KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

FORMULATION AND SHELF LIFE EVALUATION OF AVOCADO (*PERSEA AMERICANA*) FRUIT SPREAD

THIS THESIS IS PRESENTED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF MSC DEGREE IN FOOD SCIENCE AND TECHNOLOGY

BY

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DECLARATION

I hereby declare that this study is an original research work that has not been presented to any examining board or published in whole or in part for the award of a degree elsewhere.

Except for reference to other works, which have been duly acknowledged, I have personally, under the supervision of my supervisors, **Prof. J. H. Oldham and Mr. I. W. Ofosu**, undertaken this study.

As the main author of this study, I take full responsibility for all limitations that may be found herein.

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ACKNOWLEDGEMENT

To God is all the glory!!!

Special thanks go to my supervisors, Prof. J. H. Oldham and Mr. I. W. Ofosu, for their help and encouragement and most especially the patience with which they guided me. Mr. J. Y. B. Bennie, your contribution to my masters' programme in general and this project in particular cannot be quantified - I am eternally grateful. To Mr. Asare Mpere of the Plant Genetic Resources Institute, Council for Scientific and Industrial Research (Bunso, Eastern Region), I am very grateful for providing valuable information on avocado. I am highly indebted to Messrs Martey and Bismark of the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong-Akuapim for their help on the laboratory work. My heartfelt gratitude goes to all the laboratory technicians at the Biochemistry Project Laboratory, KNUST.

To Akoto Debrah Offei and Wilhelmina Annie Mensah, thanks a lot for the information you provided. To all my course mates - David, Sammy, Nicholas, Eric, Tohibu, Alfred, Pamela, Phyllis and Diana, I say it has been great knowing you all.

Last but certainly not the least, I say thank you to my parents and siblings and especially my nieces Elsa and Bella Agbey for their support and encouragement.

ABSTRACT

Avocado (Persea americana) fruit deteriorates rapidly after harvest and this necessitated the implementation of this project. The project objective was to carry out product development on the avocado fruit pulp to produce a shelf-stable avocado fruit spread, which retains the nutritional and aesthetic properties of the original fruit. A two level-factorial design with four formulation factors - masses of avocado pulp, sugar, gum and miscellaneous (mostly preservatives and acidulants) - were varied using the Design-Expert's statistical tool to give a total of eight coded products, which were quantitatively sensory evaluated on five parameters by eight trained panelists. The data obtained were modelled and used to predict the formula of the fruit spread which was used then to prepare a sample spread whose microbial, colour and peroxide shelf lives were studied by Statsgraphics centurion statistical tool. The quantitative sensory result projected a fruit spread formulation which comprised of 50 g of avocado pulp, 5 g of sugar, 9 g miscellaneous and 0.5 g of the xanthan gum based on the sensory scores of colour (0.52), taste (0.64), aroma (0.71), spreadability (0.72) and finger feel (0.80). Proximate analysis of the raw avocado fruit and the fruit spread resulted in values of 72.14 g and 60.82 g for moisture, 15.83 g and 10.08 g for fat, 5.34 g and 20.12 g for carbohydrate, 3.95 g and 6.52 g for fibre, 1.72 g and 1.39 g for protein and 0.97g and 1.87 g for ash, respectively. Energy values were calculated as 170.93 kcal for the raw avocado fruit and 176.76 kcal for the avocado fruit spread. Though the shelf life studies were modelled on three parameters, shelf life was rather concluded on microbial safety consideration as 47.50 days at a refrigeration tempearture of 5 ^{o}C, since colour and peroxide deterioration could be readily controlled by using additives.

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CHAPTER 1

1.0 INTRODUCTION

1.1 Background

The general definition of spreads include, but not limited to, spreads made from edible vegetable oil or animal fat or a combination of both such as margarine, cheese and butter and those obtained from fruits and vegetables such as jams, preserves and marmalades. It is largely known that margarine is a water-in-oil emulsion. Margarine consists of a continuous oil phase and with a finely dispersed discontinuous aqueous phase. Butter is perhaps the traditional spread developed since the inception of ancient food technology and its production technology has since not changed much. It is obtained by churning the cream that has been separated from warm cow's milk to a product consisting of unaltered fat globules and moisture droplets embedded in a continuous phase of butterfat. From the studies of Pearson (1970) and Man (2002), butter contains butterfat, water and curd which is made up of casein, lactose and mineral matter. Another variety of spread is cheese which is mainly made from curd produced from the coagulation of souring milk. The souring is done by rennin, an enzyme obtained from the inner lining of the fourth stomach of the calf (Man, 2002).

Jam, jelly and marmalade preparations constitute yet another category of spread. The basic principle of jam and marmalade manufacture is the boiling together of fruit, sugars and water. For marmalade, the peel is cooked separately prior to mixing in. Jelly manufacture involves boiling the fruit with water and then the extract after filtration is boiled with the sugars (Kirk and Sawyer, 1991).

Many researchers discuss the nutritional value of margarine and other spreads largely around two components. These are the total amount of fat and the types of fat (saturated fat, trans-fat) as components of the formulation. It has been concluded by some researchers that the saturated fatty acids in triglycerides contribute to elevated blood cholesterol levels (Keys *et al.*, 1965; Mensink *et al.*, 2003; EFSA, 2004; EFSA, 2005; IoM, 2005), which in turn has often been linked to cardiovascular diseases. It has been observed that firmer margarines contain more saturated fat (Bruijne and Bot; 1999).

Willet *et al.*, (1993), Hu *et al.*, (1997) and Hayakawa *et al.*, (2000) have also indicated a strong link between earlier death and consumption of high amounts of trans-fats which had been common in many spread formulations not quite too long ago. Trans-fats which do not occur naturally in vegetable fats are a consequence of partial hydrogenation of the oils, a requirement for some spread formulation procedures. According to some researchers Duijn, (2005) and Floter and Duijn, (2006) many industries have gradually moved away from using partially hydrogenated oils since the mid-nineties and now produce new spreads that contain less or no trans fats.

Though it has been proven that the intake of cholesterol has less effect on high blood cholesterol levels than saturated fat, the Food and Drugs Administration (FDA) of the United States of America has warned that healthy people should not consume more than 200 mg of cholesterol per day. However butter which contains high levels of cholesterol which is rapidly consumed heartily several times daily in the form of the varied spreads. Though margarine may contain no cholesterol due to their preparation procedures, the abundant saturated fat in margarine induces the bad type of cholesterol (low density lipoproteins) in the course of human metabolisms (Sebedio and Christie, 1998).

Margarines and other spreads excluding butter are however, important sources of vitamin E and, they contribute 14 % of total vitamin E intake in adults, 17 % in boys and 16 % in girls in the United Kingdom (British Nutrition Foundation, 2004). Possibly this may be the case in many affluent societies which many countries are now aspiring to become. For instance, there are various types of spread ranging from cheese, butter, margarine to fruit spreads on the Ghanaian market (Mpere, (2008), Personal communication).

Due to problems associated with consumption of such as cheeses and margarines, alternatives which can deliver the functionalities required in traditional spreads with less nutritional problems are being sought. *Persea americana* fruit also known as avocado pear or alligator pear, comes in handy. Avocado pear is a seasonal fruit and native to the tropics and sub-tropics such as tropical America, Far Asia and Cuba (Purseglove, 1968; Pamplona-Roger, 2007). Purseglove (1968) has described the flesh of the fruit of the avocado as generally pale yellowish-green and softly succulent with buttery consistency even though inferior varieties may be fibrous in nature.

It is well known that avocado fruits ripen best when they have been detached from the tree. On ripening it yields to gentle, palm pressure and can be stored in the refrigerator for several days but once the flesh is cut and exposed they tend to brown rapidly due to enzymatic browning reactions (oxidation of the iron salts) (Pamplona-Roger, 2007). It has been observed that the darkening or browning process can be lessened when the unused portion of an avocado pear is covered with a plastic wrap, removing as much air as possible. Studies have shown that avocados do not freeze well but avocado puree can be frozen, although it may be slightly watery when thawed (Morton, 1987).

Avocados are very nutritious, high in unsaturated fat and at their buttery best when used in raw preparations but when they are cooked for long periods, their delicate flavour is diminished and in some instances they can become bitter (Purseglove, 1968; Morton, 1987). Avocado is thought to promote physical beauty when applied to the skin and is therefore used in cosmetics formulations and as an aphrodisiac (Purseglove, 1968; Bergh, 1992).

1.2 Problem Statement and Justification

Avocado is a nutritious fruit that is grown widely in Ghana and consumed by many as part of their main meal. They are usually sliced and added to meals like cooked rice, boiled yam and plantain and used in salads filling or spread. However, not much has been done to process and store the fruit when it is in season and in abundance. The fruits when ripe have short life span and would discolour and rot whether refrigerated or not, and lose its flavour (Hodgson, 1950). The solutions to the problems of the short shelf life of the fruits is to eliminate the above shortcomings by presenting to consumers a processed product with an added value which will ultimately eliminate or reduce any possibility of waste during the major season, by preserving the fruit and making the fruit and its nutritional value available to many people all year round.

One way to achieve this goal is by adding value to the fruit by way of processing. This will include preservation methods that will prolong the shelf life of the fruit. Consequently, spread made from the avocado fruit has the potential of presenting to the consumer an additional variety in which the fruit can be consumed, as well as retaining the characteristic yellowish-green

colour, taste, mild and faintly nutlike flavour and rich nutritional value with functional properties.

Comparison of fresh avocados with butter, margarine and cheddar cheese shows that the fat content for 100 g are 22.2 g, 82.0 g, 81.0 g and 33.5 g respectively (Kirk and Sawyer, 1991) making the avocado a good material worth considering as a substitute for most fat based spreads.

1.3 Objectives

1.3.1 *Main Objective*

To extend the shelf life of ripe avocado fruit by processing into a fruit spread (new product development) which would retain, as much as possible, both the nutritional and aesthetic values of the fruit as a means of adding value to the raw fruit.

1.3.2 *Specific Objectives*

- To obtain the best formula for avocado fruit spread using modeling of the sensory parameters data and formulation variables.
- To study the shelf life using three deteriorating parameters of the formulated avocado fruit spread packaged in glass bottles.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Table spreads

Table spreads may be fat-based or made from fruits and vegetables. Margarines and other fat-based spreads are produced from a range of vegetable oils and fat and are used for a variety of food products. Edible oil spread is a spreadable food composed of edible oils and water soluble component such as vinegar or citric acid, in the form of a water-in-oil emulsion. Fruit and vegetable spreads means all of the recognized fruits and those vegetables recognized as suitable in making jams, including chestnuts, ginger, melon, rhubarb, tomato.

Margarine is a water-in-oil emulsion, consisting of a continuous oil phase and a finely dispersed discontinuous aqueous phase. The continuous phase contains flavour components, colouring matter, vitamins and also fat crystals. The best quality margarines are manufactured usually from vegetable oils but in recent years approximately half of all the oils used are partially hydrogenated marine oils. Animal fats are still in use in some speciality margarines. The Margarine Regulations in 1967 and the Codex standard for margarine (CAC/RS 32-1969) require all margarine to contain at least 80 percent fat of which not more than one-tenth by weight may be milk fat and not more than 16 percent water. In addition, the Margarine Regulations passed in 1967 by the American Food and Drug Administration require that any margarine sold by retail should contain in each ounce 760-940 i.u. vitamin A and 80-100 i.u. vitamin D (Kirk and

Sawyer, 1991). Butter Regulation, passed in 1966 SI No. 1074 (American Food and Drug Administration), butter shall contain not less than 80 percent milk fat, not more than 2 percent milk solids other than fat, and not more than 16 percent water.

2.2 The Avocado Plant and Fruit

The avocado tree, *Persia americana* is a tropical evergreen tree. According to Purseglove (1968) and Pamplona-Roger (2007), avocado is the only tree that produces edible fruit among the laurel family. The fruits are extremely variable in shape, size, and colour (Figure 1).

Figure 1: The Avocado fruit

The shape may range from ellipsoid through pyriform and obovate to spheroid, with the skin of the fruit also varying in texture and colour. The colour of skin ranges from yellowish-green, reddish-purple, purple- black. The outer skin may also be pliable to woody or smooth to rough (Taah *et al.,* 2003). The uses of avocado depend on the varieties available. Because the tannins in avocado result in a bitter flavour when cooked over high heat, they are usually eaten raw (Purseglove, 1968; Morton, 1987). There are three races of avocado: Guatemalan, Mexican and West Indian as represented in table 1. While each has unique features, cross-pollination permits the development of limitless varieties.

