components, including mixed-linkage (1→3) and (1→4)-D-glucan arabinoxylans, as well as cellulose. Polysaccharide-neutral β-glucan cell wall offers exceptional functional and nutritional qualities. It is particularly important in human nutrition since it achieves high viscosities at low doses. Oats' soluble fibre has been shown to lower blood cholesterol, triglyceride, and glucose levels. Oats are also rich sources of insoluble fibre, which acts as a water-holding agent and can shorten intestinal transit time when consumed in sufficient amounts. (Paton *et al.*, 1995).

2.8.4 Oats flour

Rolled oats can be ground directly into flour. Most of the particles from the groat cutting and flaking process end up in the stream of oat flour hammer mills are commonly used for grinding, however pin mills and other forms of size reduction equipment can also be employed alone or in sequence. Due to the high fat content of oat groats, corrugated rolls, which are typically used for wheat milling, are inappropriate for oat milling (Menon and Watson, 2016). Prasad *et al.* (2015) reported proximate values of moisture content, crude protein, crude fat, crude fibre, total ash and carbohydrate 10.71, 13.1, 9.2, 1, 1.8 and 74.8% respectively. Lamsal (2018) reported proximate values of moisture content, crude protein, crude fat, crude fibre, total ash and carbohydrate 11.97%, 13.56%, 9.43%, 1.18%, 1.8%, and 74.03% respectively.

"Whole oats flour is generated from clean, 100 % groats or from products derived without material loss from whole groats, by stabilizing and size reduction," according to the AACCI (American Association of Cereal Chemists International) in 2004 (Bhaduri, 2013).

2.8.4.1 Benefits of oats flour

i. Reduces heart disease risk

An epidemiologic study published in the Archives of Internal Medicine looked at the relationship between dietary fibre intake and the risk of coronary heart disease (CHD) and cardiovascular disease (CVD) in 9,776 adults. The researchers found that subjects consuming the most fibre, 20.7 g per day, had 12% less CHD and 11% less CVD compared to those eating the least amount (five grams per day) of fibre (Khanal, 1997).

ii. Lowers cholesterol

Another reason that oats flour is so great for the heart is that it has been shown to lower LDL (low density lipid) ("bad") cholesterol. Specifically, it is the beta-glucan (β-glucan) found mainly in the endosperm cell wall of oats that is believed to be responsible for decreasing total serum cholesterol and LDL cholesterol. How does it work? Well β-glucan is a highly glutinous soluble fibre so as it travels through the small intestine, it actually limits the absorption of dietary cholesterol (Mickee, 2015).

iii. Helps diabetics

Research shows that, in moderation, oats can be a healthy and helpful food for diabetics and others struggling with blood sugar issues. The aim of a 2015 scientific review was to figure out if oats intake is beneficial for diabetic patients. The researchers looked at 14 controlled trials and two uncontrolled observational studies, and the findings are quite impressive (Prasad *et al.*, 2015).

Compared with the controls, oats intake significantly reduced the concentrations of blood glucose as well as total cholesterol and LDL cholesterol. The conclusion of the review is that oats intake can benefit both blood sugar control and lipid profiles in type 2 diabetics, making it a great addition to any diabetic diet plan (Prasad *et al.*, 2015).

iv. Satiety value

Since oats flour is really just ground-up whole oats, adding it to your meals and recipes can also help you feel more satisfied after consuming it (Barro and Real, 2017).

Scientific research published in the *European Journal of Clinical Nutrition* aimed to produce a validated satiety index of common foods. Many different foods were tested, and oatmeal ended up being rated number 1 among breakfast foods and number 3 overall. A scientific review published in 2016 suggests that it's likely the beta-glucan content of oats that has such a positive effect on perceptions of satiety (Barro and Real, 2017).

2.9 Wheat

Wheat is botanically named as *Triticum vulgare*. 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 and Amos, 1983). Wheat flour is unique among all the cereal flours in that it forms an elastic mass when mixed with correct proportion of water. This unique property is due to the presence of insoluble proteins, collectively called gluten. The gluten forming proteins (glutenin and gliadin) constitute about 75-80% of the total flour proteins (Mukhopadhyay, 1990).

Glutenin gives solidity to the product whilst gliadin is the binding agent imparting the soft sticky character to the gluten. Gliadin is soluble in 70% alcohol and may be extracted from flour whereas glutenin is soluble in alcohol and water (Gorinstein *et al.*, 2002).Gluten is elastic, cohesive and rubbery and holds together the various ingredients of the dough. It has the property of holding the gases given off during fermentation and during baking. It sets in oven to form the firm, porous, open texture during baking which are necessary in the production of biscuits and crackers. Thus gluten is the necessary framework, forming the sustaining wall of the whole structure of baked products (Bohn, 1956).

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 the 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 (W. H. Smith, 1972).

The strong flour protein has long links with few bonds while weak flour protein has short links with many bonds. During cake making weak and easy to stretch, soft wheat flour is found to be better (Kim and Kim, 1999). Beside the natural quality of flour, the modifications in the flour strength can be done by various treatments. Treatment of the flour with sulphur dioxide reduces the flour strength. Heat treated flour added to untreated flour is claimed to strengthen the flour. According to Kent and Amos (1983), improvers have some effect upon the nature and character of the gluten and cause it to behave, during fermentation, like the gluten of the stronger flour.

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 (Young and Cauvain, 2006).

Lamsal (2018) 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 and (Sarwar, 2010) found that of 11.50 (wet basis), 11.3, 0.90, 0.30, 0.60, 8.9 and 86.9% respectively and (Khanal, 1997) found that of 11.97 (wet basis), 10.32, 1.02, 0.56, 0.83, 9.2 and 87.27% respectively which is as show in Table 2.6.

Table 2.6 Chemical composition of wheat

Parameter %	A	B	C
Moisture content (wb)	11.97	11.50	11.56
Crude protein	10.32	11.3	9.17
Crude fat	1.02	0.90	1.07
Crude fibre	0.56	0.30	0.45
Total ash content	0.83	0.60	0.44
Glutein	9.2	8.9	9.1
Carbohydrate	87.27	86.9	88.89

Source A = Data of Khanal (1997),

B = Data of Sarwar (2010) and

C = Data of Lamsal (2018)

2.10 Avocado

The avocado *(Persea americana)* is a tropical fruit native to Mexico and Central America, classified in the flowering plant family *Lauraceae* and widely cultivated in subtropical regions for its large, edible fruit (Koch, 2013). 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.*, 2007). Furthermore, of all commonly eaten fresh fruit, avocado has the highest level of p-sitosterol, which has been shown in clinical trials to reduce blood levels of low-density cholesterol by blocking cholesterol absorption in the intestine (Heinemann *et al.*, 2013). Thus, avocado is considered a highly desirable addition to a healthy diet. Avocado pulp is sensitive to oxidative process during postharvest storage resulting in rancidity and subsequent production of undesirable flavors and reduction in quality.

Scientific Classification

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

Source: Tanabe (2016)

The fruit is a pear shaped and is of climacteric nature. The ripened fruit has a buttery textured pulp, because of which it is also commonly known as butter fruit. The taste of the fruit is somewhat bland type, possibly attributable to its bulky fat content. Due to its bland taste, many people (in case of Nepal) dislike the fruit. But, it is much popular among the foreigners as they are well acquainted with the real secret behind the fruit.

Avocado possesses a high-quality extractable oil, which has use in human nutrition as said by Santana *et al.* (2015). Many studies and researches have confirmed avocado to be highly beneficial for human health due to its healthy fat composition. All this goodness of avocado is well conserved in avocado oil and hence presents an excellent alternative for utilization of the fruit (E. Klose *et al.*, 2015). Moreover, avocado oil was ranked as a functional food due to the presence of compounds that potentially prevent cardiovascular diseases, osteoporosis, cancer and inflammation (Salgado *et al.*, 2008). These compounds include mono unsaturated fatty acids and antioxidants such as phytosterols, tocopherols and lutein, which are present in concentrations comparable to olive oil (Wong *et al.*, 2014).

2.10.1 Historical background

The remarkable, delicious, and nutritious avocado has been known by the natives of this hemisphere for many centuries, but was not revealed to the rest of the world until the early 1500s. When the Spanish invaded the New World, the Conquistadores made the acquaintance of this delectable fruit, recorded its occurrence in many areas in their writings (first published record of the avocado was by Fernandez de Enciso in 1519), and brought seeds and plants back to Europe (primarily to Spain). At the time of the Spanish conquest, avocados were found (either wild or cultivated) from northern Mexico south through Central America into northwestern South America, south in the Andean region as far as Peru, and into the Andean region of Venezuela. From these somewhat isolated and largely semi-wild beginnings over 400 years ago, the avocado industry has gradually developed to the present situation (Zentmyer, 2002).

Avocados have long been a part of the Mexican diet. Archaeologists have found evidence of avocado consumption going back almost 10,000 years in central Mexico. Back then, humans were simply gathering and eating wild avocados. Researchers believe that humans began cultivating avocados about 5,000 years ago. Mesoamerican tribes like the Inca, the Olmec and the Maya grew domesticated avocado trees.

Sir Hans Sloane, an Irish naturalist, is believed to have coined the word “avocado” in 1696, when he mentioned the plant in a catalogue of Jamaican plants. He also called it the “alligator pear-tree.”

Henry Perrine, a horticulturist, first planted avocados in Florida in 1833. They didn’t become a commercial crop until the early 20th century, though. While they were fairly popular in California, Florida and Hawaii where they were grown, people in other states avoided avocados. They didn’t start gaining widespread popularity until the 1950s, when people started putting them in salads (Daniel., 2015).

The avocado fruit has a very high nutritional value and development of avocado orchards would be important for the diet of human beings, and also for economic reasons. However, the avocado tree is very sensitive to both climatic and edaphic factors. Low productivity due mostly to climatic factors, and poor growth due mainly to soil factors, limit the development of this unique fruit tree in wide areas of tropical and subtropical regions of the world (Ben-Yaacov *et al.*, 1992).

2.10.2 Varieties

Commonly found varieties of avocado

- Bacon
- Fuerte
- Gwen
- Hass
- Lamb Hass
- Pinkerton
- Reed
- Zutano
- Fortuna
- Dickinson
- Butter pear

Nepal government introduced avocado in 1979 AD (BS 2035) at Trisuli, Kirtipur and Sarlahi horticulture farm with five known varieties Hass, Fuerte, Ettinger, Reed and Topa topa (Atreya *et al.*, 2020). Dhankuta district is leading in Avocado farming but not yet commercialized. Due to its popularity, the local government has declared Dhankuta, as an avocado capital. In Dhankuta, avocado farming has become a key income generating sector. Currently, Dhankuta possess around 3700 bearing trees and more than 26,000 saplings were planted in last few years. Annually, Dhankuta produce around 80 tons of avocado fruit and a lot of saplings (Adhikari, 2018).

2.10.3 Cultivation

Avocados are grown in frost-free subtropical regions. Once the fruit has formed on the tree, it slowly matures (10 months), increasing in size and oil content. Avocado fruit do not ripen while they remain on the tree even once they have reached maximum maturity. If the fruits are not harvested, they can remain on the tree even when the next year's fruit is developing, and can remain on the tree for more than 18 months from flowering. Once harvested, the avocado will begin to ripen. This process involves the softening of the flesh due to endogenous pectolytic enzyme activity and, for some varieties, the coloring of the skin from green to purple-black (Lazar-Baker *et al.*, 2011). The degree of ripeness of the avocado is primarily determined by measuring the firmness of the fruit. Hence to ensure the oil content in the avocados is at the maximum for processing, the fruit should ideally be mature at harvest (Wong *et al.*, 2014).

To have optimal oil quality, avocado fruit should not be overripe and also should have minimal rots or other postharvest disorders (such as flesh greying due to long storage). The amount of oil extracted from mature and ripe avocados earlier in the season has been found to be only approximately 75% of the maximum available oil in the flesh (15% oil by fresh weight) compared to later in the season when it is possible to extract more than 90% of the available oil, this being the maximum oil yield (25% oil by fresh weight) (Wong *et al.*, 2014). Commercial avocado farming is not very difficult it requires heavy organic manure, application of nitrogen and other necessary nutrients on soil (Atreya *et al.*, 2020).

2.10.4 Morphology

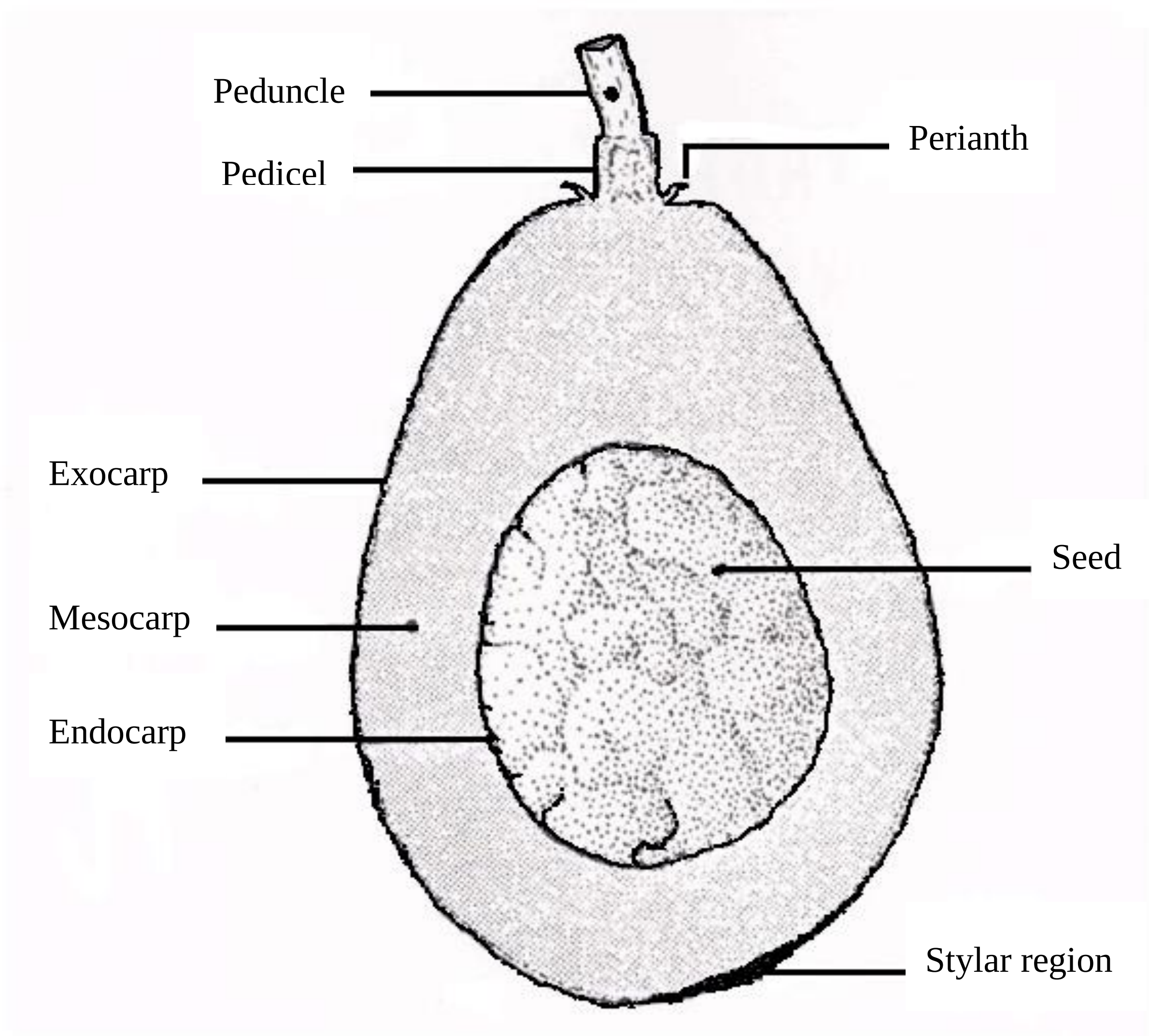

Fig. 2.2 Diagram of the median longitudinal section of an avocado fruit.