Table 1: Ecological races of Avocado and their distinctive characteristics

Characteristic	Mexican	Guatemalan	West Indian
Fruit weight (average)	98.8 g	309.8g	312.5 g
Peel thickness	Thin and very thin	Medium, thick or very thick	Thin, medium or thick
Peel texture	Membrane-like	Corky	Leathery
Skin surface	Waxy-bloom	Rough	Shiny
Leaves	Usually anise-scent	No anise-scent	No anise-scent
Fruit pulp flavour	Rich to strong	Buttery	Milk to watery

2.2.1 World Production and Consumption of Avocado

It has been recorded by Dirou (2003) that Mexico is the world's largest producer of avocado, and it supplies over a third of the world's total production of approximately 2.3 x 10^6 tons. Chile and the United States are ranked as the second and third of the world producers of avocado. According to FAO report (FAOSTAT, 2004), in 2004, Chile became the world's largest exporter but there have been significant increased exports from Mexico, Ecuador, Peru and South Africa as well. The report also suggest that Chile, South Africa, Israel and Spain trade higher shares of their output, as production is specialized and export-oriented.

On the continent of Africa however, the largest production countries are Cameroon, Zaire, South Africa, the Congo and Ivory Coast (Zentmyer, 1987), with Cameroon being the 13th in world rank producing 56 million pounds. Ivory Coast is ranked as the 24th with production levels hitting 13.5 million pounds.

In Ghana, however, it is recorded that the first planting of avocado pear occurred at *Aburi* in the year 1870, though peasant avocado pear production occurred by 1907 (Anon, 1961). There is no recorded documentary evidence of large scale cultivation of avocado in Ghana and there are no certified avocado nurseries to supply growers with grafted material. Avocado pear is regarded as an important economic crop throughout Ghana but it is mainly found in the forest zones particularly in the southern part of Ghana where the conditions are good for cultivation.

Currently, there are only a few well-established orchard productions of avocados in Ghana. Significantly, in Ghana avocado is mainly grown by smallholders, from backyard plantings and volunteer crops scattered in cocoa and other farms in most parts of the country with the exception of the Northern and Upper Regions where the crop is not found. Ghana contributes only a small percentage to the world export of the fruit because it appears the production is largely centered on internal consumption as all year round avocado fruits of different shapes, sizes and colours are offered for sale.

A variety of factors influence the consumption of avocado and such factors include income, urbanization as well as the consumption habits of the different segments of the population. Major consumers of the product appear to be those in the high and middle income bracket of the population. Increased human consumption of the fresh and processed products, as well as the result of consumers' enhanced awareness of the fruit's nutritional properties; invariably constitute the market drivers creating the increased demand. It is also believed the utilization in the cosmetic industry as a consequence of the growing demand for natural based product components is also part of the parameters driving the market.

2.3 Benefits of Avocado

Socio-economic Benefits: This economic and social importance of avocado manifests in the benefit that its cultivation gives to producers, marketers, processors, and consumers. This includes creation of by farming operations, harvest, packinghouse operations, transportation, and marketing (Téliz, 2000; APROAM, 2003) and foreign exchange earnings.

Medicinal and Health Benefits: Avocado has several medicinal and health benefits (Irvine, 1961 and Pamplona-Roger, 2007). The skin of the avocado is used as an antibiotic, for ridding the intestinal tract of parasites, and as a remedy for dysentery. The seeds are roasted and pulverized for the treatment of diarrhea and dysentery. In the powdered form, the seeds have been used as a dandruff treatment. Pieces of avocado seed are placed in tooth cavities as a toothache palliative. It is recorded that the Amazonian natives used avocado to treat gout. When the pulp of the avocado fruit is added to the diet it helps to reduce cholesterol levels (Pamplona-Roger, 2007).

Various studies have confirmed that apart from being a source of energy and vitamins, avocado also delivers specific non-nutritive physiological benefits that may enhance health. It can thus be considered as a "functional food", based on the definition of Mazza (1998). Among the nutraceutical ingredients found in avocado pulp are antioxidants, such as vitamin E or tocopherols (4.31 UI/100 g) and glutathione (17.7 mg/100 g), more than three times the amount in any other fruit). As a source of antioxidants, the vitamin E and glutathione neutralize free radicals that may damage aging cells, the heart (Rimm *et al.*, 1998; Bergh, 1992) and contribute to the development of some types of cancer, such as cancer of the mouth and pharynx (O'Toole, 2000; Heber, 2001; Pamplona-Roger, 2007). Lutein, a carotenoid, helps to protect the eye from diseases such as cataracts and reducing the risk of age related macular eye disorders. Avocados contain a high amount (248 mg/100 g) of lutein (Bergh, 1992). The level of β-sitosterol in avocado is similar to that in soy and olives. It has been demonstrated through animal studies that β-sitosterol is related to the inhibition of cancerous tumors (Heber, 2001). β -sitosterol has been shown to improve men's prostate function (Lu, 2005). As a good source of vitamin D, it is beneficial for those at risk from osteoporosis. Oleic acids and folate prevents stroke, breast cancer and lowers cholesterol levels in blood.

Nutritional Benefits: There are some notable differences in the amounts of nutrients provided by the different varieties of avocado.

Table 2: Nutritional differences between 100 g of two varieties of avocado fruit

	Carbohydrates (%)	**Protein (%)**	**Cholesterol (mg/100g)**	**Energy (calories)**	**Fat (%)**	**Saturated Fat (%)**
California	6.96	2.32	0	176.9	17.4	2.61
Florida	8.91	0.99	0	112.2	8.91	1.75

The fruit also has 60 % more potassium than bananas and is rich in B vitamins, as well as vitamins E and K (Bergh, 1992).

The smooth and creamy consistency of avocado makes it one of the first fresh fruits a baby can enjoy. Sodium- and cholesterol- free per 100 g of fresh fruits, avocados contain valuable nutrients

including 8 % of the recommended Daily Value (DV) for folate; 4 % DV for fibre and potassium, 4 % DV for vitamin E; and 2 % DV for iron. Per serving, 100 g avocados have 3.5 grams of unsaturated fats, which are known to be important for normal growth and development of the central nervous system and brain (Bergh, 1992).

When avocado is used in place of other fat in calorie-reduced diet and/or calorie-controlled diet for weight loss or weight maintenance programmes the results are very encouraging (Bergh, 1992). Some dieters avoid avocados because of the high fat content, which is about 20 %. However, two-thirds of this fat is monounsaturated, which indeed is essential to the body and the heart in particular.

It is reckoned that a food's total nutritional contribution to human needs is more critical than its calorie content. A study conducted by Slater *et al.,* (1975) indicates that one half of a 'Hass' avocado, about 80 g edible fruit, provides a substantial percentage of the daily nutritional needs of a child aged from 7 to 10 years (adult percentages are generally a little higher, especially iron for females).

Cosmetics: There are increasing concerns for the use of artificial additives and preservatives in many processed foods and cosmetic products. This generally gives avocado a major advantage as a food and as a cosmetic additive over the artificial ones because they are biodegradable. Consumers are beginning to favour basic, natural ingredients (Swisher, 1988).

2.4 Avocado Processing

Food processing is a set of techniques of food preservation, which makes of products available to the consumer (Morris *et al*., 2004). It is imperative to develop food products from avocado with increased shelf life, long enough to overcome deterioration problems and ensure effective distribution to consumers. The benefits would be enormous, for instance, promoting the creation of processing plants, leading to the generation of new jobs, and increased profits for the farmers. The development of novel technologies will result in the generation of new processes and products from avocado that may benefit developing countries.

The major problems likely to confront a food processor when developing and preserving avocado products would include controlling and maintaining an optimal ripeness state of avocado and overcoming enzymatic browning which is catalyzed by the action of polyphenoloxidase and other oxidant-reducing enzymes. Preventing or minimizing the loss of yellowish-green colour due to changes in the chlorophyll molecules at low pH values and dealing with the generation of off-flavours and loss of texture due to conventional thermal treatments must be critically looked at. Furthermore, the issue of microbial contamination present in the fruit peels as a result of inappropriate agricultural practices must be considered. The rough texture of the peel makes this contamination hard to eliminate.

It has been found that minimally processed avocado products meet the consumer demand of freshness. However, the shelf life of these products is relatively short demanding preservation factors such as a combination of additives and refrigeration. Welti-Chanes *et al.,* (1998) recommended a fruit firmness range of 800 to 1500 g for avocados to be minimally processed.
Some studies on avocado processing involved the incorporation of additives to improve the sensory quality and avoid syneresis. For instance, Stephens *et al.,* (1957) showed that freezing with nitrogen gas decreased enzymatic and microbial activities of avocados for 9 months.

Conventional thermal treatments have also been used to process avocado; though they decrease or eliminate microbial populations and inactivate enzymes they produce off-flavours that are generated depending on the processing time and temperatures (García *et al.,* 1975). It has also been demonstrated that the combination of reducing substances, organic acids, bacteriostatic substances, and sequestering agents in refrigerated products effectively delayed guacamole and avocado paste browning (Sánchez *et al.,* 1991; Dorantes-Alvarez *et al.,* 1998).

Gomez and Bates (1970) reported that freeze-drying avocado fruit achieved a moisture content less than 2.5% in samples of avocado. The result was the lowering of water activity to a level that interferes with enzymatic activity and microbial growth. Freeze-drying is considered to be a relatively expensive method because it requires freezing and vacuuming (<5 mm Hg), in order to sublimate the water present in the samples. As can be expected, such products packed with an inert gas such as nitrogen lasted for a period of nine months with high quality.

The application of high hydrostatic pressure to process avocado has also achieved some appreciable results leading to the production of some commercial products. The process destroys the microbial cellular structures, mainly their membranes and inactivating them to give a safe product (López-Malo, 1998).

Some novel technologies like the microwave thermal treatment combined with a decrease in pH and the formation of complexes of chlorophyll-zinc (Guzmán *et al.,* 2002) have been explored. This high temperature-short time treatment (HTST) achieved with microwave heating decreases the generation of off-flavours and loss of texture. Also the addition of a zinc salt prevents the loss of the green colour forming a chlorophyll-zinc complex, during the thermal process. Meanwhile, Ortiz *et al.,* (2003) have reported that pre-heating the sample with microwaves may lead to the production of a good quality product and yield of avocado oil, which is free from trans fatty acids.

2.5 Shelf Life Studies

Shelf life is the useful storage life of food. It is an important property of any food and is of interest to everyone in the food chain from producer to consumer (Steele, 2004). At the end of shelf life the food would have developed characteristic changes in taste, aroma, texture or appearance that are deemed unacceptable or undesirable (Winger, 2000; Smittle and Cirigliano, 2001). Singh (2000) explained that the underlying cause for the change may include chemical reactions, mainly due to either oxidation, or Maillard reactions (Owusu-Apenten, 2005), microbial reactions (Jay *et al.,* 2005), and biochemical reactions resulting from endogenous enzyme catalyzing reactions leading to quality loss like enzymatic browning. Other causes may be lipolysis, proteolysis (van Boekel and Walstra, 1995; van Boekel, 2007), biochemical and physical reactions resulting in coalescence, aggregation, and sedimentation which leads usually to quality loss (Walstra, 2003). Changes in texture can also be considered as physical reactions, though the underlying mechanism may be of a chemical nature (Wills *et al.,* 1981; Hindra and Baik, 2006).

As indicated earlier, shelf life represents the period of time through which a food remains safe to eat, retains its essential sensory properties and complies with the label's nutritional declarations (Steele *et al.,* 2006). Consequently, during the shelf life, a defined quality of a specified

proportion of the product remains acceptable under expected or specified conditions of distribution, storage and display. Shelf life determination estimate the time at which a consumer product is no longer usable, unfit for consumption (Villari *et al.*, 1994), or no longer has the intended sensory characteristics (Singh, 2000).

Based on the knowledge of the mechanisms of food deterioration, food scientists have developed methods of counteracting these losses in quality (Winger, 2000). The rate at which these reactions occur, the effects of temperature, water, and the myriad of other parameters have become characterized factors contributing to the science of accelerated shelf-life studies. In such studies, the rate of change is temperature dependent and it increases at higher temperatures similarly to most physicochemical reactions.

The prediction of shelf life for food products could therefore be based on the application of the principles of temperature dependent chemical reaction kinetics relating to product composition as well as environmental factors such as temperature, humidity, atmosphere, etc. Basic to any predictive use of reaction kinetics is that the relationship between the measurable changing reaction parameter and time must be linear or follow a clearly defined model. Establishing the length of time during which there is reasonable belief that the product will still be effective is an interesting statistical question. Although there are different approaches to this problem, one common method is to take samples at different lengths of time after production and construct a statistical model for one or more critical variables. The model can then be used to predict that point in time after which the probability that the product will still be effective falls below some specified threshold (Polhemus, 2005).

Preservatives are generally additives that prolong the life span of foods and drinks. Technically, preservatives are chemicals used to poison microorganisms and to prevent the food onto which it is added from fermentation and spoilage without causing any harmful effect to the consumer. The uses of chemical preservatives enhance food quality, reduce waste and enhance consumer acceptability. Friezer (1989) classifies chemical preservatives into three main types; anti microbial -such as benzoic acid, propionates; antioxidant -such as ascorbic acid, butylated hydroxyl anisole; and antibiotic -such as oxy tetracycline, nisin and lacto peroxidase.

Foods are excellent sources of nutrients and hence are excellent environments for the growth of microorganisms. Microbial growth is controlled by factors related to the food itself, or intrinsic factors, and also to the environment where the food is being stored, or what are described as extrinsic factors. Food composition is a critical intrinsic factor that influences microbial growth. If a food consists primarily of carbohydrates, spoilage does not result in major odours. Degradation of fats produce short-chained fatty acids, that render fat-based foods rancid and unpleasant (Allen and Hamilton, 1989; Prescott *et al.*, 2002).

Rancidity in fats and oils is due to autoxidation of the unsaturated fatty acids or reactions of oxygen with the unsaturated fat (Macrae *et al.*, 1993; Chandan, 1997). It is one of the major changes that occur in lipids and lipid based foods during processing, distribution and final preparation. The oxidation of lipids initiates other changes in the food and this eventually affects the food's nutritional and organoleptic qualities. Hydrolytic rancidity, for all practical purposes, does not occur during low temperature storage of foods. However, the oxidative rancidity process does occur at these temperatures because it is a chemical reaction, which requires much lower activation energies to initiate. The process of oxidative reactions during storage studies can be followed analytically by determining the peroxide value in a fat or oil by quantitating the aldehyde, n-hexanal, in food products (Prescott *et al.*, 2002).

A number of antioxidants such as ascorbic acid can be added in small amounts with a view to either completely prevent or partially retard oxidation of fat during storage and distribution (Mehlenbacher, 1960; AOAC, 1995).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Materials

Avocado (*Persia americana*) fruits used for this work were obtained from an out-grower at *Ahinsan* Estate, Kumasi. Food-grade citric acid and ascorbic acid were obtained from Sigma-Aldrich Inc., USA. Xanthan gum was obtained from BDH Chemicals Limited, UK. Sarsons malt vinegar, sugar, Annapurna iodised salt, and *Raynes* vanilla essence were obtained from *Opoku* Trading Supermarket, Kumasi. Sodium benzoate and potassium sorbate were obtained from Scharlau Chemie S.A., Spain.

3.1.1 Avocado Pulp Preparation

The avocado fruits were hand-picked at commercial maturity and kept at room temperature for three days to complete the ripening process. The ripped fruits were then washed under running potable water and subsequently soaked for 15 min in Milton's chlorinated tablets. The fruits were peeled, deseeded and cut into four wedges. These were then cut into small pieces and finally pulverized using a *Binatone* domestic food blender (5080 MP) at speed 4.

3.2 Methods

3.2.1 Formulation of avocado spread

A two-level factorial design with four factors which are the proportions of avocado pear pulp, sugar, gum and miscellaneous were varied statistically (Design-Expert, 2007) to give eight

formulations (Table 3). The miscellaneous comprised of the preservatives (sodium benzoate and potassium sorbate), citric acid, ascorbic acid, iodised salt, vinegar and vanilla essence.

Table 3: Quantities of ingredients used for the formulation of avocado fruit spreads and the coded formulations

		Factor 1	Factor 2	Factor 3	Factor 4
Run	**Labels**	**A:Avocado/g**	**B:Sugar/g**	**C:Misc. /g**	**D:Gum /g**
1	M1(654)	40	5	5	1.0
2	M2(732)	50	5	9	0.5
3	M3(754)	40	1	9	0.5
4	M4(296)	50	5	9	1.0
5	M5(254)	50	1	5	0.5
6	M6(336)	50	1	5	1.0
7	M7(963)	40	1	9	1.0
8	M8(657)	40	5	5	0.5

For each sample, the avocado pulp was poured in a bowl. The miscellaneous components were added in the order citric acid, ascorbic acid, gum, sugar, salt and preservatives. The mixture was then homogenized using *Binatone* hand mixer (CA 3090) starting at speed 1 and increasing to speed 5. Vanilla essence and vinegar were then added and thoroughly mixed into the puree. Mixing then continued until a smooth puree was achieved.

The samples were then poured into sterilized 50 ml glass bottles, pasteurized in a Martins 1700X model water bath at a temperature of 65 ^{o}C for 5 min, sealed and labeled as M1(654), M2(732), M3(754), M4(296), M5(254), M6(336), M7(963) and M8(657).

3.2.2 Sensory Evaluation of formulated spread

i) Training of sensory panel

For the quantitative descriptive test, eight panelists who have had previous training at the Food Research Institute (Council for Scientific and Industrial Research), Accra were chosen and further trained for two hours per week for a two-week period, using model products like margarine, groundnut paste and hard cheese. This was to characterize the chosen parameters for this study, which were colour, taste, aroma, spreadability and finger feel as defined in table 4.

Table 4: Definitions of sensory parameters as used in the research

Sensory parameter	**Definition and range of sensory parameter**
Colour	Yellow to Green
Taste	Uncharacteristic avocado pear taste to characteristic avocado pear taste
Aroma	Unpleasant to pleasant
Spreadability	Aggregate to easy-to-spread
Finger feel	Rough to smooth-to-touch

ii) *Sensory evaluation of formulated spread*:

This was conducted using by employing the graphic scale methodology (Larmond, 1977). A general factorial design (Design-Expert, 2007) making use of products (A) (eight of them coded from M1 to M8) shown in Table 4 and panelists (B) (eight of them coded from P1 to P8) were used to plan the serving order (Appendix A3) where each of the eight panelists sampled the eight products. The panelists were provided with unsalted cream crackers to clear the palate and water to rinse their mouths between samples.

3.2.3 Statistical Analysis

i) Fitting the data collected

The sensory evaluation data collected were loaded into the statistical software ((Design-Expert, 2007) and initially fitted into models that could explain the behaviour of the sensory parameters of the avocado spread. This involved determining the coefficients, regression- (R^2), adjusted regression- (adj R^2), prediction regression-(pred R^2), adequate precision -(adeq precision) and predicted residual error sum of squares (PRESS) of the models selected. After the fitting,

Analysis of variance (ANOVA) studies and diagnostic plots were carried out. When all the model statistics and diagnostic plots were evaluated to be good, the model graphs were obtained.

ii) Setting the limits and optimizing the selection criteria

The optimization and selection of the formulated product according to the sensory data collected was done according to Design-Expert (2007). Since avocado puree has both green and yellow portions, the colour was set at midpoint of yellowish-green which was equivalent to 0.52 (calculated from the 0.21 to 0.82 on the Larmond, (1977) 0 to 1 scale (appendix A2). The rest of the sensory parameters measured were set at maximum. The formulated products were thus set in range whereas the response variables were constrained for the optimization.

3.2.4 Analytical Methods

A series of analyses were carried out on the optimum product including proximate analysis using AOAC (1990) methods and shelf life studies based on peroxide value using methods described by Kirk and Sawyer (1991), colour of spread based on sensory evaluation and microbiological studies. The data of the shelf life studies were analyzed using the Statsgraphics Centurion software (StatPoint, 2008).

3.2.4.1 Proximate analysis of raw material and selected avocado fruit spread

The optimum sample was prepared and stored in glass bottles and kept under refrigeration conditions (5-7 ^{o}C) for the proximate and microbiological analyses. Moisture, protein, crude fat, crude fibre and ash contents were determined using standard methods (AOAC, 1990) for both the raw avocado puree and the formulated spread. All analyses were carried out in triplicates.

i) Determination of moisture content

A mass of 2.00 g of each sample was transferred into a previously dried and weighed dish (W_1). The dish plus its content (W_2) was placed in an oven (Gallenkamp, model OV 880, England) which was thermostatically controlled at 105 ^{o}C for 6 h. The dish was removed, placed in a desiccator and weighed and subsequently put back into the oven, reheated, cooled and weighed until a constant weight (W_3) was attained. The loss in weight was reported as the percent moisture content which was calculated according to the formula in Appendix B1.

ii) Determination of crude protein

Crude protein was determined using the macro Kjeldahl procedure. 2.00 g of each sample was weighed into a digestion flask and 0.5 g of selenium based catalyst added. A volume of 25 ml concentrated H_2SO_4 was added and the flask agitated to wet the entire sample. The flask was placed on a digestion burner and heated slowly for 8 h until the entire solution was clear. The sample was then cooled to room temperature and the digested sample solution transferred into a 100 ml volumetric flask and made to the mark. A volume of 25 ml of 2 % boric acid was pipetted into a 250 ml conical flask and 2 drops of mixed indicator (20 ml bromocresol green and 4 ml of methyl red) added.

The liquid from the steam trap was drained and the stopcock which drains the steam trap left opened. The conical flask and its contents were placed under the condenser. A measured volume of 10 ml of the digested sample was transferred into the steam jacket and 20 ml of 40 % NaOH was added to the decomposition flask. Distillation was run for 30 s and the distillate was titrated with 0.1 M HCl solution. To calculate for the percentage total nitrogen, the formula in Appendix B2 was used and the 6.25 factor was used to convert nitrogen to crude protein.

iii) Determination of crude fat

The Soxhlet extraction method was used in the crude fat determination. A mass of 2.00 g of the dried samples from moisture determination were transferred into a cellulose thimble. A ball of glass wool was placed in the thimble. Anti bumping granules were added to a previously dried 250 ml round bottom flask and weighed. A volume of 150 ml of petroleum spirit was added and apparatus assembled. A quick fit condenser was connected to the Soxhlet extractor and refluxed for 4 h on high heat on the heating mantle. The flask was then removed and evaporated on a steam bath. The flask with the fat was heated for 30 min in an oven (Gallenkamp, model OV 880, England)) at 103 ^{o}C, cooled in a desiccator and weighed. The formula in Appendix B3 was used to calculate for the crude fat.

iv) Determination of crude fibre

The defatted sample from crude fat determination was transferred to a 750 ml Erlenmeyer flask, 0.5 g asbestos added followed by 200 ml of boiling 1.25 % H_2SO_4. The flask was immediately placed on hot plate and connected to the condenser. After 30 min the flask was removed and the contents filtered through linen cloth in funnel and washed with boiling water until washings were no longer acidic. The charge and asbestos were washed back into the flask with 200 ml boiling 1.25 M NaOH. The flask was connected to the condenser and boiled for 30 min. The contents were filtered through linen cloth and washed thoroughly with boiling water.

The residue was transferred to Gooch crucible and the remaining particles washed into the crucible with 15 ml alcohol. The crucible and contents were dried for 1 h at 100 ^{o}C, cooled in a desiccator and reweighed. The crucible was ignited in an electric furnace (Muffle furnace size 2, England) at 600 ^{o}C for 30 min, cooled and reweighed. The percentage crude fibre was calculated using the formula in Appendix B4.

v) Determination of ash content

A mass of 2.00 g of each sample was transferred to a previously ignited and weighed crucible and placed in a preheated furnace (Muffle furnace size 2, England) at 600 ^{O}C for 2 h. The crucible was removed, allowed to cool in air and placed in a desiccator whilst still hot. The sample was allowed to cool and weighed. The formula in Appendix B5 was used to calculate the percentage fat content.

vi) Determination of carbohydrate

Total carbohydrate was determined by subtracting the amount of ash, protein and fat from the total dry matter. That is, percentage carbohydrate = 100 - (moisture + protein + crude fat + crude fibre + ash).

vii) Determination of energy

Energy content of the products was calculated by Atwater's method (AOAC, 1990).

$$\sum(protein \times 4 + carbohydrate \times 4 + fat \times 9)$$

3.2.5 Shelf life Analysis

For shelf-life studies, samples from the same batch of the optimum sample were divided into two and stored under refrigeration and ambient conditions. The shelf life determination was performed based on three parameters - peroxide value, deterioration of the yellowish-green colour and microbial load, run for a period of six consecutive weeks and sampled weekly for analysis.

i) Determination of Peroxide Value

Peroxide value (Kirk and Sawyer, 1991; Maforimbo, 2002) was determined by first extracting the avocado oil according to the method described by Ikhuoria and Malik (2007), with the appropriate modifications. In this method, a volume of 100 ml of chloroform was added to 50 g of the sample, stoppered and shaken thoroughly with a Griffin Flask Shaker (M009B) for 30 min and the procedure repeated five times. The extract was collected in a conical flask and left in a fume chamber for most of the chloroform to evaporate and the oil was siphoned using a syringe.