Source**:** Storey (2004)

Avocado is a single seeded berry, if the fruit is examined cutting longitudinally, it can be seen that the exocarp is the skin or rind. It may be very thin as in Mexican race or thick and almost woody as in some of the large Guatemalan race fruits. The mesocarp is fleshy and makes up the bulk of the pericarp. The inner layer is endocarp which, is thin, often not well differentiated from the mesocarp, and sometimes imperceptible. In some soft ripe avocados, it may adhere to the outer seed when the seed is removed from the fruit, giving the seed a sort of frosty appearance. In the inner side, there lies a large stone (seed) which may not be suitable for edible purposes as shown in Fig. 2.2 (Storey, 2004).

2.10.5 Chemical composition

Fresh avocado is rich in moisture. Besides, fat is the second important constituent of dried fruit. A very small quantity of carbohydrate is also present in the fruit. Protein, in the other hand, is also an important constituent. Apart from these, the fruit is rich in vitamins and minerals. Table 2.7 gives a general idea about the chemical constituents of avocado fruit:

Table 2.7 Proximate constituents of avocado (wet basis)

Constituents	%
Moisture	73.6
Protein	1.7
Fat	22.8
Carbohydrate	0.8
Minerals	1.1

Source: DFTQC (2012)

Avocado is rich in vitamins and minerals. Among the Vitamins, Vitamin A and Vitamin C are dominant (Watnick, 2009). Abundant minerals are found in the following concentration as shown in Table 2.8.

Table 2.8 Mineral composition of avocado

Minerals	Concentration (mg/kg)
Calcium	24
Phosphorous	105
Iron	1.11
Potassium	975
Sodium	14

Source: (Watnick, 2009)

2.10.6 Nutritional components of Avocado

2.10.6.1 Dietary fibre

Avocado fruit carbohydrates are composed of about 80% dietary fibre, consisting of 70% insoluble and 30% soluble fibre (Marlett and Cheung, 1997). Avocados contain 2.0 g and 4.6 g of dietary fibre per 30 g and one-half fruit, respectively (USDA, 2011). Thus, moderate avocado consumption can help to achieve the adequate intake of 14 g dietary fibre per 1000 kcal as about one-third this fibre level can be met by consuming one-half an avocado. Avocado fruit is rich in both soluble and insoluble fibre (J. Smith *et al.*, 1983).

2.10.6.2 Sugar

Compared to other fruits, avocados contain very little sugar (USDA, 2011). One-half an avocado contains only about 0.2 g sugar (e.g., sucrose, glucose, and fructose). The primary sugar found in avocados is a unique seven-carbon sugar called D- mannoheptulose and its reduced form, perseitol, contributes about 2.0 g per one-half fruit but this is not accounted for as sugar in compositional database as it does not behave nutritionally as conventional sugar and is more of a unique phytochemical to avocados (Meyer and Terry, 2008; Shaw *et al.*, 1980). Preliminary D-mannoheptulose research suggests that it may support blood glucose control and weight management (Roth *et al.*, 2009). The glycemic index and load of an avocado is expected to be about zero.

2.10.6.3 Fat

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) (Bao *et al.*, 2011). The lipid content in avocados is higher than in other fruits. The fat content of the avocado depends upon different factors such as variety of fruit, maturity, moisture content etc (Tango *et al.*, 2004). Orhevba and Jinadu (2011) found that from their research that the fat content, protein content, total ash content, crude fibre, carbohydrate, was found to be 12.18%, 0.94%, 1.54%, 6.9%, and 7.4% respectively. 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 (Wang *et al.*, 2015). These lipids are also used to make emulsions of water and lipids, and have a wide variety of applications in food, pharmaceuticals, and cosmetics industries

(Ranade and Thiagarajan, 2015). Oleic acid is the principal fatty acid in avocado, comprising 45% of its total fatty acids (Melo *et al.*, 2019) and during the ripening process, palmitic acid content decreases and oleic acid content increases (Carvalho *et al.*, 2015). In terms of its total fat content and fatty acid composition, avocado oil is considered to be similar to olive oil (Swisher, 1988). Other fattyacids present include palmitic and palmitoleic acids with smaller amounts of myristic, stearic, cinolenic, and arachidonic acids. However, the compositions of these fatty acids largely depend on the cultivars, stage of maturity, and part of the fruit and geographic location of plant growth (Duarte *et al.*, 2016).

2.10.6.4 Vitamins

Vitamins such as β-carotene, tocopherol, retinol, ascorbic acid, thiamine, riboflavin, niacin, pyridoxine, and folic acid are also abundantly found in avocado, which are of great importance for overall health and well-being (Dabas *et al.*, 2013; Duarte *et al.*, 2016). Carotenoids, including lutein, zeaxanthin, and α- and β-carotene found in the pulp of the avocado are potent free radical scavengers (Dabas *et al.*, 2013; Dreher and Davenport, 2013). The lutein content of avocado is higher than any other fruit, which comprises about 70% of its total carotenoid content. The color of avocado pulp is predominantly attributed to the higher content of xanthophylls (lutein and zeaxanthin). Seasonal variations in the phytochemical profile of avocado especially carotenoids, tocopherol, and fatty acid content have also been reported. Due to their fat-soluble nature, these bioactive compounds have been shown to promote vascular health (Dreher and Davenport, 2013).

2.10.6.5 Bioactive Components

In addition to the important major compounds, avocado contains substantial amounts of bioactive compounds such as phytosterols**,** especially in the lipid fraction, and the main representative is the p-sitosterol (Santos *et al.*, 2014). Diets rich in phytosterols can lead to the reduction of the total cholesterol and LDL cholesterol (Lottenberg *et al.*, 2002). Phytosterol is a substance of vegetable origin whose structure is very similar to cholesterol.

Its mechanism of action in the body involves the inhibition of intestinal cholesterol absorption and decreased hepatic cholesterol synthesis. According to Brufau *et al.* (2008), it acts on total plasma cholesterol and LDL cholesterol without affecting HDL and blood triglycerides. The benefit of cholesterol reduction also comes from replacing saturated by unsaturated fats, which promote a decrease in total cholesterol and LDL and an increase in

HDL levels (Salgado *et al.*, 2008).

The P-sitosterol in avocados also has a special effect on immunity, contributing to the treatment of diseases such as cancer, HIV and infections. In relation to cancer, it works by suppressing carcinogenesis and in HIV by strengthening the immune system (Patrick, 2002). This compound enhances lymphocytes proliferation and natural killer cell activity, which inactivates invading microorganisms (Bouic *et al.*, 1996). Avocado also has a carotenoid named lutein that helps protect against prostate cancer and eye diseases such as cataracts and muscular degeneration (Krinsky and Johnson, 2005).

2.10.6.6 Pigments

Pigments are important contributors to the appearance and healthful properties of both avocado fruits and the oils extracted from these fruits. Carotenoid and chlorophyll pigment concentrations in the skin and three sections of the flesh (outer dark green, middle pale green, and inner yellow flesh nearest the seed) and anthocyanin concentrations in the skin of Hass avocado during ripening at 20°C. Carotenoids and chlorophylls identified in the skin, flesh, and oil were lutein, R-carotene, β-carotene, neoxanthin, violaxanthin, zeaxanthin, antheraxanthin, chlorophylls a and b, and pheophytins a and b with the highest concentrations of all pigments in the skin.

Chlorophyllides a and b were identified in the skin and flesh tissues only. As the fruit ripened and softened, the skin changed from green to purple/black, corresponding to changes in skin hue angle, and a concomitant increase in cyaniding 3-O-glucoside and the loss of chlorophyllide a. In flesh tissue, chroma and lightness values decreased with ripening, with no changes in hue angle. The levels of carotenoids and chlorophylls did not change significantly during ripening. As fruit ripened, the total chlorophyll level in the oil from the flesh sections remained constant (Ashton *et al.*, 2006).

2.10.6.7 Enzymes

Being fruit it is home for many enzymes such as polyphenoloxidase, lipase, peroxidase, invertase, protease, lysozyme, amylase, catalase, as well as oxidative enzyme etc. Polyphenoloxidase is pronounced with the change of coloration that the fruit presents when it has contact with oxygen. The enzymes lipase and amylase had greater stability and activity in water (Oropeza, 2018).

2.10.7 Anti-nutritional components of avocado

Avocado contains high nutritive values and health benefit, however it is linked with the anti-nutritional factor. Some of the anti- nutrition factors found in avocado are hydrogen cyanide, tannins, alkaloids, phytates and oxalates. In the research of Anhwange *et al.* (2015), they found that the hydrogen cyanide, tannins, alkaloids, phytate and oxalate in the avocado as 0.02 mg / 100 g, 0.11 mg / 100 g, 0.0097 mg / 100 g, 0.25 mg / 100 g and 0.02 mg / 100 g respectively and the values were within the permissible limit. Udousoro and Akpan (2014) found that the high temperature treatment shows the significant decrease in the activity of the anti- nutrition factor. Since muffin was treated in the baking temperature at 215 ºC for 20±3 min the anti- nutrition factor will tends to remain in within the limit.

2.10.8 Benefits of avocado puree

According to Sengupta (2018), benefits of avocado are mentioned below:

i. Anti-inflammatory properties

Arthroritis discomfort can be relieved with avocados' anti-inflammatory qualities. Additionally, it is rich in omega-3 fatty acids, which are known to ease joint discomfort by lubricating joints and reducing inflammation. According to Polunin (1997), "The fats of this fruit are unique. They include phytosterols, plant hormones such as campesterol, beta-sitosterol, and stigmasterol, that help keep inflammation under control. It also contains polyhydroxylated fatty acids (PFAs), which are anti-inflammatory".

ii. Regulates blood pressure

Avocados may also help you maintain a healthy blood pressure level. Avocados are high in potassium and low in sodium, which helps to keep blood pressure in control. A healthy blood pressure can help prevent heart attacks and strokes.

iii. Good for heart

Avocados are also high in antioxidants and monounsaturated fats, which assist to protect the heart and lower the risk of stroke. Avocado consumption can also help to lower LDL and HDL cholesterol levels, as well as triglyceride levels in the blood.

iv. Boosts fertility

Avocados help in boosting fertility and better the chances at conceiving too. It is also linked to increasing the success of IVF Treatment.

v. Good for eyes

Avocados may improve your vision if you include them in your diet. Avocados are high in beta-carotene and antioxidants, both of which are good for the eyes. They also include the minerals lutein and zeaxanthin, which aid to preserve strong eyesight, night vision, and reduce UV light damage. Vitamin A is also beneficial in lowering the risk of macular degeneration, which occurs as people age. Avocados include monounsaturated fatty acids, which aid in the absorption of fat-soluble antioxidants like beta-carotene.

vi. Good source of folate

Avocados contain a lot of folate. Avocados are essential for pregnant women because folate promotes good foetal development. Folate has also been linked to a lower risk of depression in some studies. Homocysteine build-up is prevented by folate. This chemical tends to obstruct blood flow and the supply of nutrients to the brain.

vii. Improves digestion

Avocados are rich in fibre, which may help reduce constipation, improve digestion, and improve colon health. Fibre adds weight to the stool, which helps to maintain bowel regularity and improve digestion.

viii. Powerhouse of nutrients

Avocado is a powerhouse of many essential nutrients and minerals. It is rich in vitamin K, B5, B6, B3, E and C. They are also dense in potassium, folate, magnesium, manganese copper, iron, zinc and phosphorous.

ix. Benefits for weight loss

Avocado's rich fibre content could also help you lose a few pounds. For every 100 gram serving of avocado, there are 7 gram of fibre. Because it makes you feel full, you're less likely to binge eat later. Avocados are very low in carbohydrates, which aids in weight loss.

x. Helps absorb nutrients

Avocados improve the absorption of plant-based nutrients. The fat-soluble nutrients include a number of vitamins and Because of this, they must be combined with fats in order to be absorbed and utilized by the Avocados are a good source of plant nutrients such as vitamin A, D, E, and K.

xi. Keeps the skin healthy

As a result, avocados are high in vitamin C (17% of the recommended daily consumption) and vitamin E (17% of the recommended daily consumption), both essential for keeping healthy and glowing skin. It contains phytosterols with the same penetrating ability as lanolin and effective sunscreen properties that make it suitable for skin products. The cosmetic and nutritional value of avocado oil is evident (Human, 1987).

Part III

Materials and methods

3.1 Raw materials

3.1.1 Avocado

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

3.1.2 Oats

Rolled oats named ‘D lite’ was brought from the local market of Dharan.

3.1.3 Wheat flour

Wheat flour named ‘Fortune’ was brought from local market of Dharan.

3.1.4 Butter

Standard Amul butter was used.

3.1.5 Sugar

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

3.1.6 Baking powder

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

3.1.7 Egg

Egg was brought from local market of Dharan.

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 avocado puree used to prepare muffin.

3.2.2 Formulation of recipe

The recipe formulation for the avocado puree incorporated muffin was carried out as given in Table 3.1. The amount given is on parts basis.

Table 3.1 Recipe formulation for muffin

Ingredients	A	B	C	D	E
Wheat flour	25	25	25	25	25
Oats flour	75	75	75	75	75
Sugar	60	60	60	60	60
Butter	65	48.75	32.5	16.25	0
Avocado puree	0	16.25	32.5	48.75	65
Baking powder	1.42	1.42	1.42	1.42	1.42
Egg	57	57	57	57	57
Water	31	31	31	31	31

For the preparation of muffin egg was beaten for 2 min, creaming of shortening and sugar for 10 min. Along with these ingredients water, composite flour (75% oats and 25% wheat) 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). Here the modification is in the shortening. Different proportion of butter and avocado puree are used for muffin preparation of muffin as suggested by Design Expert 13.

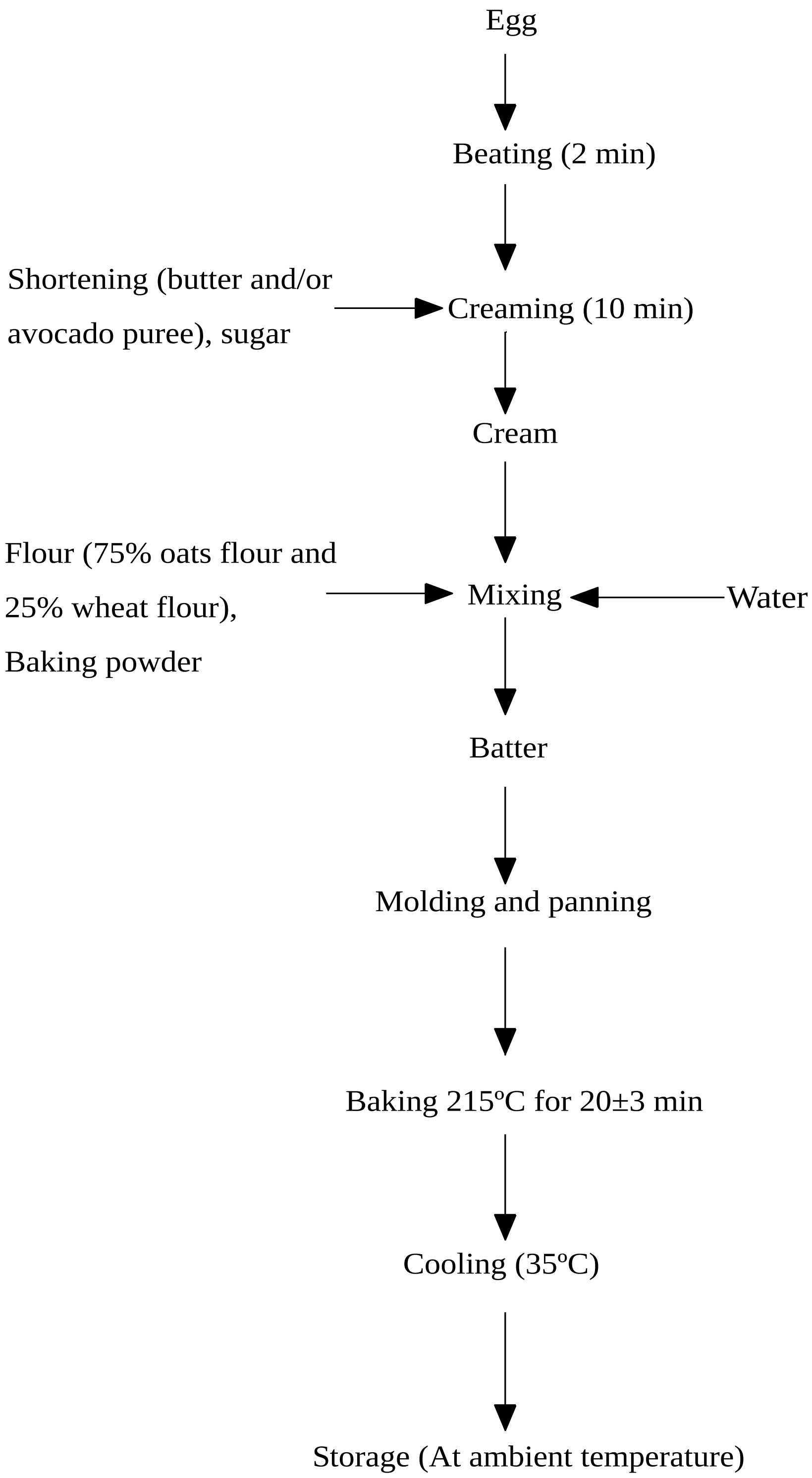

Fig. 3.1 Flow chart of avocado puree incorporated muffin.

3.3 Analysis of raw material and product

3.3.1 Physical parameter analysis

3.3.1.1 Color and surface

Color and surface was determined by visual inspection method. The oats flour and wheat flour were spread on separate tray and color and surface was diligently examined. Similarly, the color of the muffin samples were 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 oats flour such as phenolics, anthocyanins, tannins, carotenoids, and xantophylls (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 (Schirmera *et al.*, 2012). Similarly, the fat replacer content also affect the loaf volume , the loaf volume decreased with the incorporation of apple pomace as fat replacer (Samkaria, 2018) similarly, loaf volume decreased with the addition of mushroom powder (Farooq *et al.*, 2021) and the loaf volume increased with the addition of bitter melon (Sang-Hee, 2014).

$$\text{Specific loaf volume} = \frac{\text{Volume of the muffin}}{\text{Weight of the muffin}}$$

3.3.1.3 Weight loss (WL)

According to (Ureta *et al.*, 2013) weight loss, WL, was calculated as the percentage difference between initial and final product weight (wet basis), W_i and W_f, respectively,

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

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 (MohdJusoh *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 (McWilliams, 2001). A desirable muffin should have a uniform cell structure without tunnels (Halliday and 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 (AOAC, 2005).

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

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, W_1 = weight of beaker

W_2 = 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).

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

3.3.2.4 Crude fibre

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

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

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, W_1 = weight of empty crucible

W_2 = weight sample + crucible before ashing

W_3 = weight of samle + 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 *et al.*, 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.

3.3.4 Statistical analysis

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

3.3.5 Microbiological analysis

Total Plate Count (TPC) was determined by pour plate technique on Plate Count Agar (PCA) medium (incubated at 30°C/48 h). Coliform count was determined by pour plate technique on MacConkey medium (incubated at 37°C/48 h) (AOAC, 2005). The permissible limits of bakery product: total colony count < 2.0×10^5 CFU/g, coliform count < 200 CFU/g (WHO, 1994).

3.3.6 Acceptability period of muffin

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 standard quantity of different muffin formulation with different proportion of avocado puree with butter. As muffin is, a product widely favoured and consumed by 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 oats flour

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

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

Parameters %	Wheat flour	Oats flour
Moisture (wb)	11.53±0.377	8.4±0.016
Crude protein (db)	10.18±0.055	13.34±0.024
Crude fat (db)	1.13±0.021	9.38±0.024
Crude fibre (db)	0.45±0.008	1.48±0.019
Total ash (db)	0.46±0.021	1.53±0.018
Gluten (db)	9.65± 0.082	-
Carbohydrate (db)	87.74±0.023	74.27±0.024

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

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%, 9.65% and 87.74% respectively as given in Table 4.1.

Sarwar (2010) reported respective proximate values were 11.50 (wet basis), 11.3, 0.90, 0.30, 0.60, 8.9 and 86.9% respectively and Khanal (1997) found that of 11.97 (wet basis), 10.32, 1.02, 0.56, 0.83, 9.2 and 87.27% respectively 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, 1980) i.e. 13% max. , lower than the value obtained by (Khanal, 1997; Lamsal, 2018) but slightly higher than the valve 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 is greater than that obtained by (Sarwar, 2010), lower than that obtained by (Khanal, 1997) and equal 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 (1980), the maximum limit of total ash is 0.5% as the obtained value was 0.46%. which was lower than obtained by (Khanal, 1997; Sarwar, 2010) and slightly higher 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 oats 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 8.4%, 13.34%, 9.38%, 1.48%, 1.53%, and 74.27% respectively as given in Table 4.1.

Prasad *et al.* (2015) reported proximate values of moisture content, crude protein, crude fat, crude fibre, total ash and carbohydrate 10.71, 13.1, 9.2, 1, 1.8 and 74.8% respectively. Similarly, Lamsal (2018) reported proximate values of moisture content, crude protein, crude fat, crude fibre, total ash and carbohydrate 11.97%, 13.56%, 9.43%, 1.18%, 1.8%, and 74.03% respectively.

The moisture content of the oats flour was found to be 8.4 % which is lower than the valve obtained by (Lamsal, 2018; Prasad *et al.*, 2015), it is lower than that suggested by

(DFTQC, 2012) i.e. 10.7%. The moisture content of the cereal varies according to the maturity stage, climatic condition, variety, storage condition and packaging material (Lamsal, 2018). The sample taken was kept in airtight zip lock bag. In our case, timely switching off of the electric appliance and fluctuation of temperature in the college appeared to be a problem in continuing the heating of oven removing the moisture. The crude protein content of oats flour was found to be 13.34% which is slightly lower than suggested by (DFTQC, 2012) i.e. 13.6%, higher than the valve obtained by (Prasad *et al.*, 2015) and lower than the valve obtained by (Lamsal, 2018) and suggested by (Zhao *et al.*, 2014). This might be due to the loss of nitrogenous material during digestion which eventually reduced the final protein content. The crude fat content of oats flour was found to be 9.38% which is higher than that suggested by (DFTQC, 2012) i.e. 7.6% but lower than that suggested by (Zhao *et al.*, 2014). The obtained value was slightly lower than that obtained by (Lamsal, 2018). The fat content of oats flour depends on different factors like moisture content, geographical condition, climatic condition, maturity, variety of oats (Lamsal, 2018). This shows that the variety of oats affect the fat content. The crude fibre of oats flour was found to be 1.48%

4.2 Chemical composition of avocado puree

The proximate composition of the sample avocado is shown in Table 4.2

Table 4.2 Proximate composition of avocado paste

Parameters	%
Moisture content (wb)	72.64 ± 0.067
Total dry matter (db)	27.36 ± 0.067
Crude protein (db)	3.826 ± 0.040
Crude fat (db)	78.74 ± 0.288
Crude fibre (db)	11.69 ± 0.153
Total ash (db)	2.607 ± 0.087
Carbohydrate (db)	3.167 ± 0.219

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

Proximate analysis of avocado for various parameters like moisture content (%), crude protein (%), crude fat (%), crude fibre (%), ash (%), and carbohydrate (%) (dry basis except moisture content) were found to be 72.64%, 3.826%, 78.74%, 11.69%, 2.607%, 3.167% respectively as given in Table 4.2. The values in the wet basis of given parameter are 72.64%, 1.06%, 21.55%, 3.19%, 0.68% and 0.91% respectively.

Moisture content of the avocado sample (72.64%) was found to be slightly lower that that suggested by (DFTQC, 2012) i.e. 73.6%, within the range of 70-79% as suggested by (Santana *et al.,* 2015).The moisture content of fruits varies according to maturity stage, the sample taken was assumed to be matured but it might have been in early maturity stage. This might be the reason behind low level of moisture content. Moisture content of the fruit is determined at low temperature (70-75℃) for a longer period of time (15h) (AOAC, 2005). In our case, timely switching off of electric appliance in college appeared to be the problem in continuing the heating of oven to remove the moisture. Thus, low moisture content might have been reported due to this technical difficulty. Also, moisture content of fruit depends upon different environmental conditions, such as variety of fruit, harvesting time, maturity. etc. (Santana *et al.*, 2015)

Crude fat content in the avocado was determined to be 21.55% (wb). This is a considerable amount of a fat to be present in any of the fruits. According to food composition table issued by (DFTQC, 2012), avocado contains 22.8% fat. So, comparing to this figure, the avocado under study contained slightly lesser amount of fat. The fat contain of avocado depends upon different factors such as variety of fruits, maturity, moisture content etc. (Tango *et al.*, 2004). In another study, the fat content in the avocado fruit was determined to be 12.18% (Orhevba and Jinadu, 2011). This shows that, the fruit type directly affect the fat content.

Protein content was determined using the micro-Kjeldhal method. Comparing the result of protein with other studies, it (1.06%) was slightly lower, 1.7% as per (DFTQC, 2012), but higher than 0.94% as per (Orhevba and Jinadu, 2011). According to the (USDA, 2011) National Nutrient Database, avocado contain 1.99% of protein. Some loss of nitrogenous material had might occur during the digestion of the sample. This might have reduced the final protein content. Besides, the protein content in the fruit was determined to be significant comparing to other different fruits. According to Slater *et al.* (2007), avocado contained maximum up to 2.4% protein depending upon the different cultivars.

Total ash content was determined using the dry ashing method. Ash represents all the minerals that do not volatize at ashing temperature (AOAC, 2005). The total ash was determined to be 0.74% which is lower than reported by (Orhevba and Jinadu, 2011) (1.54%) and (DFTQC, 2012) (1.1%). But it falls within the range (0.4-1.68%) reported by FAO (1989). The lower value may be due to the difference in a variety and some experimental errors. Higher ash content of the fruits signifies higher mineral content and vice versa.

Crude fibre in the avocado fruit was found to be 3.19% in wet basis. This is a significant amount of fibre in any fruit. The crude fibre in the fruit was found to be 6.9% by (Orhevba and Jinadu, 2011). Comparing this figure, the fruit under study contained slightly less amount of crude fibre. According to J. Smith *et al.* (1983), avocado fruit is rich in both soluble and insoluble fibre. So, some sort of variations had been observed in proximate constituents of the avocado fruit.

Carbohydrate in the fruit was estimated to be 0.91%. According to Orhevba and Jinadu (2011), the carbohydrate content of the fruit was 7.4%.Comparing to this, the estimation was too low. It may be due to the difference in variety of the fruit. But, according to DFTQC (2012), carbohydrate in avocados are estimated to be 0.8%, which is around our result. Also, carbohydrate content was determined by difference method which may have shown such huge variation it is easy but not accurate method for the carbohydrate determination. Conclusively, environmental conditions, variety of fruit, experimental errors, can be considered as the causes of the variations (Santana *et al.*, 2015).

4.3 Effect of avocado puree on the physical parameters of muffins

The effect of avocado puree on the physical parameters of muffins are given below:

4.3.1 Volume of the muffins

The change in the muffins with the incorporation of avocado puree is shown in Fig. 4.1

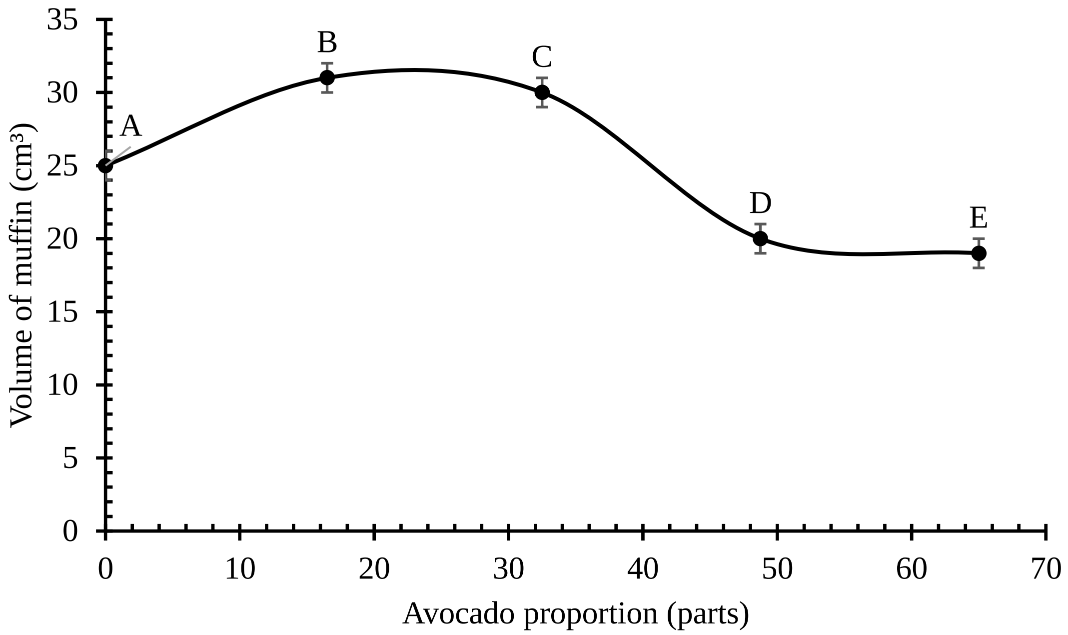

Fig. 4.1 Effect of avocado puree on the volume of the muffin

*Values are the means of the three determinations. Vertical error bars represent standard deviation.

From the Fig. 4.1 it is seen that the incorporation of the avocado puree 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 to rise in the 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 (Schirmera *et al.*, 2012). The decreased in the volume of Sample D and Sample E might be due to the dilution of protein, baking powder and fat by the moisture of avocado puree. 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 of Marina *et al.* (2016).