The peroxide value was then determined by weighing 3 g of the extracted oil into a stoppered conical flask and 10 ml of chloroform added and the fat dissolved by vortexing after which 15 ml of glacial acetic acid and 1 ml fresh saturated aqueous potassium iodide was added. The flask was then stoppered and shaken for 1 min and placed in the dark for 5 min, topped with 75 ml of distilled water, mixed and titrated with 0.01 M sodium thiosulphate solution using 1 % soluble starch solution as indicator. The peroxide value was obtained using the equation in Appendix C1.

ii) Colour determination of sample formulation

The essence of the colour determination was not to extract the chlorophyll but to measure the colour as it appeared in the fruit spread. A specified quantity of distilled water was added to dilute the product so it could be measured volumetrically. For the weekly capturing of colour, exactly 2.00 g of the sample spread was mixed thoroughly with 5 ml of distilled water and a drop pipette was used to place six drops of the diluted sample on filter paper (Machery-Nagel MN 615) and dried in a desiccator for 30 min.

The filter paper was then scanned using an HP Scanjet 2400 at 1200dpi onto an Acer laptop TravelMate series 4021WLCi. In addition to the filter paper measurement, 50 g of the sample spread were placed in petri dishes, photographed using a digital camera (564 MCD) and printed on a printer (HP 3900, UK). Eight trained panelists were then made to measure the colour intensity on a sensory evaluation card with a scale running from 1 to 10 (Appendix C2) at the sixth week of sampling.

iii) Microbial load

A sub-sample mass of 1.00 g of the sample fruit spread was prepared by mixing with 1 ml of distilled water. Six test tubes labelled 10^{-1} to 10^{-6} were then filled with 9 ml of Ringers solution (used as diluent) after which 1 ml of the mixture was pipetted into the first tube labeled 10^{-1}. The 10^{-2} dilution was subsequently obtained by taking 1ml of the 10^{-1} dilution solution into the 10^{-2} labeled tube and the rest of the serial dilutions were similarly done.

Using a fresh sterile pipette tip for each dilution, 1 ml each of the six dilutions was aseptically added to six test tubes containing molten plate count agar at 45 ^{o}C. This was mixed by rotating the tubes between the palm taking care to avoid the formation of bubbles. The agar was then aseptically poured into fresh petri dishes, which were labeled 10^{-1} to 10^{-6}, allowed to solidify and incubated in inverted positions at 37 ^{o}C for 24 h and an electronic colony counter (Fisher 2CB) was used to count the colonies formed.

3.2.5.1 Shelf life Data Analysis

The results obtained for the shelf life analysis (peroxide value, deterioration of the greenish yellow colour and microbial load) were loaded into the Statsgraphics Centurion software (Stat point, 2008) and analyzed by fitting the data to models and studying their polynomial regression. The polynomial order was deemed adequate where the largest regression- (R^2), statistics (interpreted as the percentage of the variability in the response variable), which has been explained by the model and corresponding to a p-value (0.05) of the highest order term of the polynomial. At this point it was considered that the highest order term was statistically significant at the 95 % confidence level. The regressions were therefore subsequently used to calculate the shelf-life of the product. The activation energy (Ea) (or the deterioration energy) of

the fruit spread, which represented the energy that must be overcome in order for spoilage reactions to occur, was also calculated by first determining the Q_{10} according to the Labuza (1984) procedures.

i) Determination of Q10 according to Labuza method

A simple way to express this acceleration is to use the Q_{10} concept (Labuza, 1984). The parameter Q_{10} is the increase in the rate of the reaction when the temperature is increased by 10 ^{o}C (18°F). van Boekel (2005) indicated that the Q_{10} parameter shows the temperature dependence of a reaction as the factor by which the reaction rate is changed when the temperature is increased by 10 ^{o}C. This value can be calculated from the data of most storage tests where the product has been stored at two or more temperatures regardless of whether or not they are 10 °C apart.

Using the formula;

$$Q_{10} = \frac{Shelf - life\ [T°\text{C}]}{Shelf - life\ [T\ \ + 10°\text{C}]}$$

proposed by Labuza (1979), the Q_{10} parameter was calculated for the peroxide and colour values (Appendix B6) since these two parameters constitute the chemical reactions. The values obtained were then used to calculate for the activation energy for the two parameters (Appendix B7).

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Fitting the model for the sensory parameters

A summary of the statistics of the regressions fitted showed the standard deviation, the R^2 and adjusted R^2, predicted R^2 and the predicted residual error sum of squares (PRESS). These were the indicators of how well the sensory models fitted the data. For goodness-of-fit, low standard deviations, R^2 near 1 and relatively low PRESS were desired (Design-Expert, 2007).

For colour (Table 5), the predicted R-squared of 0.36 was in reasonable agreement with the adjusted R-squared of 0.51 therefore the regression model for colour was significant ($p= 0.0001<0.05$). However, since there were negative predicted R-squared values for taste (-0.22), aroma (-0.01), spreadability (-0.13) and finger feel (-0.38), it implied their regression models were not significant ($p>0.05$) and this implied the overall means were better predictors of the sensory responses. According to Myers and Montgomery (2000), adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable; therefore the ratios of 9.31, 4.15, 5.45, 4.74 and 4.21 obtained for colour, taste, aroma, spreadability and finger feel respectively indicate an adequate signal suggesting that their models could be used to navigate the design space.

Table 5: A summary of the statistics of the analysis of variance for the sensory parameters

	Standard deviation	**Mean**	**PRESS**	**R-Squared**	**Adj R-Squared**	**Pred R-Squared**	**Adeq Precision**

Colour	0.11	0.50	0.98	0.62	0.51	0.36	9.31
Taste	0.15	0.56	1.96	0.28	0.08	-0.22	4.15
Aroma	0.11	0.53	1.09	0.41	0.24	-0.01	5.45
Spreadability	0.16	0.60	2.10	0.33	0.15	-0.13	4.74
Finger feel	0.11	0.70	1.11	0.19	-0.04	-0.38	4.21

Since the R^2 for all the responses were significantly below 1 and the differences between the adjusted R^2 and the predicted R^2 were greater than 0.2 (Myers and Montgomery, 2000), it presupposes that the means of the sensory parameters were better predictors rather than their response regressions. Therefore it was not necessary to generate any response models but rather the mean plots for the sensory parameters.

Table 6 describes the F-values and p-value prob >F of the sensory parameters. For colour, the model F-value of 5.78 implies the model is significant and that there is only a 0.01 % chance that a model F-value this large could occur due to noise. For the taste, however, the model F-value of 1.39 implies the model is not significant and there is a 19.2 % chance that a model F-value this large could occur due to noise. On the other hand, taking aroma into consideration, the model F-value of 2.43 implied the model is significant and that there is only a 1.1 % chance that a model F-value this large could occur due to noise. Similarly for spreadability, the model F-value of 1.77 implies there is a 7.14 % chance that a model F-value this large could occur due to noise. The model F-value of 0.81 for finger feel implies the model signal is not significant relative to the noise and that there is a 65.50 % chance that a model F-value this large could occur due to noise.

Table 6: A summary of the F-values and p-values of the sensory parameters

Sensory Parameter	**F-values**	**p-values**

Colour	5.78	0.0001
Taste	1.39	0.192
Aroma	2.43	0.011
Spreadability	1.77	0.0714
Finger feel	0.81	0.6550

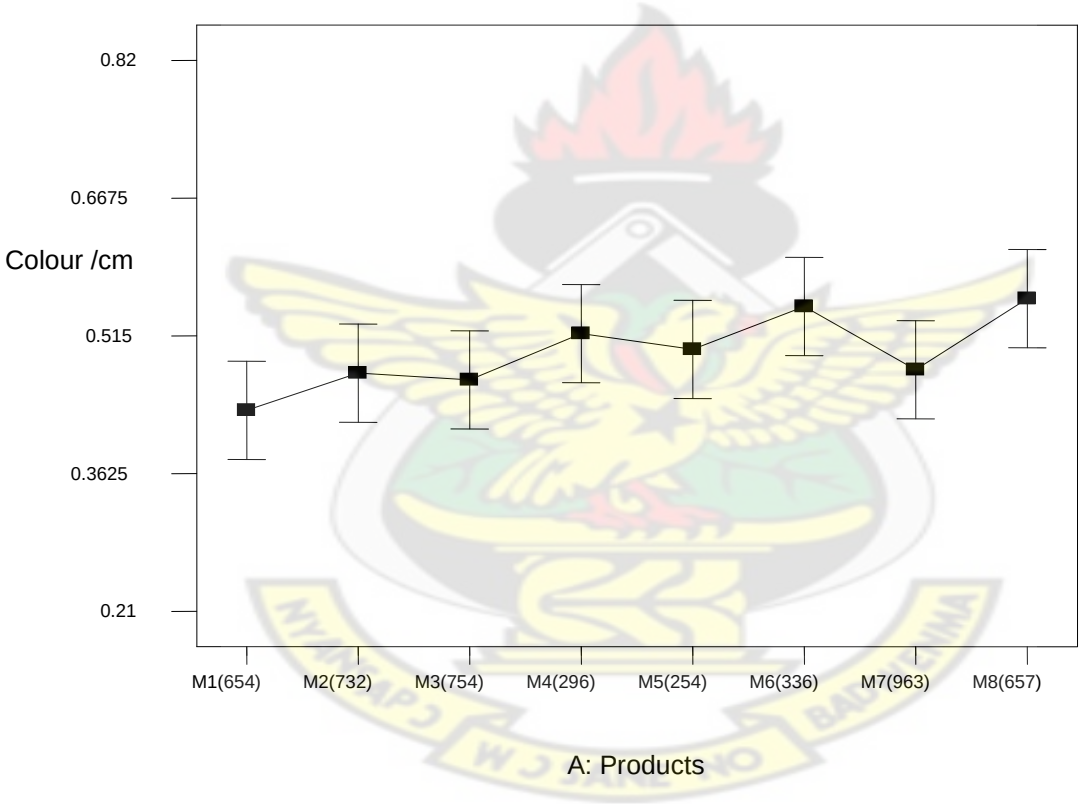


Figure 2: Measurements of the yellowish-green colour of avocado fruit spread evaluated by panellists and calculated as the means and displayed using confidence intervals.

From Figure 2, the means for colour ranged between 0.36 and 0.67 on a scale of 0.0 - 1.0 cm and they were evenly distributed. The average scores for samples M1 (654) and M6 (336) and sample

M1 (654) and sample M8 (657) were statistically significant ($p<0.05$). However, the rest of the products did not show any significant differences ($p>0.05$). In terms of the yellowish-green colour of the avocado fruit spread, the panelists rated sample M8 (657) as the best and sample M1 (654) as the least acceptable. The implication is that sample M8 (657) retained most of the yellowish-green colour of the avocado fruit after processing. This could be so probably because the quantities of the anti-oxidants (citric acid and ascorbic acid) and xanthan gum used significantly reduced the deterioration of the yellowish-green colour of the avocado fruit spread.