4.3.2 Weight of the muffins

The change in weight of muffins with incorporation of avocado puree is shown in Fig. 4.2

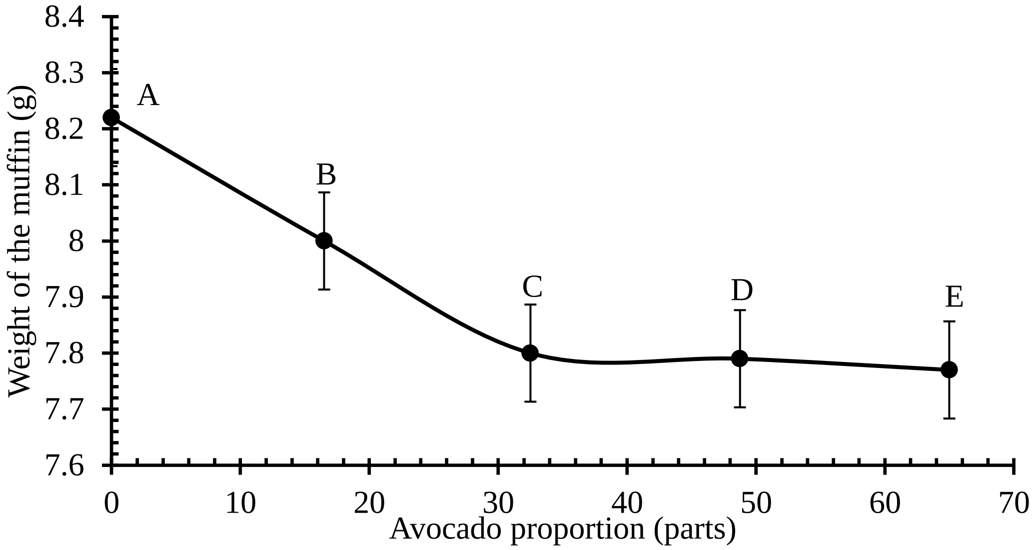

Fig. 4.2 Effect of avocado puree on the weight of the muffins

From the Fig. 4.2 it is seen that the incorporation of the avocado puree decreased the weight gradually. With respect to the control (sample A) the weight of Sample B and Sample C, decreased significantly, it may be due to adequate leavening action, the free water easily evaporates during baking making the muffin puffy and decrease in weight. Since, moisture content in the avocado puree is higher than in butter.

With respect to sample C in sample D and E, there is insignificant change in the weight which might be due to the dilution of the leavening agents in batter and the free water release during baking. Due to which there might be insufficient leavening of the batter as the batter was found pasty causing less water evaporation during baking also, which might be the reason for slightly decrease in weight of muffins sample D and sample E comparing with sample C.

4.3.3 Specific loaf volume

The incorporation of the avocado puree to certain level increased the specific loaf volume and then the specific loaf volume gradually decreased. The LSD shows that formulations A, B, C, D and E are significantly different among themselves at 5% level of significant which is clear from Fig. 4.3. Sang-Hee (2014) found similar result when adding bitter melon was added to muffin. Lamsal (2018) also find similar trend with the oat flour incorporated muffin. Farooq *et al.* (2021) also found that the loaf volume decreases with the addition of mushroom powder.

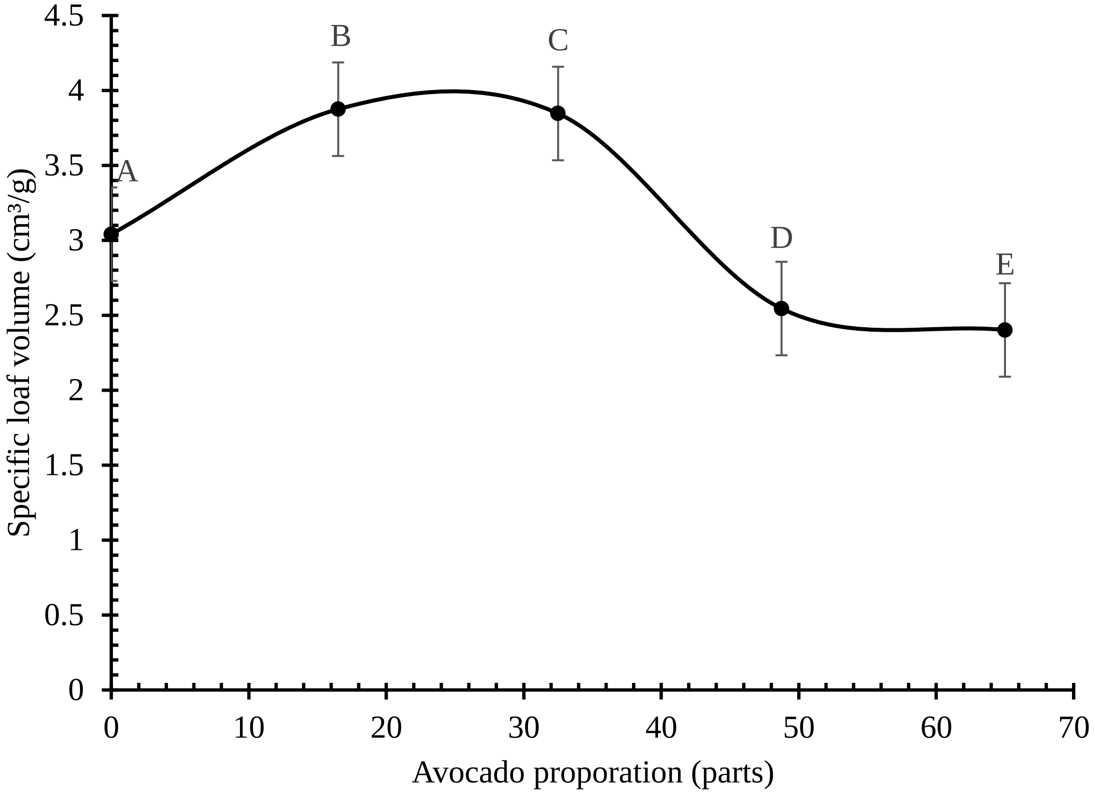

Fig. 4.3 Effect of avocado puree on the weight of the muffins

4.3.4 Cell uniformity and size

The cell size and uniformity of sample A and sample C was observed. The muffin with a uniform cell structure without tunnels is desirable (Halliday and Noble, 1946). The porosity depends on the number and size of pores (Ureta *et al.*, 2013). The sample A has a large air space except that there is almost uniform cell structure which is as shown in Fig. 4.4.

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

Similarly, the sample C have more homogeneous and smaller size pores with significant amount of larger air space, due to which the volume of sample C muffin is higher than sample A muffin. In both samples A and C there was no formation of tunnels. The photograph and binary image of product C is shown in Fig. 4.5.

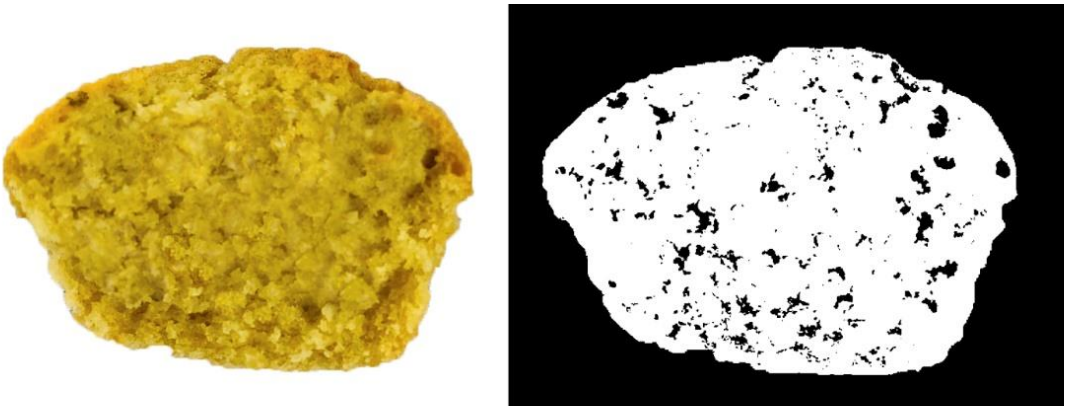

Fig. 4.5 Photograph and binary image of sample C muffin cross-sectional area

4.4 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 oats muffin with avocado puree as fat replacer formulation. Panelists are those who have tasted muffins. The ANOVA and LSD table for sensory evaluation are presented in the Appendix B.

Here A (65 parts butter and 0 part avocado puree), B (48.75parts butter and 16.25 part avocado puree), C (32.5 parts butter and 32.5 parts avocado puree), D (16.25 parts butter and 48.75 parts avocado puree), E (0 part butter and 48.75 parts avocado puree).

4.4.1 Appearance

The mean sensory score for appearance were found to be 8.000, 7.723, 7.545, 5.273 and 4.273 for the muffin formulation A, B, C, D and E respectively. Statistical analysis showed that partial substitution of butter with avocado puree had significant effect ($p < 0.05$) on the appearance of the different muffin formulations. The sample A, B and C were not significantly different to each other but significantly different to other, which is shown graphically in Fig. 4.6. The sample A, B and C got highest score than sample D and E. The preference of the muffin was up to 32.5 parts substitution i.e. (32.5 parts butter and 32.5 parts avocado puree) was observed. B and C were not significantly different to A but the score was slightly low.

A similar result was observed in muffin with avocado puree added as a fat replacer, in which full-fat replacement lowered the appearance score as compared to control (A) (Othman *et al.*, 2018). Similarly, in cake with cantaloupe added as a fat replacer, full-fat replacement lowered the appearance score as compared to the control product (Hussien, 2016).

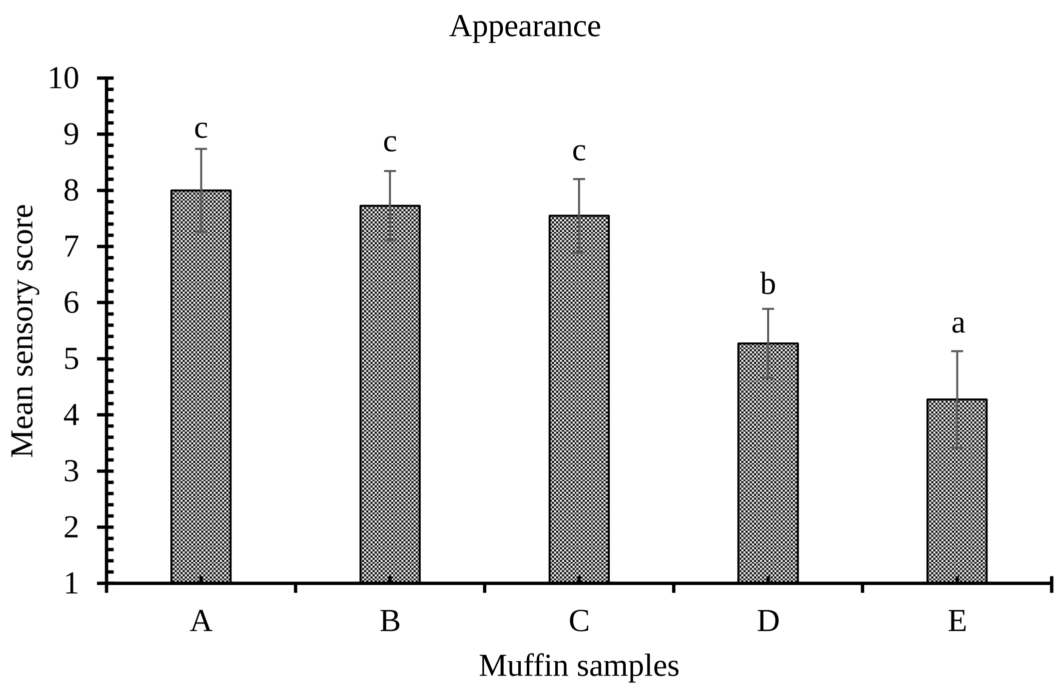

Fig. 4.6 Mean sensory scores for appearance of muffins of different formulations. Bars with similar alphabets at the top are not significantly different.

4.4.2 Color

The mean sensory score for color were found to be 7.909, 7.727, 7.727, 5.634 and 4.365 for the muffin formulation A, B, C, D and E respectively. Statistical analysis showed that partial substitution of butter with avocado puree had significant effect ($p < 0.05$) on the color of the different muffin formulations. The sample A, B and C were not significantly different to each other but significantly different to other, which is shown graphically in Fig. 4.7. The sample A, B and C got highest score than sample D and E. Sample A was the most while sample E was the least accepted by panelists. Sample B and sample C scored slightly lower than A, but showed no significant difference among all formulation.

A similar result was observed in muffin with avocado puree added as a fat replacer, in which full-fat replacement lowered the color score as compared to control (sample A) (Othman *et al.*, 2018). Similarly, in cake with cantaloupe added as a fat replacer, full-fat replacement lowered the color score as compared to the control product (Hussien, 2016).

The color of the fat replacer used greatly affected the crumb color of final products, with color changes either desirable or undesirable to the panelists. In this study, the greenish-yellowish color of avocado puree produced undesirable and detectable color changes in muffins.

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 oats flour such as phenolics, anthocyanins, tannins, carotenoids, and xantophylls (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, browing, and caramelization reaction in which reducing sugar and protein plays the vital role (Serna-Saldivar, 2012).

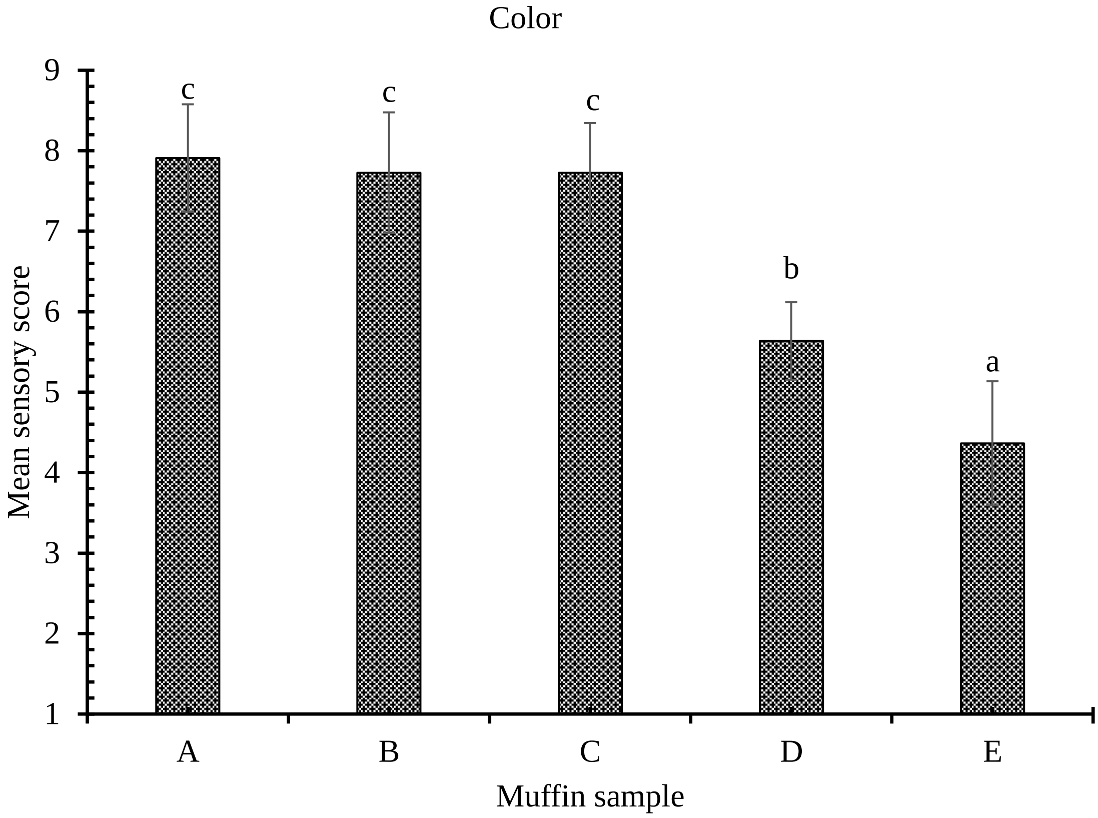

Fig. 4.7 Mean sensory scores for color of muffins of different formulations. Bars with similar alphabets at the top are not significantly different.

4.4.3 Aroma

The mean sensory score for aroma were found to be 7.909, 7.636, 7.455, 5.273 and 4.455 for the muffin formulation A, B, C, D and E respectively. Statistical analysis showed that partial substitution of butter with avocado puree had significant effect ($p < 0.05$) on the aroma of the different muffin formulations. The sample A, B and C were not significantly different to each other but significantly different to other, which is shown graphically in Fig. 4.8. The sample A, B and C got highest score than samples D and E.

Sample A had the highest while sample E recorded the lowest score. Avocado incorporation in muffin did not significantly affect the panelists' acceptance with respect to the aroma, except for samples D and E. This is because avocado does not have a strong aroma, and thus it does not affect much the overall aroma of muffin. There was an unpleasant aroma of the egg in sample E, it may be due to under baking and high moisture content.

A similar result was observed in muffin with avocado puree added as a fat replacer, in which full-fat replacement lowered the aroma score as compared to control (A) (Othman *et al.*, 2018). Similarly, in cake with cantaloupe added as a fat replacer, full-fat replacement lowered the aroma score as compared to the control product (Hussien, 2016).

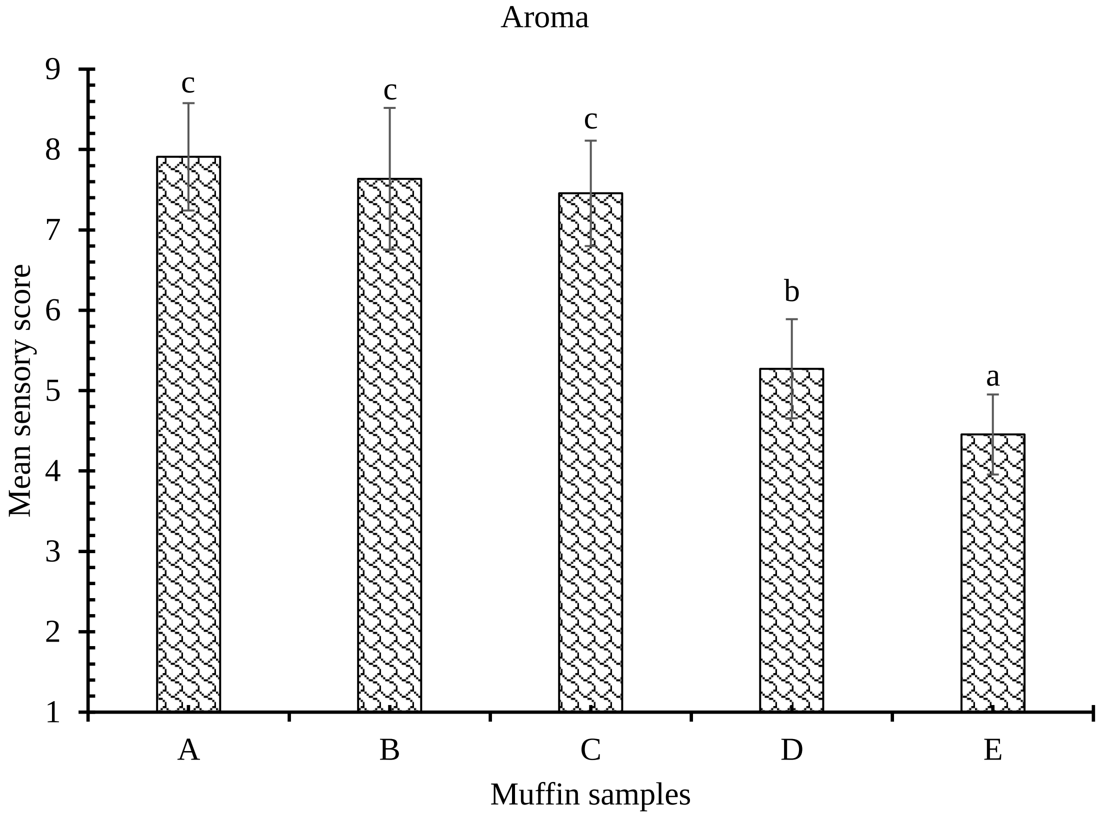

Fig. 4.8 Mean sensory scores for aroma of muffins of different formulations. Bars with similar alphabets at the top are not significantly different

4.4.4 Texture

The mean sensory score for texture were found to be 8.091, 7.818, 7.273, 5.455 and 4.273 for the muffin formulation A, B, C, D and E respectively. Statistical analysis showed that partial substitution of butter with avocado puree had significant effect ($p < 0.05$) on texture of the different muffin formulations. The sample A, B and C were not significantly different to each other but significantly different to other, which is shown graphically in Fig. 4.9. The sample A, B and C got highest score than sample D and E.

Sample A had the highest while sample E recorded the lowest score. Avocado incorporation in muffin did not significantly affect the panelists' acceptance with respect to the texture, except for samples D and E. The texture of muffins became more compact and harder due to less incorporation of air in cell with increased level of fat replacement (Samkaria, 2018).

Fat gives the softer texture and make the muffin porous (Bennion and Bamford, 1973). As the fat content of the butter is greater than avocado, control sample (sample A) was richer in fat content than that of optimum sample (sample C).

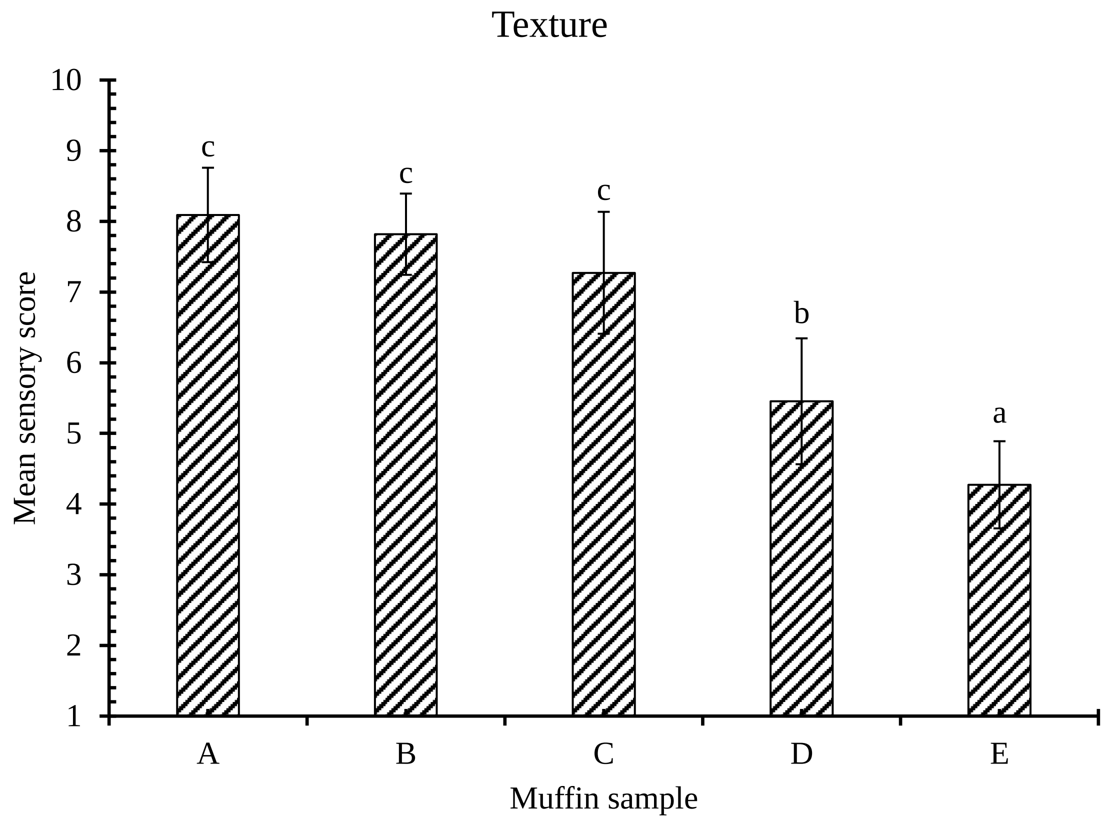

Fig. 4.9 Mean sensory scores for texture of muffins of different formulations. Bars with similar alphabets at the top are not significantly different

4.4.5 Taste

The mean sensory score for taste were found to be 8, 7.636, 7.636, 5.727 and 4.364 for the muffin formulation A, B, C, D and E respectively. Statistical analysis showed that partial substitution of butter with avocado puree had significant effect ($p < 0.05$) on the taste of the different muffin formulations. The sample A, B and C were not significantly different to each other but significantly different to other, which is shown graphically in Fig. 4.10. The sample A, B and C got highest score than sample D and E. Similar trend was seen in the research of Samkaria (2018) with apple pomace as fat replacer.

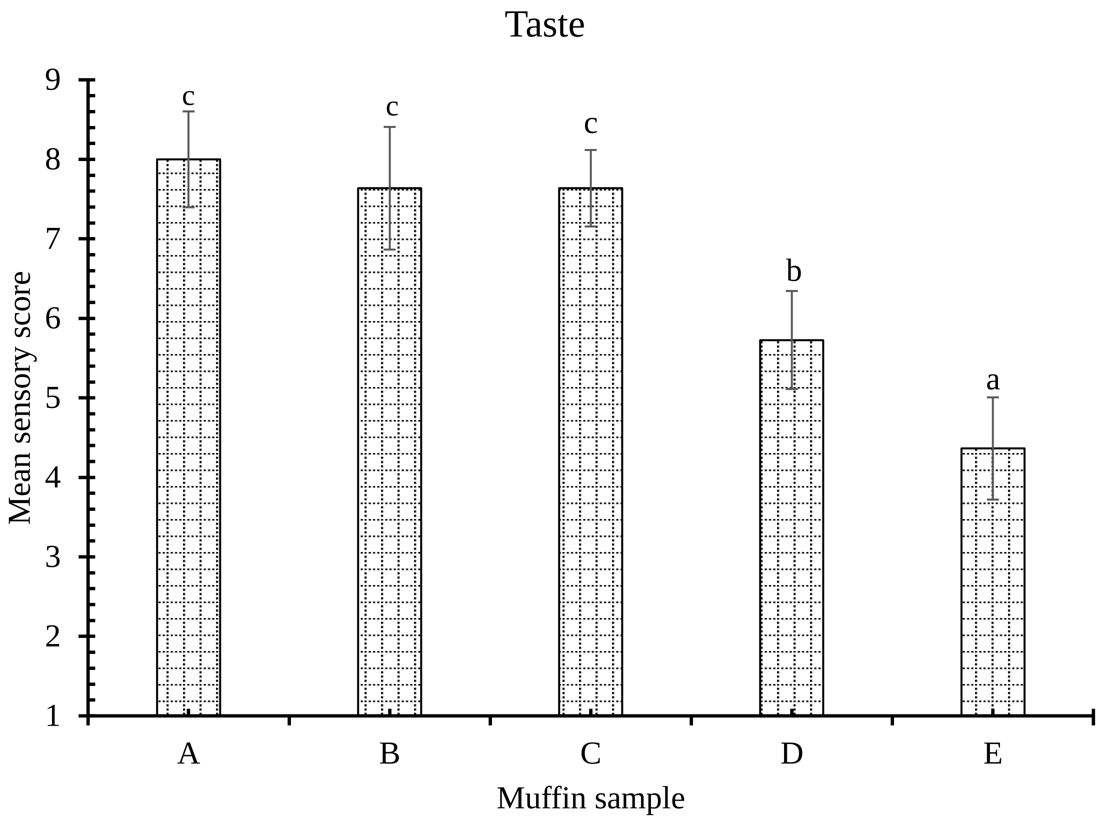

Fig. 4.10 Mean sensory scores for taste of muffins of different formulations. Bars with similar alphabets at the top are not significantly different

4.4.6 Overall acceptability

The overall score for different muffin samples were obtained as 8.091, 7.727, 7.636, 5.545, 4.364 respectively. Statistical analysis showed that partial substitution of butter with avocado puree had significant effect ($p < 0.05$) on overall of the different muffin formulations. The sample A, B and C were not significantly different to each other but significantly different to other, which is shown graphically in Fig. 4.11. The sample A, B and C got highest score than sample D and E. A was the most while E was the least accepted by panelists. B and C scored slightly lower than A, but showed no significant difference among all formulation.

The appearance, color, aroma, taste and texture of the sample A, B and C was very much liked. With respect to the control i.e. sample A, sample B and sample C got high score in terms of overall acceptability as shown in Fig. 4.9. Therefore, overall acceptability of the muffin up to 32.5 parts (in 65parts) substitution of butter by avocado puree sample C was found to be significantly superior based on the sensory characteristics of muffins.

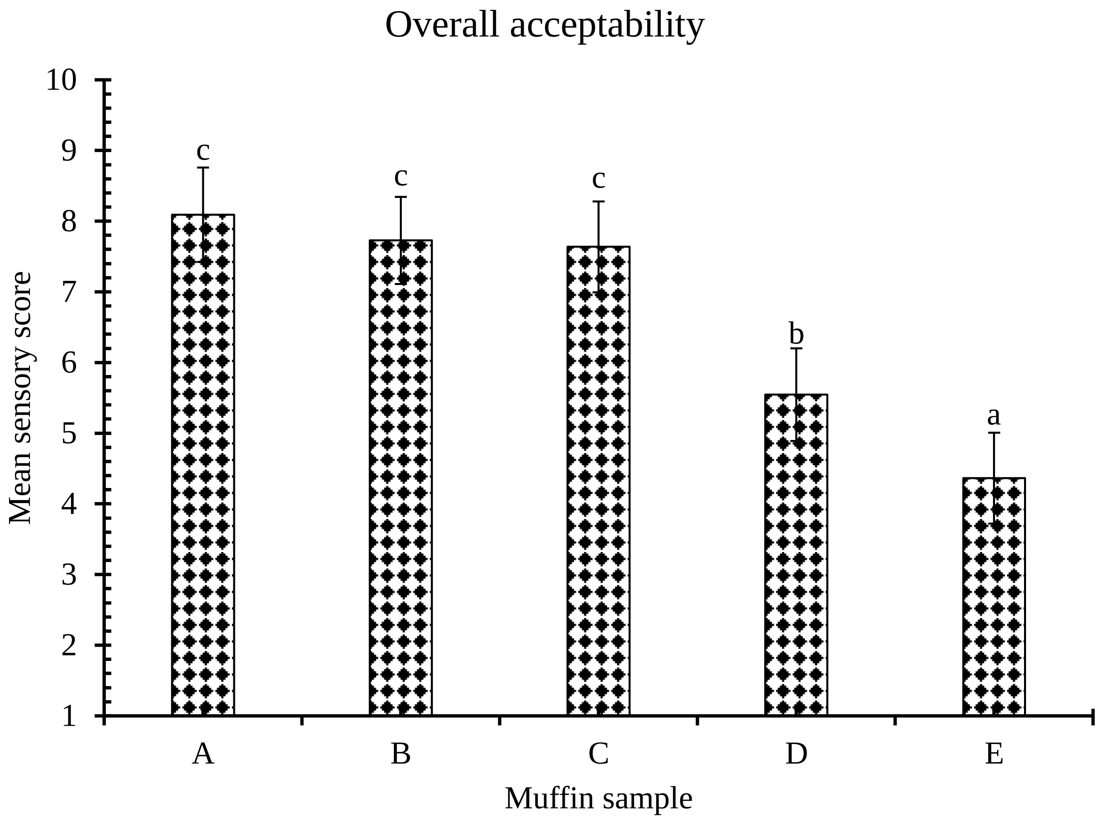

Fig. 4.11 Mean sensory scores for overall acceptability of muffins of different formulations with similar alphabets at the top are not significantly different