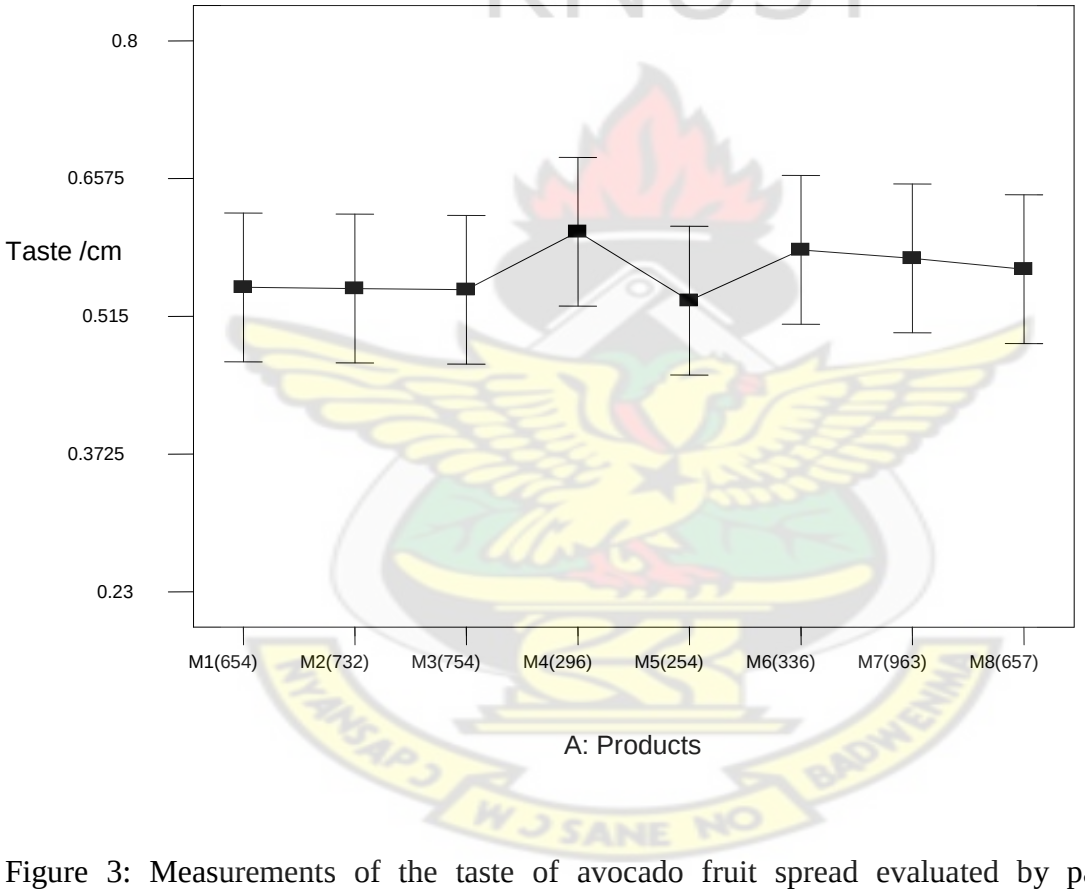


Figure 3: Measurements of the taste of avocado fruit spread evaluated by panellists and calculated as the means and displayed using confidence intervals.

The average scores for taste (Figure 3) ranged between 0.52 and 0.66. In general, there was no significant difference among all the product samples. The scores for the first three samples M1 (654), M2 (732) and M3 (754) approximately remained constant. There was a gradual decrease in the scores from sample M6 (336) to sample M8 (657). Sample M5 (254) and sample M4 (296)

were respectively rated as the least preferred and most preferred in terms of taste by the panelists but the values were however statistically insignificant as indicated in Figure 3.

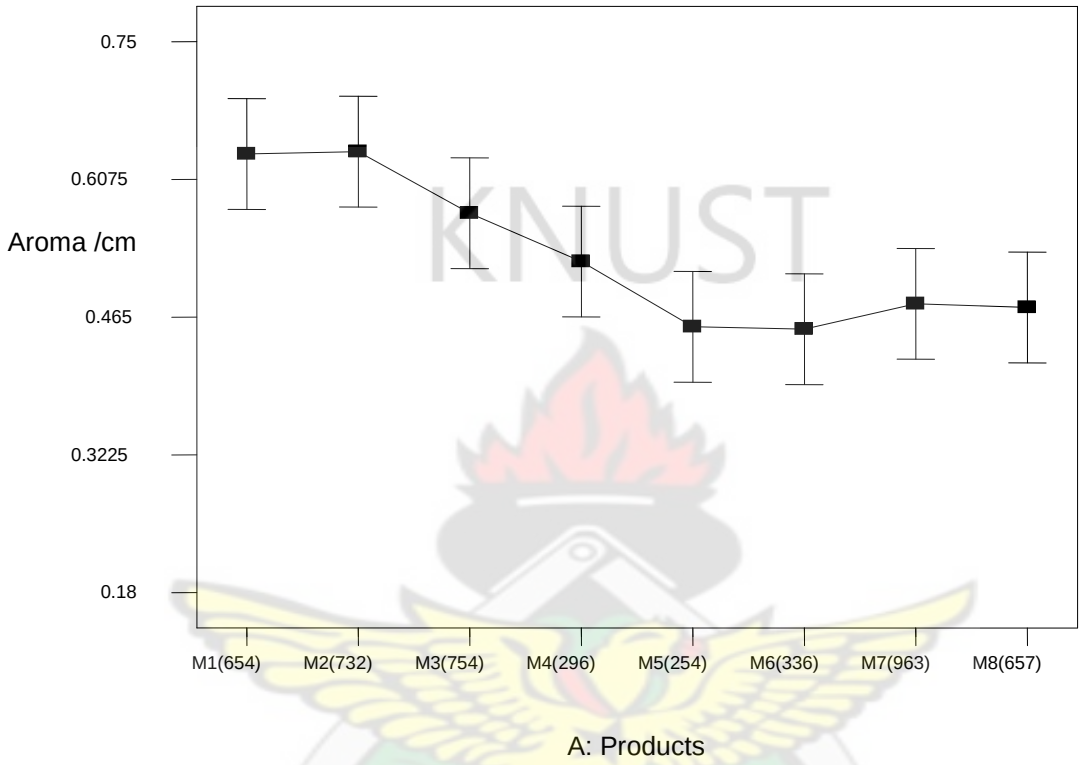


Figure 4: Measurements of the aroma of avocado fruit spread evaluated by panellists and calculated as the means and displayed using confidence intervals.

The average scores for aroma had a wide range from 0.47 to 0.75. There was a general decrease in the average scores from sample M2 (732) to sample M8 (657). Sample M2 (732) was rated as the best in terms of aroma. Sample M6 (338), on the other hand, received the lowest rating by the panelists in terms of its aroma as shown in Figure 4. Samples M1 (654) and M2 (732) was significantly different compared to samples M5 (254) to M8 (657), however, the rest of the products did not show any significant differences.

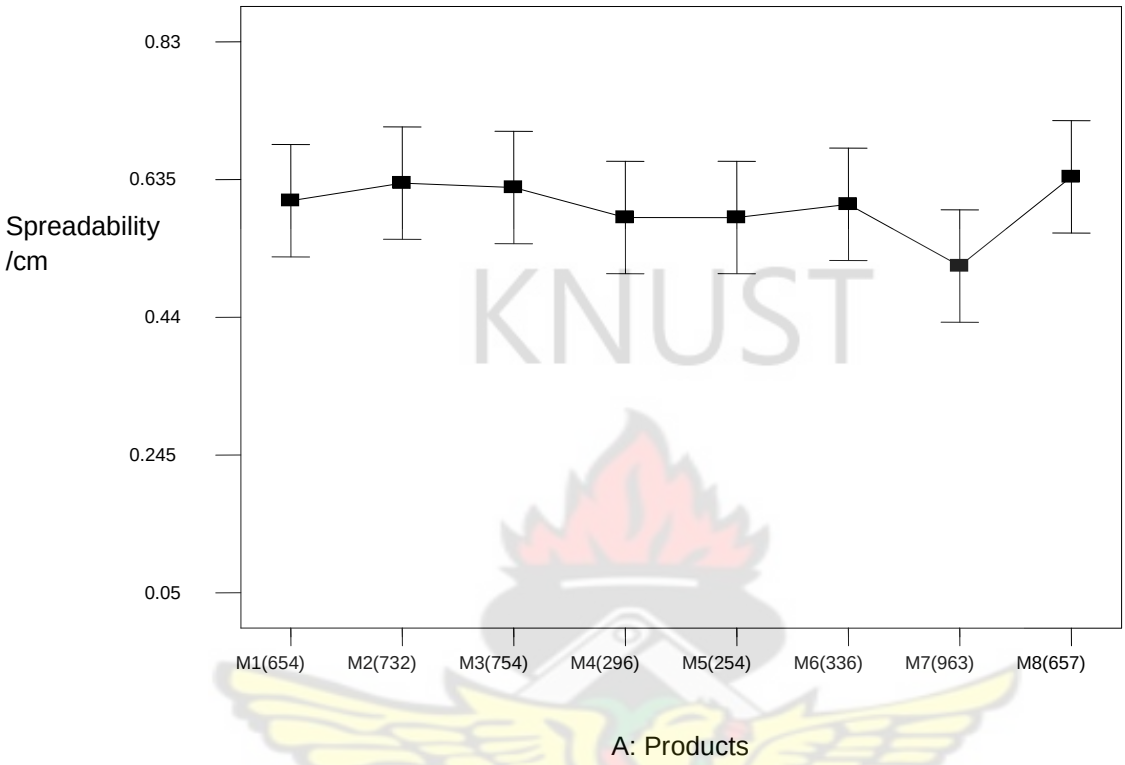


Figure 5: Measurements of the spreadability of avocado fruit spread evaluated by panellists and calculated as the means and displayed using confidence intervals.

As shown in Figure 5, the average scores for spreadability ranged between 0.44 and 0.83. Sample M7 (963) was adjudged the least spreadable by the panelists as against sample M8 (657) being the best in terms of spreadability. However, there was no significant difference in the products in terms of spreadability.

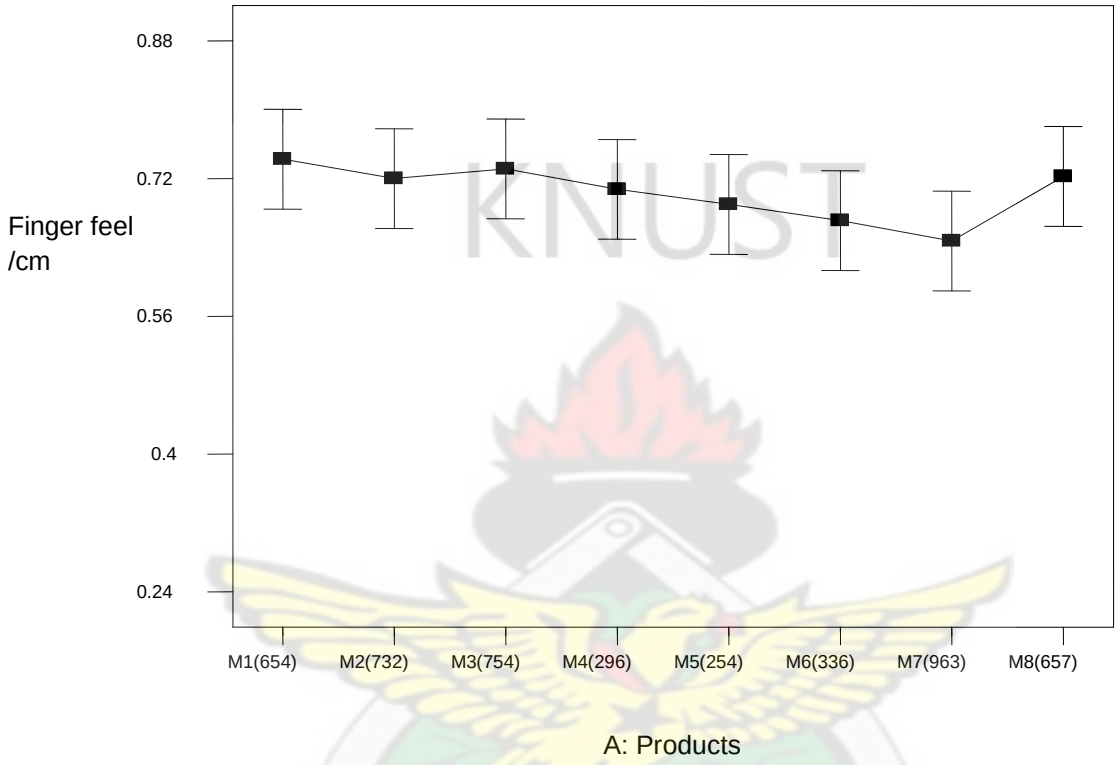


Figure 6: Measurements of the finger feel colour of avocado fruit spread evaluated by panellists and calculated as the means and displayed using confidence intervals.

As indicated in Figure 6, the range for the average scores for finger feel was between 0.72 and 0.88. There was a gradual score decrease from sample M1 (654) to sample M7 (963) after which it increased sharply in sample M8 (657). Sample M1 (654) was adjudged the most preferred according to the panelists, whilst sample M7 (963) was the least. There was no significant difference between the samples in terms of finger feel.