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. 4.12

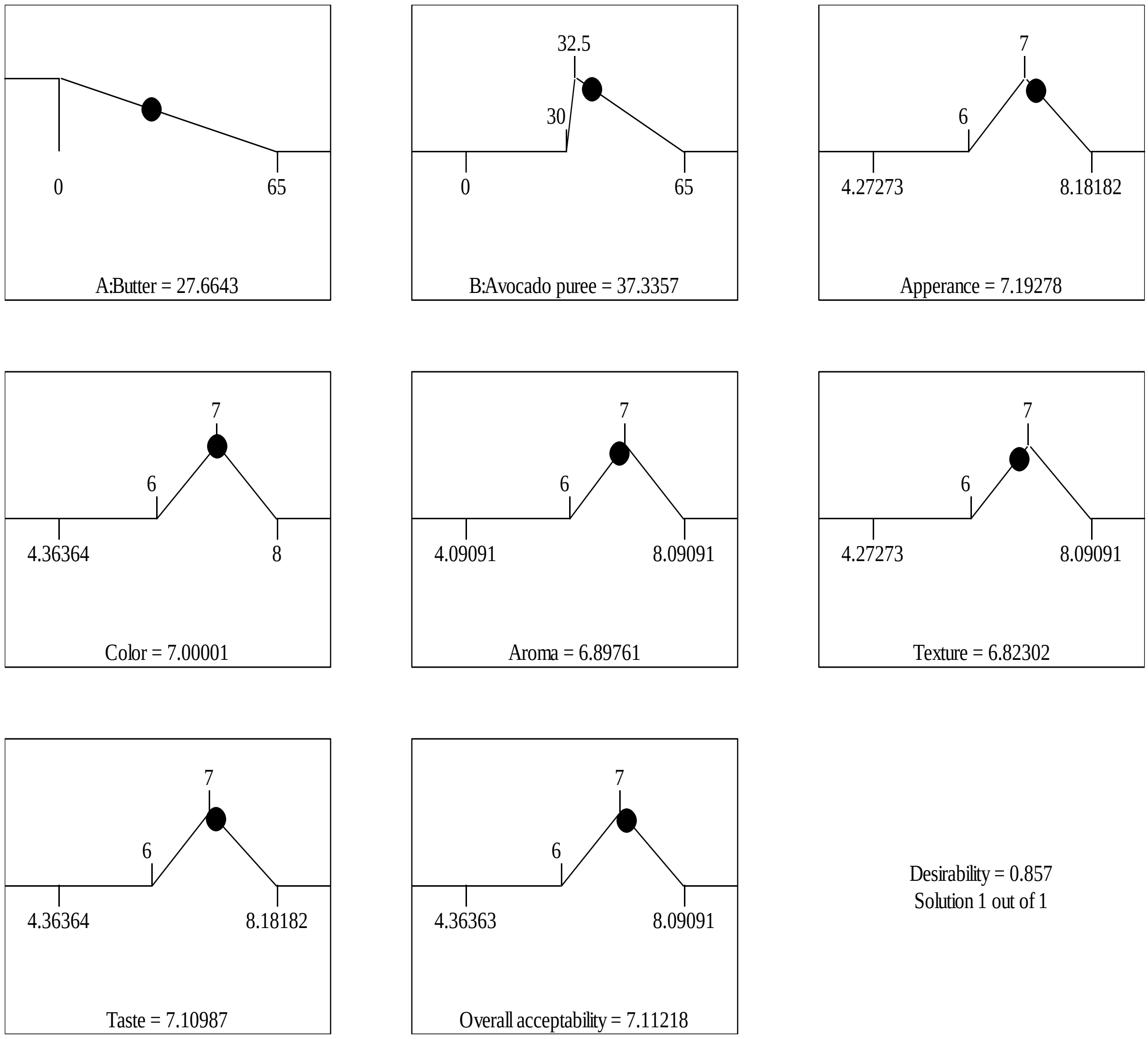

Fig. 4.12 Avocado puree and butter portion with the sensory quality and desirability

4.5 Proximate composition of products

Thus from statistical sensory analysis, the substitution of butter by 32.5 parts avocado paste had no significant different with the control sample. So, sample C was found to be the best muffin sample containing 32.5 parts butter and 32.5 parts avocado paste in 65 parts shortening required. The proximate composition of sample C and control muffin (sample A) were presented in Table 4.3.

Table 4.3 Proximate composition of product

Parameters	Sample A (Control)	Sample C (Best)
Moisture % (wet basis)	22.311^{a}±0.086	27.988^{b}±0.233
Crude Protein % (dry basis)	12.168^{a}±0.326	12.624^{a}±0.046
Crude Fat % (dry basis)	27.975^{a}±0.030	19.761^{b}±0.157
Crude Fibre % (dry basis)	0.596^{a}±0.025	1.139^{b}±0.032
Total ash % (dry basis)	2.102^{a}±0.033	2.451^{b}±0.003
Carbohydrate % (dry basis)	57.159^{a}±0.347	64.026^{b}±0.206
Crust moisture % (wet basis)	14.817^{a}±0.117	18.062^{b}±0.076
Crumb moisture % (wet basis)	26.271^{a}±0.197	34.542^{b}±0.308
Weight loss (%)	13.243^{a}±0.400	16.747^{b}±0.125
Crust/Crumb ratio	0.528^{a}±0.005	0.660^{b}±0.009
Caloric value (kcal/100 g)	514.791^{a}±0.0499	468.441^{b}±0.941
Fat reduction (%)	-	29.362
Caloric value reduction (%)	-	9.004

*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, protein, fat, crude fibre, ash and carbohydrate of sample A were found to be 22.311%, 12.168%, 27.975%, 0.596%, 2.102% and 57.159% respectively and that of sample C were found to be 27.988%, 12.624%, 19.761%, 1.139%, 2.451% and 64.026% 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 C with the moisture content 22.311% and 27.988% respectively. Moisture content increased significantly ($p < 0.05$) in muffins incorporating avocado puree as compared to control. The high moisture content in Sample C can be explained by the high moisture content of avocado puree when used as a substitute for butter. Similar trends were observed for the muffin with avocado paste(Othman *et al.*, 2018). The crumb moisture content and crust moisture content of sample A were found to be 26.271% and 14.817% respectively and that of sample C were found to be 34.542% and 18.062% respectively. The weight loss and crust/crumb ratio of sample A were found to be 13.243, 0.528 respectively and that for sample C were found to be 16.747 and 0.660 respectively. The higher moisture content makes it prone to microbial attack but it also gives the characteristic firmness to the muffins.

Protein content in muffins showed an insignificant increment ($p > 0.05$) with the incorporation of avocado puree, ranging from 12.168% to 12.624%. Previous studies confirmed a similar trend in which incorporation of a fat replacer increased the protein content of baked goods, either significantly or insignificantly (Othman *et al.*, 2018; Samkaria, 2018; Sang-Hee, 2014)

The muffin incorporating avocado puree up to 32.5 parts i.e. Sample C was accepted. After the incorporation of avocado puree there was the significant decrement of fat content from 27.975% to 19.761% at 5% level of significant. The lower fat content was due to the replacement of shortening with avocado puree. Previous studies also showed a similar trend, in which fat replacement reduced the fat content progressively in baked products (Othman *et al.*, 2018; Samkaria, 2018; Sang-Hee, 2014).

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 2.102% in oats muffin (Sample A) to 2.451% in the avocado puree incorporated oats muffin (Sample C).

It was observed that crude fibre content is also significantly increased in avocado puree incorporated muffin which was due to higher crude fibre content in avocado puree than that of butter. The crude fibre ranged from 0.596% in oats muffin (Sample A) to 1.139% in the avocado puree incorporated oats muffin (Sample C).

Carbohydrate content significantly increased ($p < 0.05$) following the increment of avocado puree incorporation, from 57.159% (sample A) to 64.026% (Sample C). Avocado puree incorporation contributed to the increase in carbohydrate content in muffin formulation since other ingredients were kept at a constant amount. Caloric values of muffins decreased significantly ($p < 0.05$), owing to the reduction in fat content in muffins. This is because fat contributes the highest caloric value (9 kcal/100 g) as compared to protein and carbohydrate. Thus, reducing the fat content will simultaneously reduce the caloric value. Similar observation was seen in the research of Othman *et al.* (2018).

The muffin incorporating avocado puree was accepted up to 32.5 parts avocado (in 65 part shortening) incorporation i.e. sample C, with 29.362% fat reduction and 9.004% lower calorie content compared to the full-fat counterpart. The nutritional value also increased significantly or insignificantly.

4.6 Anti- nutritional factor of avocado puree influencing muffin samples

There are many anti- nutritional components in the avocado. Although they are found within the permissible limit as concluded by Anhwange *et al.* (2015). The high temperature inactivates the anti- nutritional component and also lowers the concentration of oxalate and phytates specially as noted by Udousoro and Akpan (2014). The concentration of anti-nutritional component was low in the avocado puree and high temperature treatment i.e. baking (muffin preparation) ultimately lowers its amount, below than the permissible limits.

4.7 Shelf life evaluation of the muffin

The shelf life of the muffin was studied for 12 days with triplicate samples. The samples were stored in ambient temperature (25 ± 3 ℃) and refrigerated condition (4 ± 1 ℃). The acid value, peroxide value of the extracted fat, total plate count, coliform test yeast and mold and moisture content of the product was evaluated from the date of manufacture up to 12 days as follows:-

4.7.1 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 1.02 at initial which reached 1.29, 1.802, 2.033, 3.814 within 2, 4, 6, and 8 days respectively. Similarly, for sample C acid value was 1.435 at initial which reached 2.145, 3.451 and 5.164

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 C respectively. The change in the acid value of the sample A and sample C is shown in Fig. 4.13. The acid value of sample C was greatly increased earlier than that of sample A. It might be due to 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.

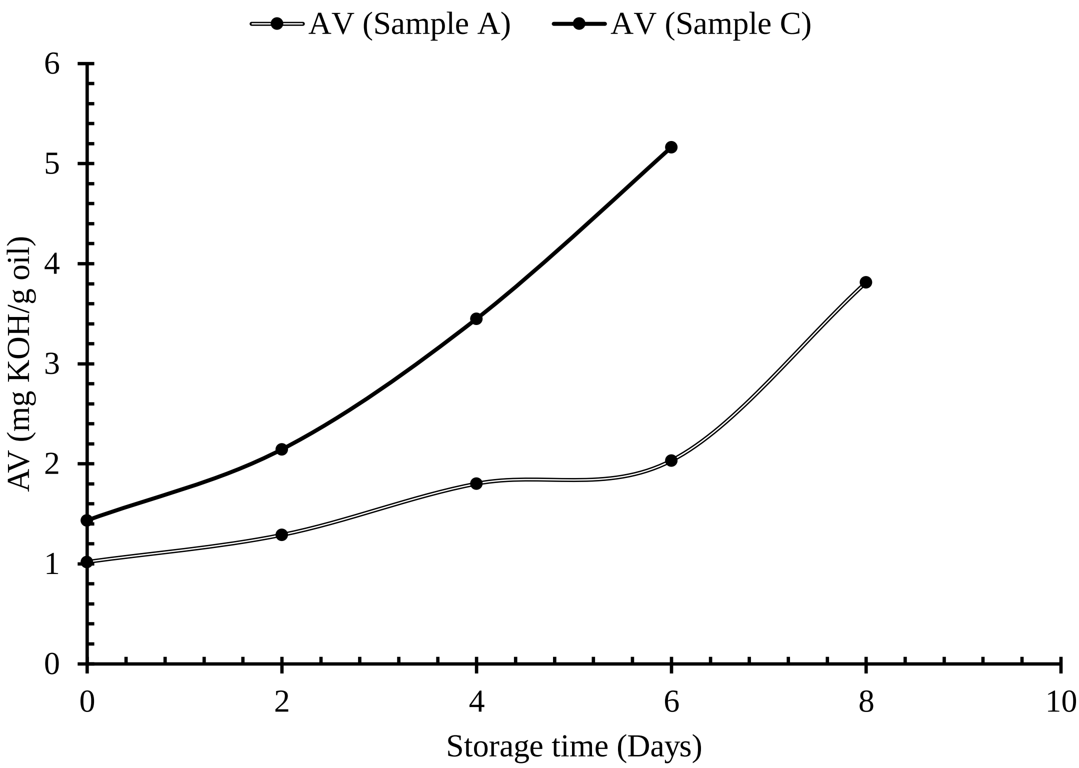

Fig. 4.13 Change in acid value during storage at ambient temperature of sample A and sample C

Likewise, at the refrigerated temperature the acid value of sample A was observed to be 1.02 at initial which reached 1.061, 1.231, 1.632, 1.782, 2, 2.432 within 2, 4, 6, 8, 10, and 12 days respectively. Similarly, for sample C acid value was 1.435 at initial which reached 1.685, 2.144, 2.955, 3.643, 4.982 and 5.12 within 2, 4, 6, 8, 10 and 12 days respectively but the acid value was below the unacceptability level of 6 mg KOH/mg of oil as described DFTQC (2018) till 12th day of analysis for sample A and sample C. The change in the acid value of the sample A and sample C is shown in Fig. 4.14.

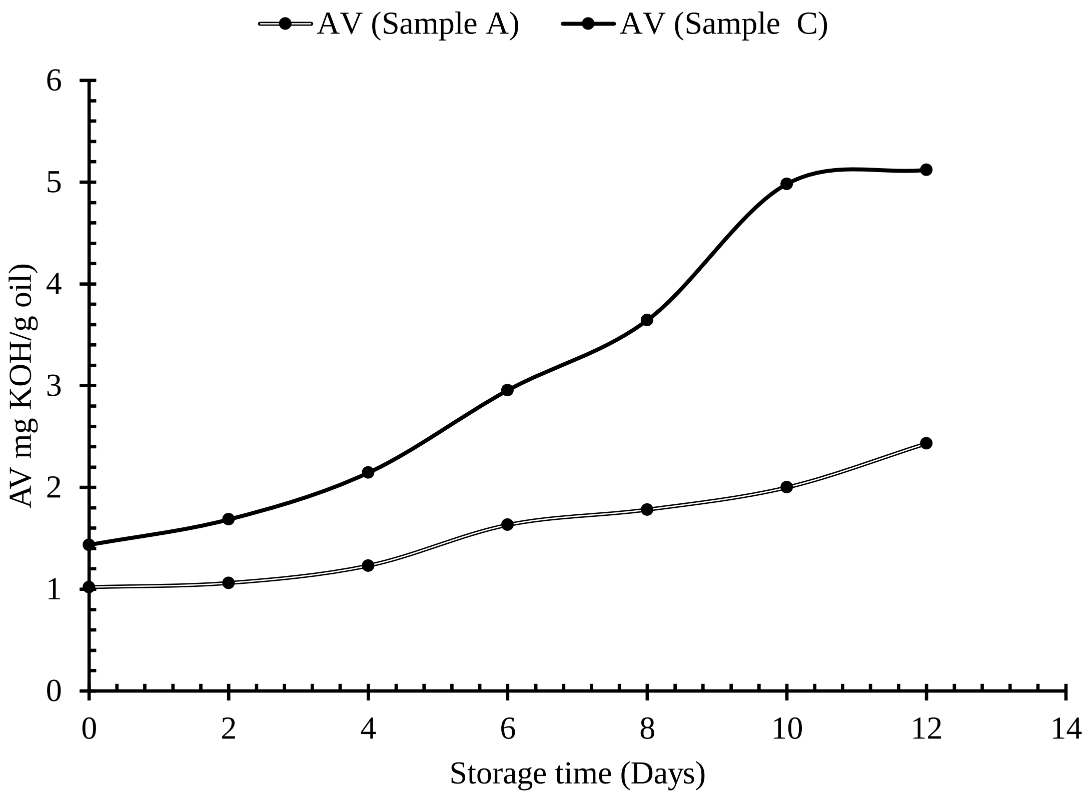

Fig. 4.14 Change in acid value during storage at refrigerated temperature of sample A and sample C

4.7.2 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 the sample A at ambient temperature was observed to be 2.605 at initial which reached 2.906, 3.3, 3.785 and 4.327 within 2, 4, 6, 8 days respectively. Similarly, for sample C peroxide value was 1.561 at initial which reached 2.253, 3.248 and 4.924 within 2, 4 and 6 days respectively but the PV obtained was far below the unacceptable level of maximum 10 $MeqO_2$ /kg fat as described by (DFTQC, 2012; Mukhopadhyay, 1990) till the last date of analysis. The rapid increase in the peroxidase value of sample C earlier might be due to the presence of high amount of unsaturated fatty acid in avocado puree than butter. The increase amount of unsaturated fatty acid is prone to rancidity. The change in peroxide value of the sample A and sample C in ambient temperature is shown in Fig. 4.15.

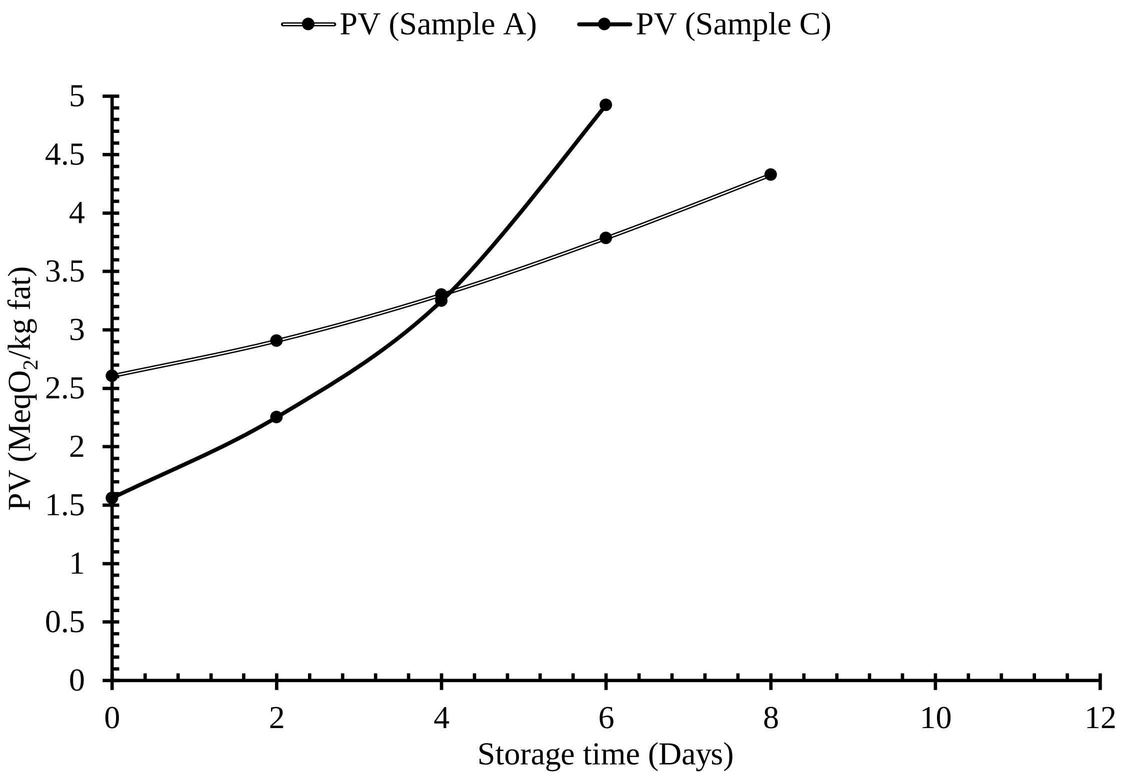

Fig. 4.15 Change in peroxide value during storage at ambient temperature of sample A and sample C

Likewise, at the refrigerated temperature the peroxide value of sample A was observed to be 2.634 at initial which reached 2.645, 2.698, 2.856, 3.155, 3.213 and 3.561 within 2, 4, 6, 8, 10, and 12 days respectively. Similarly, for sample C peroxide value was 1.561 at initial which reached 1.925, 2.366, 3.163, 3.762, 4.152 and 4.612 within 2, 4, 6, 8, 10 and 12 days respectively but the peroxide value was below the unacceptability level of 10 $MeqO_2$/kg fat as described by (DFTQC, 2012; Mukhopadhyay, 1990) till 12th day of analysis for sample A and sample C. The change in the peroxide value of the sample A and sample C in refrigerated temperature is shown in Fig. 4.16.

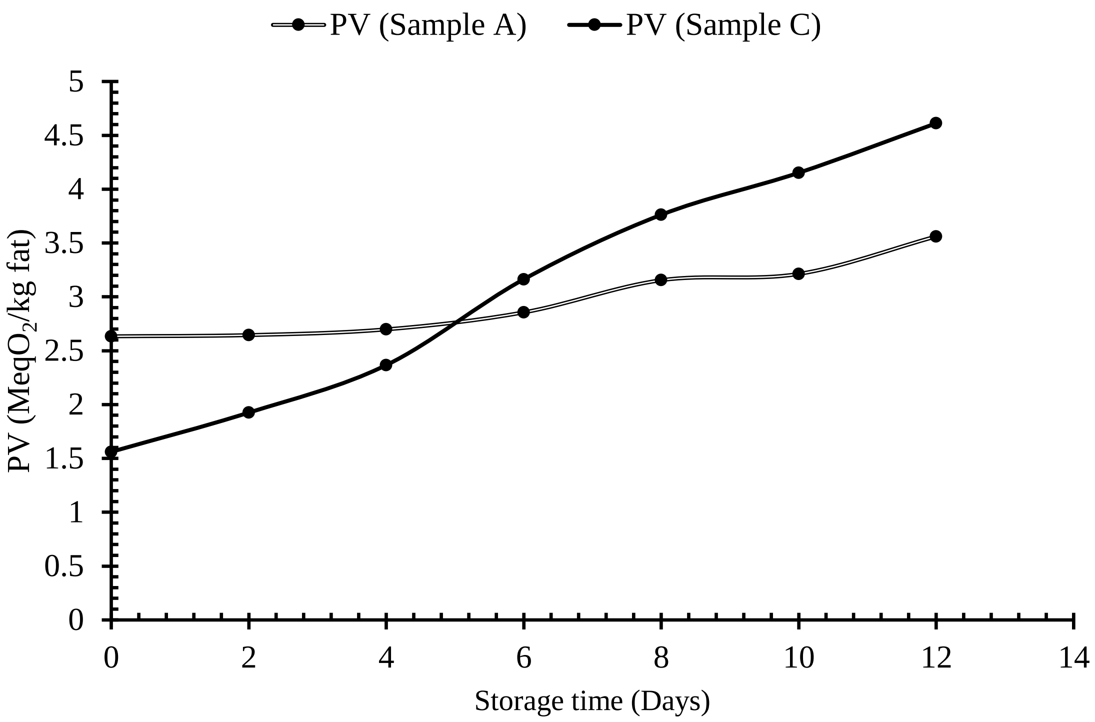

Fig. 4.16 Change in peroxide value during storage at refrigerated temperature of sample A and sample C

4.7.3 Change in moisture content

At ambient temperature the moisture content of the sample A was observed to be 22.31 at initial which reached 20.782, 20.103, 18.683 and 18.096 within 2, 4, 6 and 8 days respectively. Similarly, for sample C the moisture content was observed to be 27.988 at initial which drops to 23.309, 22.191 and 18.779 within 2, 4 and 6 days respectively but the moisture content was within 15-30% as described by Sain *et al.* (2014) till 8^{th} and 6^{th} day of analysis for sample A and sample C respectively. The change or decreasing trend of moisture content of sample A and sample C at ambient temperature is shown in Fig. 4.17

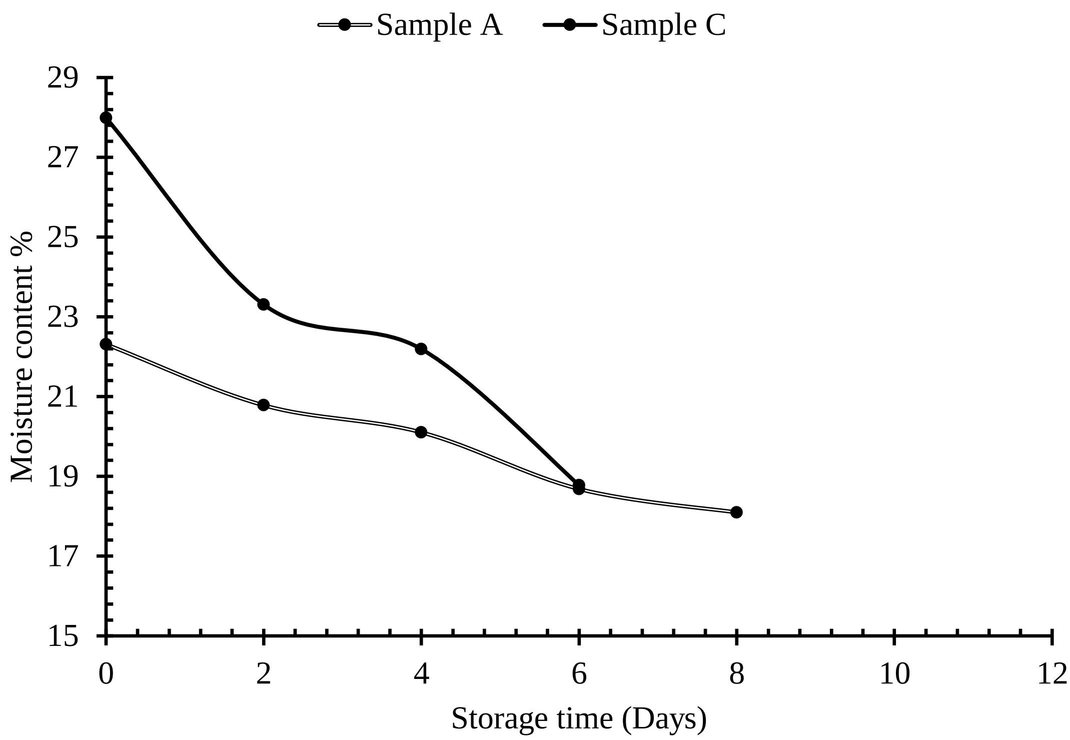

Fig. 4.17 Change in moisture content during storage at ambient temperature of sample A and sample C

Likewise, at refrigerated temperature the moisture content of the sample A was observed to be 22.31 at initial which reached 20.061, 18.654, 17.266, 15.949, 15.162 and 15.054 within 2, 4, 6, 8, 10 and 12 days respectively. Similarly, for sample C the moisture content was observed to be 27.988 at initial which drops to 24.138, 21.077, 18.989,17.263, 16.626 and 16.093 within 2, 4, 6, 8, 10 and 12 days respectively. The change or decreasing trend of moisture content of sample A and sample C at refrigerated temperature is shown in Fig. 4.18. Similar observation was seen in the research of (Grillo *et al.*, 2014). The observed data is within the range as described by Sain *et al.* (2014) till 12 days of analysis.

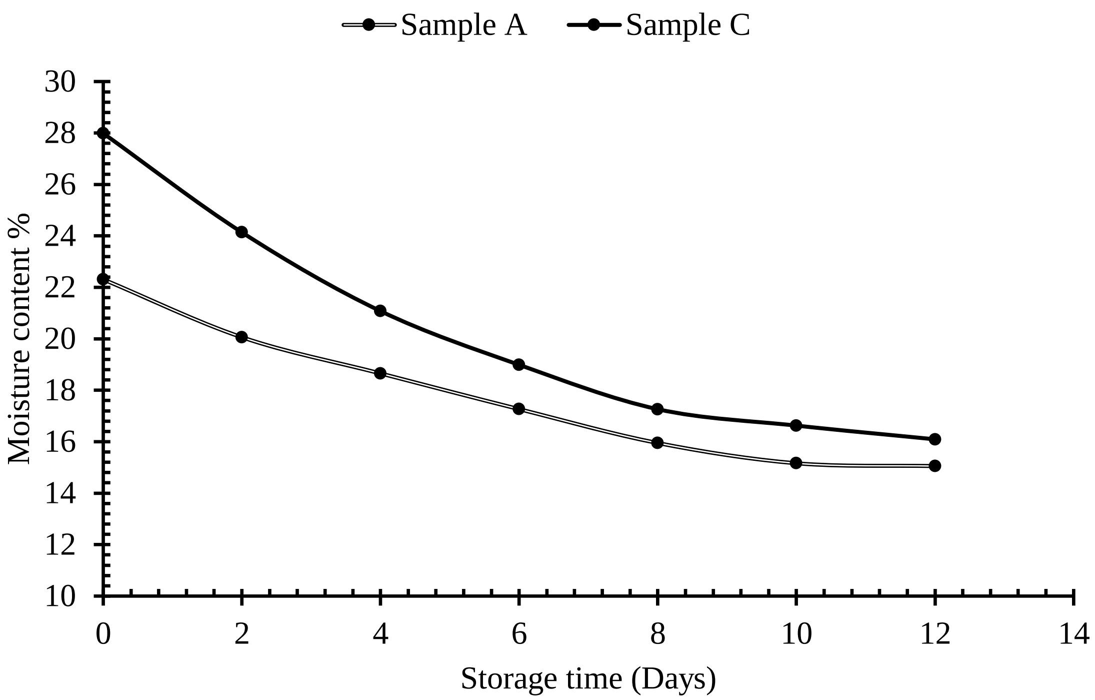

Fig. 4.18 Change in moisture content during storage at refrigerated temperature of sample A and sample C

4.7.4 Change in microbial count

At ambient temperature the total plate count of the sample A was observed to be 1.3×10^3 CFU/g at initial which reached 2.4×10^4, 9×10^4 and 1.5×10^5 and 2.1×10^5 CFU/g within 2, 4, 6 and 8 days respectively. Similarly, for sample C the total plate count was observed to be 1×10^3 CFU/g at initial which reached to 3×10^4, 1.91×10^5, 2.3×10^5 CFU/g within 2, 4 and 6 days respectively. Here, the sample A crossed the permissible limit in 8th day and sample C crossed in 6th day where the permissible limit is less than 2×10^5 CFU/g (WHO, 1994). The coliform in the MacConkey agar was nil. The microbial count in the sample C increased earlier than sample A. It might be due to the high moisture content which in turn increased the water activity. The increase in the total plate count of sample A and sample C is as shown in the Fig. 4.19.