Producing avocado fruit spread with the preferable sensory parameters that maintained the nutritional properties were the principal aims of this project. In order to achieve the aims, the sensory parameters were constrained as shown in Table 7. For colour, the constraint was set at midpoint value of 5.2 representing the yellowish-green colour of avocado fruit. The constraints for the other five sensory parameters were set at maximum since the optimum characterisics of these sensory parameters were desired.

Table 7: A constraint table showing responses and the goals set for optimizing the avocado fruit spread

NAME	GOAL	LOWER LIMIT	UPPER LIMIT
Colour	target = 0.52	0.21	0.82
Taste	maximize	0.23	0.80
Aroma	maximize	0.18	0.75
Spreadability	maximize	0.05	0.83
Finger feel	maximize	0.24	0.88

The lower and upper limits described the smallest and highest scores assigned to each of the sensory parameters by the 8 panelists. The lower and upper limits for the products M1(654) and M8(657) respectively. The scores for the lower limits of the sensory parameters ranged between 0.05 for product spreadability to 0.24 in its finger feel. The upper limits had scores ranging from 0.75 in product aroma to 0.88 in finger feel. At the end of the sensory evaluation optimisation process, sample M2(732) was rated the best. Sample M2(732) which has composition of 50 g avocado puree, 5 g sugar, 9 g miscellaneous and 0.5 g xanthan gum was thus formulated and used for the rest of the project.

4.2 Proximate analysis of avocado fruit and the selected avocado fruit spread

Results of proximate analysis are shown in Table 8.

Table 8: Proximate analysis and the energy values of raw avocado fruit and the selected avocado fruit spread determined on a 100 g basis.

Parameters	Raw Avocado	Avocado Spread
Moisture /g	72.14±0.17	60.82±0.24
Fat /g	15.83±0.45	10.08±0.36
Total Carbohydrate /g	5.34±0.13	20.12±0.19
Crude Fibre /g	3.95±0.07	6.52±0.16
Crude Protein/g	1.72±0.06	1.39±0.07
Ash /g	0.97±0.03	1.87±0.04
Energy Value /kcal	170.93±0.66	176.76±0.79

The raw avocado fruit and the avocado fruit spread contained high amounts of moisture. High moisture content favours the growth and proliferation of microorganisms as reported by Adegoke *et al.,* (1992) thus reducing the shelf life of the raw avocado and the avocado fruit spread. The moisture content of the raw avocado fruit, 72.14 g/100 g, was comparable to that obtained by (74.20 %) Pamplona-Roger, (2007) and other fruits such as raw garden egg 73.46 % (Akaninwor and Arachie, 2002), banana (71.00 g), medlars (74.50 g) and passion fruit (73.30 g) (Food Standards Agency (FSA), 2007). The moisture content of the raw avocado fruit was however higher than the value of 69.00 g reported by FSA (2007) for raw avocado, and 68.84 % for banana and 66.36 % for avocado pear reported by Akaninwor and Arachie (2002). In general, most fruits contain about 75.00 % moisture or more with the seeds having much less moisture (Hawthorn, 1981; Pamplona-Roger, 2007).

The lower moisture content of the avocado fruit spread compared with the raw avocado may be due to the addition of other solutes such as sugar, salt and the gum. Prescott *et al.,* (2002) reported that by adding solutes, water can be made less available. The water content or moisture of a food affects its physical as well as chemical properties such as the structure, appearance and

taste of the food product. These properties become important in determining the food's susceptibility to spoilage, shelf life and the processing and packaging conditions required.

Fat is one of the major constituents of both the raw avocado and its spread. In the raw avocado, the fat content was 15.83 g and that of the avocado spread was 10.08 g. Earlier reports on fat contents have shown that fruits such as avocado and coconut have high levels of fat whilst those of banana, pawpaw and mango are on the low side (Ihekoronye and Ngoddy, 1985; Rice *et al*., 1986; Akaninwor and Arachie, 2002; Pamplona-Roger, 2007; Afolabi, 2008). The fat content of the avocado fruit spread was similar to that reported by the United States Department of Agriculture (USDA) (2005) nutritional database for guacamole but lower than that of salad cream, peanut butter, margarine (including planta margarine) and cheddar cheese (FSA, 2007). Morton (1987) also reported that avocado has high lipid content - from 5 to 25 % - depending on the cultivar. Nevertheless, the fat content of the fruit spread was significantly reduced compared to the fresh avocado fruit.

The carbohydrate content was 20.12 g in the avocado fruit spread and 5.34 g in the raw fruit indicating that the addition of sugar and gum resulted in significantly higher carbohydrate content in the fruit spread. Akaninwor and Arachie (2002) reported that the carbohydrate content of most fruits and seeds gave values ranging from 2.97 g to 4.00 g. FSA (2007) reported negligible values for carbohydrates in both butter and margarine. It can be implied from the above that the avocado fruit spread is a good source of carbohydrate.

The crude fibre content of the raw fruit was found to be 3.95 g. This was comparable to that obtained by FSA (2007) for banana and guava, which were 3.4 g and 3.6 g respectively. This value was also higher than the values reported by FSA (2007) and Morton (1987) for raw avocado fruit. In the fruit spread, the fibre content increased to 6.52 g and this can be explained by the addition of the gum. This is similar to the value of 6.6 g for guacamole reported by the USDA (2005). High dietary fibre of the fruit indicated the presence of reasonable quantity of trapped water because bound water can be held by the hydrophilic polysaccharides of the fibre. This water is unavailable and has very low chemical activity.

The crude protein content gave generally low values – 1.72 g for the raw fruit and 1.39 g for the fruit spread. Fruits have been reported to provide comparatively little protein (Hawthorn, 1981). It can thus be deduced that the avocado fruit spread is a poor source of protein and should be used in conjunction with other foods rich in protein.

As shown in Table 8, the ash content was 0.97 g in the raw fruit and 1.87 g in the processed product. Morton (1987) reported ash in raw avocado ranges from 0.46 to 1.68 g. The relatively higher ash value in the processed product in this project might be as a result of the salt, sodium benzoate and potassium sorbate.

Since both the raw fruit and the processed products had high food energy, they could be used to supplement the daily energy intake of consumers (Nkafamiya *et al.*, 2007).

4.3 Shelf life characteristics of the preferred avocado fruit spread

Shelf life represents the period of time through which food products remain safe to eat and retain their essential sensory properties and comply with the label's nutritional declarations (Steele *et al.*, 2006; Doughari *et al*., 2007**).** Many products have a limited shelf life because as soon as they are produced, changes in their wholesomeness begin to occur and after some period, the product loses its wholesomeness and therefore must be removed from the shelf (Prescott *et al.*, 2002). Labuza (1982) explained that the principal mechanisms involved in the deterioration of processed foods are related to microbiological spoilage sometimes accompanied by pathogen development, chemical and enzymatic activities causing breakdown of lipid, colour, odour, flavour and texture and finally moisture and or vapour migration producing changes in texture, water activity and flavour.

Polhemus (2005) deduced that in predicting shelf life, the largest number of weeks for which the degrading parameter has reached 90 % must be calculated. The degrading parameters in this case were microbial counts, peroxide values and natural yellowish-green colour intensity.

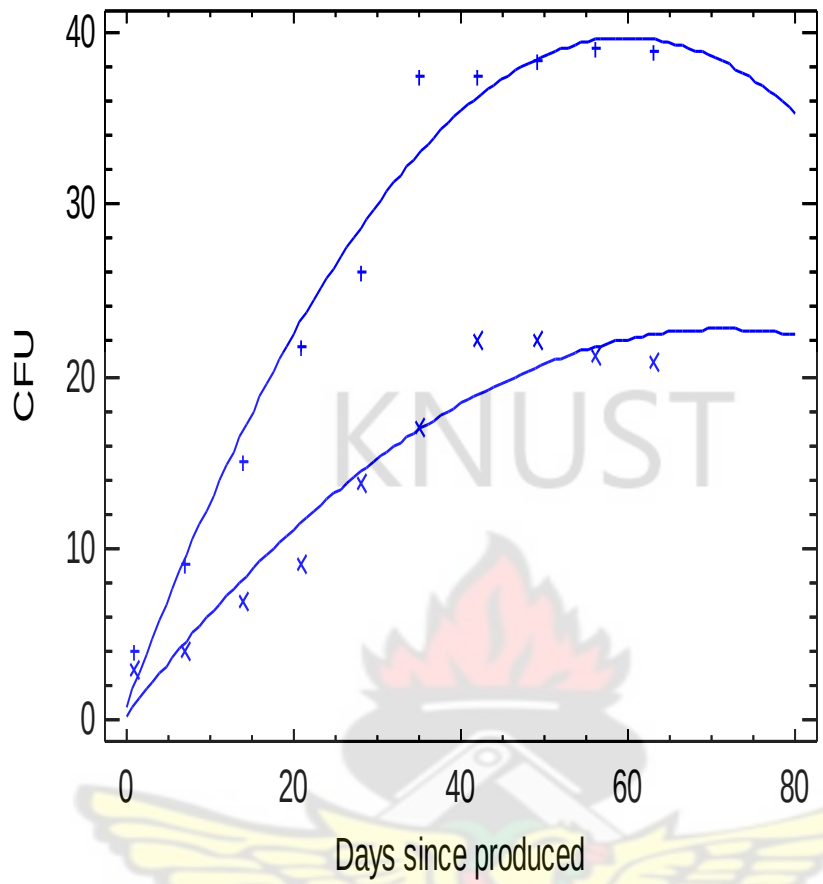
4.3.1 Microbial load of avocado fruit spread

Tables 9 and 10 (Appendix C3) show the total viable count (TVC) for the samples stored at the two different temperatures, refrigeration and ambient conditions. The growth pattern followed the normal microbial growth curve (Prescott *et al*., 2002). There was a steady rise in the microbial counts as the days increased until it tapered off and subsequently declined (Figure 8) for the two curves studied. As expected, the refrigerated samples deteriorated slowly compared to the samples stored at ambient conditions which deteriorated rapidly. Mathematically, the models for the deterioration of the avocado fruit spread followed a quadratic equation (1) with R^2 value of 0.989 for the sample stored under ambient conditions, but followed a polynomial of the 3rd order (2) with R^2 value of 1 for the one stored at refrigeration condition:

$$y = -0.50x^2 + 9.8x - 7.71 (1)$$

$$y = -0.089x^3 + 1.28x^2 - 2.22x + 4.05 (2)$$

The Australian/New Zealand Food Authority Guide to the Food Safety Standards, ANZFA (2001) sets the limit for total viable count for packaged fruit and vegetable products at 100 cfu/ml. Therefore 90 % of the 100 cfu/ml limit set by the ANZFA is 90 cfu/ml. But from Figure 7, the shelf life was deduced from the point at which the curve tapered off since the growth was no longer following a linear progression hence the 90 cfu/ml recommended could never be reached. Following from the Polhemus (2005) deduction, the shelf life was determined at a cfu/ml value of 90 % of tapering off at 38 total cfu/ml. This was calculated to be 34.2 total cfu/ml. Using the *locate* button in the Statsgraphic Centurion to locate 34.2 cfu/ml on the graph gave a shelf life of 37.49 days (5.35 weeks). For the refrigerated samples, the curve tapered off at 22.8 total cfu/ml; 90 % of which gave a total cfu/ml of 20.52 and a shelf life of 47.50 days (6.78 weeks).



Key: , +—+ = ambient, x—x = refrigerated

Figure 7: Polynomial regression of total cfu/ml at ambient and refrigerated conditions and days since produced.

4.3.2 Peroxide value of avocado fruit spread

Peroxide value (PV) measures the formation of intermediate hydroperoxides in milliequivalents of active oxygen per kilogram of sample. Fresh oils have values less than 10 mEq/kg (Pearson, 1970) and values below 10 characterized the majority of conventional food grade oils (Codex Alimentarius, 1993).