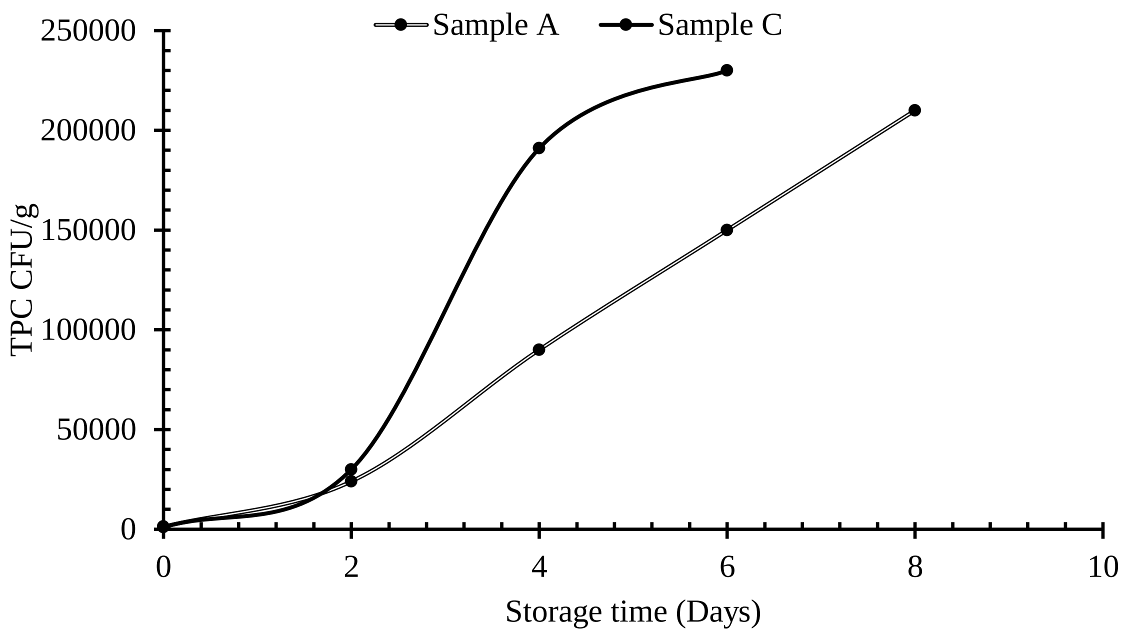

Fig. 4.19 Change in total plate count during storage at ambient temperature of product A and product C

Likewise, at refrigerated temperature the total plate count of the sample A was observed to be 1.3×10^3 CFU/g at initial which reached 2.3×10^3, $5. \times 10^3$, 1.1×10^4, 5.4×10^4, 1.2×10^5 and 1.9×10^5 within 2, 4, 6, 8, 10 and 12 days respectively. Similarly, for sample C the total plate count was observed to be 1×10^3 CFU/g at initial which reached to 3×10^3, 5.7×10^3, 1.8×10^4, 9.2×10^4, 1.9×10^5 and 2.2×10^5 within 2, 4, 6, 8, 10 and 12 days respectively. The change in total plate count of sample A and sample C at refrigerated temperature is shown in Fig. 4.20. Here, the sample A has not crossed the permissible level whereas sample C crossed the permissible level till the 12th day of analysis. The coliform in the MacConkey agar was nil till last day of analysis. In the refrigerated condition there was no drastic increment in the colony count till day 4th which might be due to the lower temperature after that the microbial number increased significantly. The increase in the total plate count of sample A and sample C is as shown in the Fig. 4.20

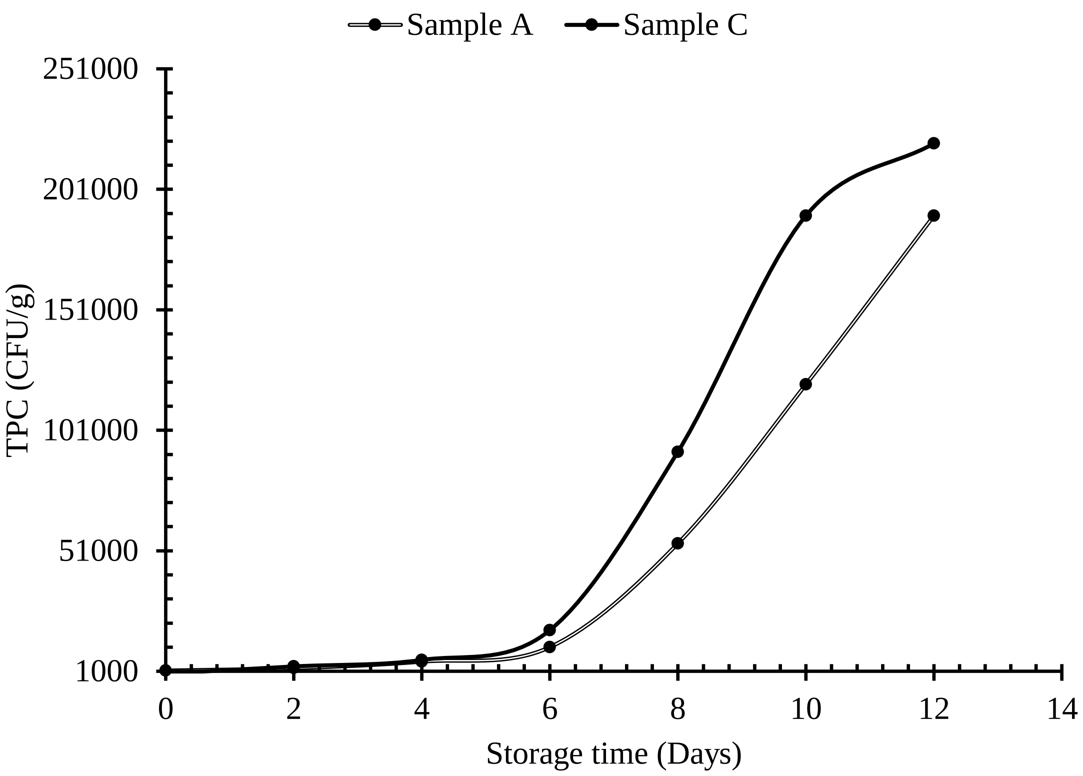

Fig. 4.20 Change in total plate count during storage at refrigerated temperature of sample A and sample C

4.7.5 Shelf life of the product

Sample A and sample C 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 C was fit to consume for 4 days respectively. Similarly, at refrigerated temperature sample A was fit for consumption for 12 days and sample C was fit to consume for 10 days respectively. It was seen that the muffins were unfit to consume in terms of microbial aspect (total plate count). There were no colonies of coliform. Coliform might got eradicated during baking. No colonies of yeast and mold was found in day 6 and day 12 for sample A and day 4 and day 10 for sample C at ambient and refrigerated temperature respectively. Similar observation was seen in the research of Lamsal (2018).

4.8 Cost evaluation of products

The total cost associated with the control and best product was calculated and the cost of oats muffin (sample A) and avocado puree oats muffin (sample C) was NRs. 50.2498 and NRs. 41.929 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 reduced by 16.559% with the replacement of butter by avocado puree.

Part V

Conclusions and recommendations

5.1 Conclusions

On the basis of the research, 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 fibre, total ash content and carbohydrate of wheat flour, oats flour and avocado puree were found to be in acceptable level.
2. Different physical properties were analysed 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 avocado incorporated oats muffin seemed to be enhanced in the case of fibre, ash content and protein content. The fat content of avocado incorporated oats muffin is reduced to 29.362% and caloric valve reduction is 9.004%.
4. There was no significant difference in the sensory quality of muffin up to 32.5 parts substitution of avocado puree.
5. The chemical and microbiological analysis of product shows acceptability of avocado puree 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 avocado incorporated oats muffin is reduced by 16.559%.

5.2 Recommendation

The experiment can be further continued with the following recommendations:

1. Entrepreneur can utilize avocado puree substituting the butter up to 32.5 parts to increase the nutritional value of general muffin without hampering consumer’s acceptance.
2. Texture of the prepared muffin can also be analysed using texture meter.

Part VI

Summary

Muffins are a sweet, high-calorie baked food that customers admire for their exquisite flavor and soft texture. Avocado (*Persea american*) is a tropical fruit, produced annually. However, it has not been commercialised in the context of Nepal. Avocado puree contains a high amount of soluble fibre, vitamins, minerals, folates, and potassium, as well as a distinctive lipid composition. As a result of which, avocado is used more frequently and commercially as a fat replacer in muffin preparation. The current research focuses on a formulation that can increase the value of avocado.

For the preparation of avocado puree incorporated muffin, Response surface methodology was used. Five different muffin formulation namely A, B, C, D, and E with the avocado puree parts 0, 16.25, 32.5, 48.75 and 65 respectively. Sensory evaluation was carried out based on appearance, color, flavor, taste, texture and overall acceptability. The data obtained were statistically analysed using two way ANOVA (no blocking) at 5% level of significance. Sample C got the highest mean sensory score after the sample A. The proximate analysis for moisture, crude protein, crude fat, crude fibre, total ash and carbohydrate were found to be 27.988%, 12.624%, 19.761%, 1.139%, 2.451% and 64.026% and 72.64%, 3.826%, 78.74%, 11.69%, 2.607%, and 3.167% and 8.4%, 13.34%, 9.38%, 1.48%, 1.53% and 74.27% of sample C and avocado puree respectively. The loaf volume decreases with the increase in avocado puree proportion.

The AV, PV and moisture content of sample C at ambient temperature and refrigerated temperature at day 0 was found to be 1.435 mg KOH/g oil and 1.561 meq O_2/kg fat and 27.988% respectively. At ambient temperature, AV, PV and moisture content of sample C reached 5.164 mg KOH/g oil, 4.924 meq O_2/kg fat and 18.779% on day 6. Similarly, at refrigerated temperature, AV, PV and moisture content of sample C reached 5.12 mg KOH/g oil, 4.612 meq O_2/kg fat and 16.093% on day 12. The sample was further analysed for prediction of shelf life based on TPC (bacteria, yeast and mold). No colony of coliform were found from day 0 to day 12. The cost of sample C was found to be NRs.41.92 per 100 g, which is 16.559% less than the cost of sample A including overhead cost and profit of 10%.

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Appendices

Appendix A

Sensory Analysis Score Card

Name of the panelist: Date:

Name of the product: Avocado puree incorporated muffin

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

Any comment

Appendix B

ANOVA results of sensory analysis

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Muffin_type	4	124.2545	31.0636	53.22	<.001
Panelist	10	3.9273	0.3927	0.67	0.742
Residual	40	23.3455	0.5836		
Total	54	151.5273			

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Muffin_type	4	111.7455	27.9364	68.75	<.001
Panelist	10	8.1091	0.8109	2.00	0.060
Residual	40	16.2545	0.4064		
Total	54	136.1091			

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Muffin_type	4	108.5455	27.1364	62.19	<.001
Panelist	10	7.6364	0.7636	1.75	0.103
Residual	40	17.4545	0.4364		
Total	54	133.6364			

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Muffin_type	4	119.7455	29.9364	50.20	<.001
Panelist	10	5.7818	0.5782	0.97	0.484
Residual	40	23.8545	0.5964		
Total	54	149.3818			

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Muffin_type	4	108.2909	27.0727	59.80	<.001
Panelist	10	3.7091	0.3709	0.82	0.612
Residual	40	18.1091	0.4527		
Total	54	130.1091			

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

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Muffin_type	4	117.2000	29.3000	68.14	<.001
Panelist	10	5.7091	0.5709	1.33	0.249
Residual	40	17.2000	0.4300		
Total	54	140.10			

Appendix C

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

	Sample A	*Sample C*
Mean	22.31066667	27.98833333
Variance	0.011041333	0.081590333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-32.31102832	
P(T<=t) one-tail	3.25756E-05	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	6.51513E-05	
t Critical two-tail	3.182446305	

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

	Sample A	*Sample C*
Mean	12.168	12.624
Variance	0.159523	0.003136
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-1.958332453	
P(T<=t) one-tail	0.094646862	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.189293725	
t Critical two-tail	4.30265273	

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

	Sample A	*Sample C*
Mean	0.596	1.138666667
Variance	0.000919	0.001537333
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	-18.96488101	
P(T<=t) one-tail	2.27674E-05	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	4.55348E-05	
t Critical two-tail	2.776445105	

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

	Sample A	*Sample C*
Mean	27.97466667	19.761
Variance	0.001352333	0.037027
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	72.61878662	
P(T<=t) one-tail	9.47869E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.000189574	
t Critical two-tail	4.30265273	

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

	Sample A	*Sample C*
Mean	2.102	2.450667
Variance	0.001629	1.23E-05
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-14.90639627	
P(T<=t) one-tail	0.002235141	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.004470282	
t Critical two-tail	4.30265273	

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

	Sample A	*Sample C*
Mean	57.15933	64.02567
Variance	0.18018	0.045658
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-25.0257	
P(T<=t) one-tail	7E-05	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.00014	
t Critical two-tail	3.182446	

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

	Sample A	*Sample C*
Mean	23.55333333	34.995
Variance	0.000124333	0.020523
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-137.917042	
P(T<=t) one-tail	2.62845E-05	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	5.2569E-05	
t Critical two-tail	4.30265273	

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

	Sample A	*Sample C*
Mean	12.56266667	16.12433333
Variance	0.000492333	0.001281333
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-146.4798535	
P(T<=t) one-tail	3.50779E-07	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	7.01559E-07	
t Critical two-tail	3.182446305	

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

	Sample A	*Sample C*
Mean	514.7913	468.4413
Variance	0.003745	1.329074
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	69.5384	
P(T<=t) one-tail	0.000103	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.000207	
t Critical two-tail	4.302653	

Table C.10 t-test (two- sample assuming unequal variance) for weight loss of the best sample (sample C) with control (sample A)

	Sample A	*Sample C*
Mean	13.24333333	16.74706667
Variance	0.240202333	0.023508013
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	-11.81756739	
P(T<=t) one-tail	0.003542253	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.007084505	
t Critical two-tail	4.30265273	

Table C.11 t-test (two- sample assuming unequal variance) for crust/crumb ratio of the best sample (sample C) with control (sample A)

	Sample A	*Sample C*
Mean	0.528333333	0.660333
Variance	3.03333E-05	0.000108
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	-19.41549738	
P(T<=t) one-tail	0.000149232	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.000298465	
t Critical two-tail	3.182446305	

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	60	25	1.5
Oats Flour	450	75	33.75
Sugar	80	60	4.8
Avocado	250	0	0
Butter	850	65	55.25
Egg	390	57	22.23
Baking Powder	125	1.42	0.1775
Raw material cost			117.708
Processing and labour			11.7708
(10% of raw material cost)			
Profit (10%)			12.9479
Grand total cost			129.478
Average weight of muffin batter		283.42	
Number of muffin formed		24	
Per piece weight of muffin (g)		11.809	
Per piece cost muffin			5.9344
Total cost of muffin (Rs/100g)			50.2498

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

Particulars	Cost (Rs/kg)	Weight in a lot (g)	Cost(Rs)
Wheat flour	60	25	1.5
Oats flour	450	75	33.75
Sugar	80	60	4.8
Avocado	250	32.5	8.125
Butter	850	32.5	27.625
Egg	390	57	22.23
Baking powder	125	1.42	0.1775
Raw material cost			98.2075
Processing and labour			9.82075
(10% of raw material cost)			
Profit (10%)			10.80283
Grand total cost			118.8311
Average weight of muffin batter		283.42	
Number of muffin formed		24	
Per piece weight of muffin (g)		11.809	
Per piece cost of muffin			4.9513
Total cost of muffin (Rs/100g)			41.928

Appendix E

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. MacConkey medium
ix. Plate count agar
x. Alcohol
xi. Sodium thiosulphate
xii. Potassium dichromate
xiii. Phenolphthalein

Color plates

P1: Panelist performing sensory

P2: Weighing avocado puree

P3: Creaming