Tables 11and 12 in Appendix C3 represented the peroxide values obtained for the 63 days that the samples were under observation. It was observed that as the number of days of storage increased, there was a rise in peroxide values with a steady rise for samples stored under ambient

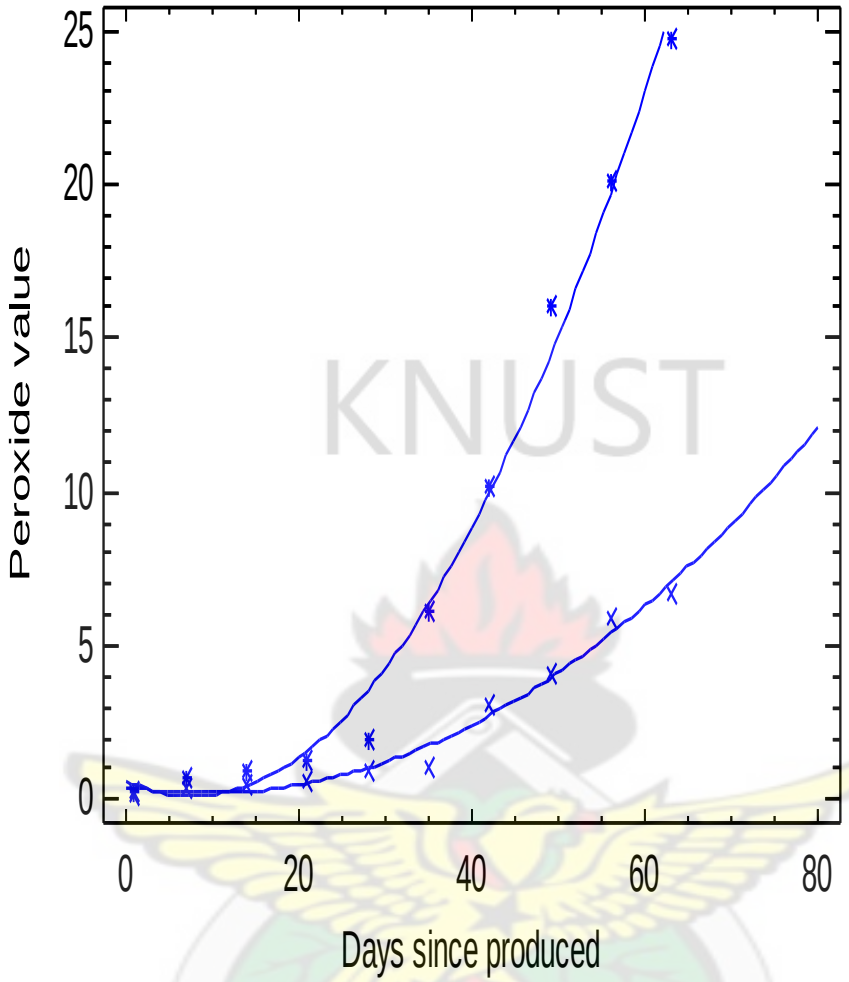
conditions and a slower gradual increase for samples stored under refrigeration conditions. The data obtained from this project were to be expected since the rate of increase of peroxide concentration for the refrigerated samples were lower than the rate of increase for the ambient samples (Figure 8). Pearson (1970) stated that a rancid taste was noticeable when the peroxide value was between 20 and 40 mEq/kg in oily products but based on the data obtained from this project, the refrigerated and ambient samples for this project the limit for rancidity was pegged at 10 mEq/kg since a value of 10 was a reasonable point where the two curves could be well compared. Furthermore, based on Polhemus (2005) deductions; 90 % of 10 mEq/kg that equaled 9 mEq/kg was used as the limit. Then using the Statsgraphics Centurion *locate* button to locate 9 mEq/kg gave a shelf life of 70.15 days (10.02 weeks) and 40.26 days (5.75 weeks) for refrigerated and ambient samples, respectively.

The rise in peroxide values as a measure of the shelf stability followed a quadratic regression equation for samples stored under both ambient (3) and refrigeration (4) conditions:

$$y = 0.4089x^2 - 1.6958x + 1.824 \quad (3)$$

$$y = 0.1158x^2 - 0.5321x + 0.8087 \quad (4)$$

R^2 values of 0.992 and0.985 were recorded for samples stored under both ambient and refrigeration conditions respectively.



*Key *—* = ambient, x—x = refrigerated*

Figure 8: Polynomial regression of peroxide value at ambient and refrigerated conditions and days since produced

The shelf life determined in this research was 70.15 days when the spread was refrigerated as against 40.26 days for spreads kept at ambient conditions. Maskan *et al.,* (2007) has evaluated the shelf life of margarine samples coded as A and B as 145 and 134 days respectively using a similar parameter as peroxide value but the values determined in this project were much lower, probably due to the lower stability of the unsaturated fatty acids and lower antioxidants content.

4.3.3 Colour of avocado fruit spread

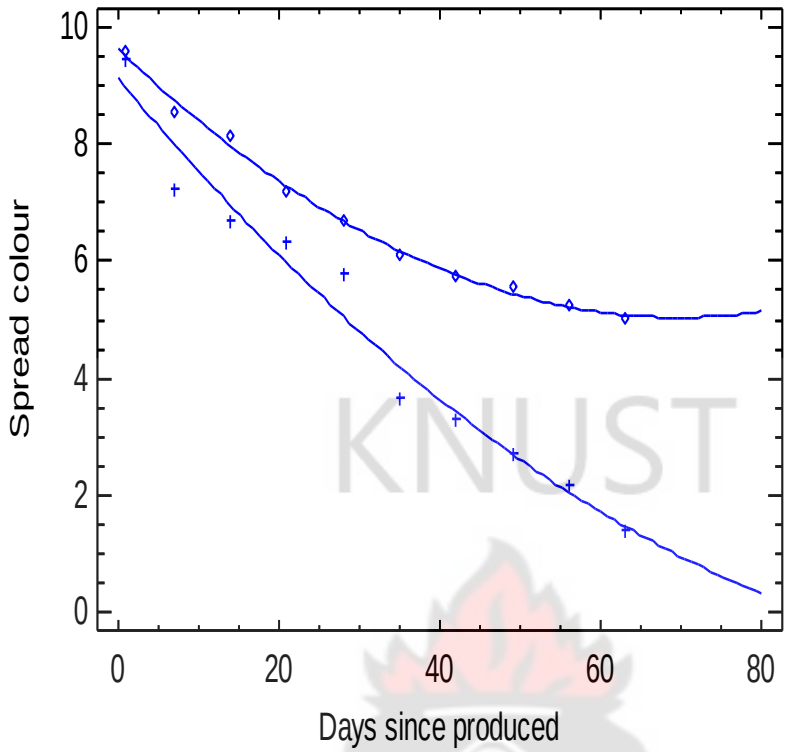
Tables 13 and 14 in Appendix C3 show the values for colour obtained for the 63 days that the samples were under observation. The study indicated that as the number of days increased, the yellowish-green colour of the avocado fruit spread generally decreased (Figure 9). The rate of decrease followed the same trend as in peroxide and microbial determinations. The scale used during the sensory descriptive test for colour determination ranged from 1 to 10, indicating colour shades from yellow to green. For this study, the midpoint of the highest colour intensity was used in calculating for the shelf life reflecting the yellowish green colour of the avocado flesh. The highest value for the two curves from the study was 9.5, half of which gave 4.75. With reference to Polhemus (2005) deduction for shelf life determination, 90 % of 4.75 gave 4.275, which eventually gave a shelf life of 69 days (9.86 weeks) and 30.1 days (4.3 weeks) for refrigerated and ambient samples respectively.

The deterioration of the yellowish-green colour of the avocado fruit spread followed a linear regression for samples stored under ambient conditions (5) but followed a quadratic equation for samples stored under refrigeration conditions (6):

$$y = -0.8388x + 9.4907 \quad (5)$$

$$y = 0.0431x^2 - 0.9708x + 10.459 \quad (6)$$

For colour, the samples stored under ambient conditions and those stored under refrigeration conditions both had R^2 value of 0.999.



Key: +—+ = ambient, ◦—◦= refrigerated

Figure 9: Polynomial regression of yellowish-green spread colour at ambient and refrigerated condition and days since produced

Taoukis *et al.,* (2001) determined the shelf life of tomato ketchup as 260 days in glass at 30 ^{o}C but the shelf life reduced to 150 days at 40 ^{o}C. The retention of the inherent colour of fresh vegetables is often used as a quality indicator and has a substantial impact on consumer acceptance (Roura *et al.,* 2000). The green and violet colours of most vegetables are contributed by chlorophyll and anthocyanins (Weichmann, 1987; Lazcano *et al.,* 2001); therefore the degradation of colour in the avocado fruit spread could most likely be related to the degradation of chlorophyll, which often has a higher degradation rate at higher temperatures and availability of oxygen compared to lower temperatures (Taoukis *et al.,* 2001). The presence of stronger anti-browning agents such as cysteine and food colouring agents could mitigate these occurrences in commercial ventures.

The resulting shelf lives for the peroxide value, spread colour and total colony forming units at ambient and refrigerated temperatures were as recorded in the Table 15.

Table 15: Shelf lives of the responses total colony forming units, peroxide value and spread colour at ambient and refrigerated conditions recorded as days since produced

RESPONSES	DAYS SINCE PRODUCED
Total CFU/ML(Ambient)	37.49
Total CFU/ML(Refrigerated)	47.50
Peroxide Value (Ambient)	40.26
Peroxide Value (Refrigerated)	70.15
Spread Colour (Ambient)	30.10
Spread Colour (Refrigerated)	69.00

4.4 CALCULATION OF Q_{10} AND ACTIVATION ENERGY OF THE CHEMICAL REACTIONS (COLOUR AND PEROXIDE VALUE)

Richardson and Finley (1985) deduced that chemical deterioration of packaged food products can be grouped into three mechanisms; non-enzymatic browning, enzymatic degradation and oxidation of lipids, and that all three can take place concurrently in food systems and are accelerated to some degree by rising storage temperatures. Chemical deterioration reactions require a certain amount of energy to get started. This is called Activation Energy (Ea), which is the energy barrier that molecules need to overcome to be able to react, measured in kcal/mol (Petrou *et al*., 2002; van Boekel, 2007). The higher the activation energy is for a reaction, the greater the acceleration with increases in temperature.

For peroxide the Q_{10} calculated from the shelf life values obtained for peroxide for samples stored under refrigeration (5 ^{o}C) and ambient (26.5 ^{o}C) conditions gave 1.74 calculated as shown in Appendix B6.

The activation energy was then calculated for the products using the formula derived by Hough *et al*., (2006) in the determination of the shelf life of *ricotta* cheese. The temperature in Kelvin for the avocado spread stored at 5 ^{o}C is T (^{o} K) = 278 ^{o}K and that for the product stored at 26.5

^{o}C (average of 25-28 ^{o}C) is [T (^{o} K) + 10] = 296 ^{o}K. Then using the Q_{10} value, Ea value of 8.80 kcal/mol was obtained (Appendix B6).

For the shelf life values obtained for colour stored under the two temperature conditions, a Q_{10} value of 2.29 was achieved (Appendix B7). Ea value of 13.17 kcal/mol was obtained for colour as shown in Appendix B7. Q_{10} and Ea values for peroxide and colour are shown in table 16.

Table 16: Q_{10} and Ea for peroxide and avocado fruit spread colour responses

Responses	**Days since produced**	**Q_{10}**	**Ea : Kcal/mol**
Peroxide Value (Refrigerated)	40.26	1.742	8.80
Peroxide Value (Ambient)	70.15		
Spread Colour (Refrigerated)	69.00	2.29	13.17
Spread Colour (Ambient)	30.10		

CHAPTER 5

5.0 CONCLUSION

A two-level factorial was used to formulate avocado fruit spread using four factors at specific levels as: 50 g of avocado pulp, 5 g of sugar, 9 g miscellaneous and 0.5 g of the xanthan gum. Sample M2(732) which was the most preferred had its responses evaluated as: Colour (0.52), Taste (0.64), Aroma (0.71), Spreadability (0.72) and Finger feel (0.80).

For the microbial load, the shelf life was determined to be 47.50 days and 37.49 days for the samples stored at refrigeration and ambient conditions, respectively. For the peroxide value, the shelf life was 70.15 days and 40.26 days for the samples at refrigeration and ambient conditions, respectively. The colour of the sample provided a shelf life of 69.00 days and 30.10 days for the products kept under refrigeration and ambient conditions, respectively.

For most food products, microbial load is the principal food safety concern. For this project, therefore, it was submitted that the shelf life should be contingent on the safety issue-microbial load-whilst the quality issues-peroxide values and colour-would guide the final/commercial presentation of the product.

From the results obtained, the shelf stability of the avocado fruit spread was determined to be 47.50 days under refrigeration conditions, which is significant considering the short shelf life of the fresh fruit.

Q_{10} and activation energy (Ea) values of 1.74 and 8.80 kcal/mol respectively were obtained for the chemical deterioration due to increasing peroxide values. For chemical deterioration due to decreasing colour intensity, values of 2.29 and 13.17 kcal/mol were obtained for Q_{10} and activation energy (Ea) respectively.

6.0 RECOMMENDATIONS

1. Colour enhancers and an improved combination of anti-oxidants, example cysteine can be used to extend the period within which the colour deteriorates. Methods of retaining the green colour due to chlorophyll could be studied such as replacing the magnesium with zinc.
2. The vitamin content and functional properties of the confectionery spread can also be determined as part of the nutritional content determination in relation to its functional and nutraceutical properties.
3. Water activity (a_w) can be measured as an additional approach of determining the shelf life of the product as water activity can affect growth of microorganisms and lipid oxidation.
4. Further studies could be mounted to observe the effect of opening and closing at refrigerating temperatures in a subsequent research.
5. A food product label as shown in Appendix D1 could be developed when the product is commercialized.

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APPENDIX

Appendix A1: Sensory Evaluation Product Attitude Survey

To match your preferences, usage and sensory skills to the samples to be evaluated, please complete the questionnaire. All information will be maintained confidential.

Name:……. Year:……

Tel.:……. Date:…….

Gender:……

Age: 20 – 25 ☐ 26 – 30 ☐ Over 30 ☐

Married ☐ Single ☐

Please indicate which, if any, of the following foods disagree with you (allergy, discomfort, etc):

Avocado (pear) ☐ Sugar ☐ Salt ☐ Flavour ☐

Emulsifying agents ☐ Vinegar ☐ Preservatives ☐

Please indicate if you are on a special diet:

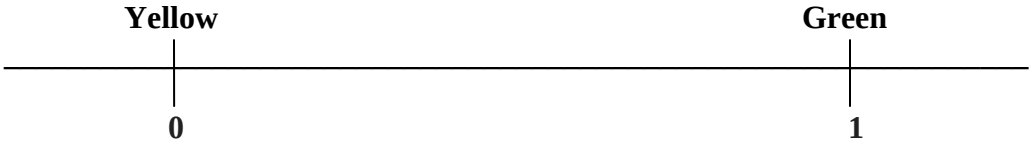
Diabetic ☐ Low salt ☐ High Calorie ☐ Low calorie ☐

No special diet ☐ Others (specify) …….

Appendix A2: Graphic Scale for Sensory Evaluation

SAMPLE No. ….

1. Colour

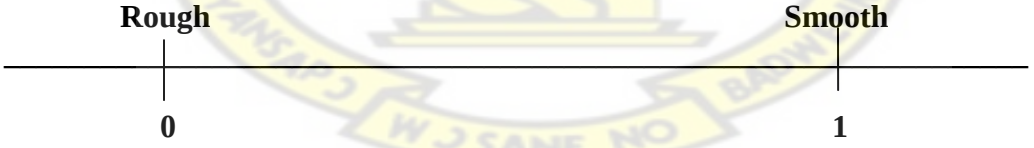


2. Taste

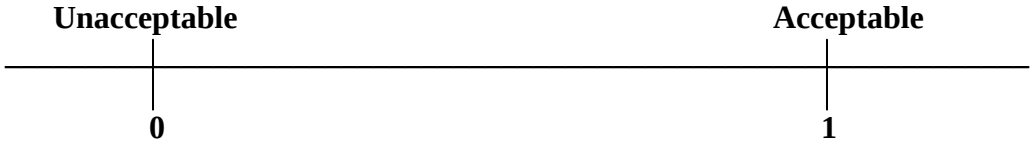
3. Aroma

4. Spreadability

5. Finger feel



6. Overall acceptability



Appendix A3: Research design for serving order

	Factor 1	Factor 2
Run	A:Products	B:Panelist
1	M1(654)	P1
2	M2(732)	P1
3	M3(754)	P1
4	M4(296)	P1
5	M5(254)	P1
6	M6(336)	P1
7	M7(963)	P1
8	M8(657)	P1
9	M1(654)	P2
10	M2(732)	P2
11	M3(754)	P2
12	M4(296)	P2
13	M5(254)	P2
14	M6(336)	P2
15	M7(963)	P2
16	M8(657)	P2
17	M1(654)	P3
18	M2(732)	P3
19	M3(754)	P3
20	M4(296)	P3
21	M5(254)	P3
22	M6(336)	P3
23	M7(963)	P3
24	M8(657)	P3
25	M1(654)	P4
26	M2(732)	P4
27	M3(754)	P4
28	M4(296)	P4
29	M5(254)	P4
30	M6(336)	P4
31	M7(963)	P4
32	M8(657)	P4
33	M1(654)	P5
34	M2(732)	P5
35	M3(754)	P5
36	M4(296)	P5
37	M5(254)	P5
38	M6(336)	P5
39	M7(963)	P5
40	M8(657)	P5
41	M1(654)	P6
42	M2(732)	P6
43	M3(754)	P6
44	M4(296)	P6
45	M5(254)	P6
46	M6(336)	P6
47	M7(963)	P6
48	M8(657)	P6
49	M1(654)	P7
50	M2(732)	P7
51	M3(754)	P7
52	M4(296)	P7
53	M5(254)	P7
54	M6(336)	P7
55	M7(963)	P7
56	M8(657)	P7
57	M1(654)	P8
58	M2(732)	P8
59	M3(754)	P8
60	M4(296)	P8
61	M5(254)	P8
62	M6(336)	P8
63	M7(963)	P8
64	M8(657)	P8

Appendix B1: Formula used for Moisture determination

$$\frac{W_2 - W_3}{W_2 - \mathrm{W}_1} \times 100$$

where:
W_1 is the weight of dish
W_2 is the weight of dish and wet sample
W_3 is the weight of dish and dried sample.

Appendix B2: Formula used for Protein determination

$$\frac{100 \times (V_{A-}V_B) \times N_A \times 0.01401 \times 100}{W \times 10}$$

where:
V_A is the volume in ml of standard acid used in titration
V_B is the volume in ml of standard acid used in blank
N_A is the normality of acid (HCL) and
W is the weight in grams of the sample.

Appendix B3: Formula used for Crude fat determination

$$\frac{Weight\ of\ flask\ and\ fat - Weight\ of\ flask}{2} \times 100\%$$

Appendix B4: Formula used for Crude fibre determination

$$\frac{(X - Y)}{W} \times 100$$

where:
X is the weight of crucible and dried sample before ashing
Y is the weight of the crucible and sample after ashing
W is the weight of the sample used in the fat determination.

Appendix B5: Formula used for Ash determination

$$\frac{C - A}{B - A} \times 100$$

where:
A is the weight of the crucible
B is the weight of crucible and raw sample
C is the weight of crucible and dried sample.

Appendix B6: Formula used for Q_{10} and Ea calculation for peroxide value

$$Q_{10} = \frac{\text{Shelf-life } [T°\text{C}]}{Shelf - life[T \ + 10°\text{C}]}$$

$$Q_{10} = \frac{10.02\ weeks}{5.75\ weeks}$$

$$Q_{10} = 1.74$$

$$\text{Ea} = \log(Q_{10}) \times \text{T}(^{\text{o}}\text{K}) \times [\text{T}(^{\text{o}}\text{K}) + 10] \times 4.57 \times 10^{-4}$$

$$Ea = \log(1.74) \times 278 \times 288 \times 4.57 \times 10^{-4}$$

$$Ea = 8.80 kcal / mol$$

Appendix B7: Formula used for Q_{10} and Ea calculation for colour

For the shelf life values obtained for colour stored under the two temperature conditions, a Q_{10} value of 2.29 was achieved, calculated as:

$$Q_{10} = \frac{9.86\ weeks}{4.3\ weeks}$$

$$Q_{10} = 2.29$$

Ea value of 13.17kcal/mol was obtained for colour. This was calculated as:

$$E_a = \log(2.29) x 278 x 288 x 4.57 x 10^{-4}$$

$E_a = 13.17 \text{kcal /mol}$

Appendix C1: Formula used for Peroxide value determination

$$\frac{(V - V_0)T}{M} \times 10^3 mEq/Kg$$

where
V-V_0 is the titre value,
T is the exact molarity of the sodium thiosulphate solution
M is the mass in grams of the sample.

Appendix C2: Scale for Colour intensity measurement

Table 9: Results for total viable count of formulated pear spread stored at refrigeration conditions.

Days of sampling	**Total viable count per gram of sample stored under refrigeration conditions (5^0C)**			
	Sample A	Sample B	Sample C	Mean
Day1	3	2	4	3
Day7	4	5	3	4
Day 14	5	7	9	7
Day 21	8	9	10	9
Day 28	12	13.4	16	13.8
Day 35	16.3	16	19	17.1
Day 42	22	21	23	22
Day 49	22	21.1	23	22.1
Day 56	20	21	22.2	21.2
Day 63	20.2	20.5	22	20.9

Table 10: Results for total viable count of formulated pear spread stored under ambient conditions.

Days of sampling	**Total viable count per gram of sample stored under ambient conditions (20-23^{0}C)**			
	Sample A	Sample B	Sample C	Average
Day1	3	5	4	4
Day7	11	7	9	9
Day 14	17	15	13	15
Day 21	22	19	24	21.66
Day 28	22	23	27	26
Day 35	38	34	40	37.33
Day 42	38.2	34.1	40.2	37.5
Day 49	39	35	41.2	38.4
Day 56	41	36	40	39
Day 63	41	35.4	40	38.8

Table 11: Peroxide value of avocado spread stored under refrigeration conditions

DAYS OF SAMPLING/ TITRE VALUES	T 1	T2	T3	AVERAGE
Day1	0.2	0.2	0.2	0.2
Day7	0.4	0.4	0.4	0.4
Day 14	0.5	0.5	0.6	0.53
Day 21	0.5	0.6	0.6	0.57
Day 28	0.8	0.9	1.0	0.9
Day 35	1.0	1.0	1.1	1.03
Day 42	3.0	3.1	3.2	3.1
Day 49	3.9	4.1	4.2	4.07
Day 56	5.7	5.9	6.1	5.88
Day 63	6.4	6.9	6.8	6.7

Table 12: Peroxide values of avocado spread stored at ambient conditions

DAYS OF SAMPLING/ TITRE VALUES	T 1	T2	T3	AVERAGE
Day1	0.2	0.3	0.2	0.23
Day7	0.8	0.8	0.7	0.77
Day 14	1	0.9	1	0.97
Day 21	1.4	1.2	1.2	1.27
Day 28	1.9	1.9	2	1.93
Day 35	6.2	6.1	6.1	6.13
Day 42	10.1	10.2	10.3	10.2
Day 49	16	16.2	16	16.07
Day 56	20	20.1	20.1	20.067
Day 63	24.8	24.7	24.8	24.77

Table 13: Results for colour determination of avocado samples kept under refrigeration conditions

Days of sampling	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Panelist 5	Panelist 6	Panelist 7	Panelist 8	Mean
Day1	9.6	9.8	9	9.4	9.6	9.8	9.9	9.5	9.575
Day7	8.8	9	8.6	7.8	8.1	8.5	8.7	8.9	8.55
Day 14	8.3	8	8.4	8.1	7.6	8.2	8.4	8	8.125
Day 21	7.6	7.1	7.5	7.3	6.9	7.7	6.6	6.8	7.1875
Day 28	6.9	6.4	6.8	7	6.3	6.5	7	6.7	6.7
Day 35	6.2	5.9	6.5	6.1	5.8	6.1	6.3	6	6.1125
Day 42	5.8	6.1	5.9	5.1	5.6	5.8	5.9	5.7	5.7375
Day 49	5.7	5.9	5.7	5	5.3	5.5	5.8	5.5	5.55
Day 56	5.5	5.7	5.4	4.7	5.1	5.1	5.4	5.2	5.2625
Day 63	5.3	5.5	5.1	4.6	4.8	4.9	5.1	4.8	5.0125

Table 14: Results for colour determination of avocado samples kept under ambient temperatures

Days of sampling	Panelist 1	Panelist 2	Panelist 3	Panelist 4	Panelist 5	Panelist 6	Panelist 7	Panelist 8	Mean
Day1	9.9	9.7	8.9	9.7	9.5	9.3	9	9.7	9.4625
Day7	7.8	6.9	6.8	7.7	7	7.2	7.3	7.1	7.225
Day 14	6.6	6.8	6.5	6.7	6.4	6.9	6.9	6.8	6.7
Day 21	6.4	6.1	6.5	6	6.3	6.4	6.3	6.5	6.3125
Day 28	6	5.7	6.1	5.5	5.9	5.8	5.6	5.7	5.7875
Day 35	3.9	3.6	3.5	3.8	3.7	3.5	3.9	3.4	3.6625

Day 42	3.5	3.1	3.4	3.2	3.5	2.9	3.2	3.5	3.2875
Day 49	2.4	2.5	2.8	3.1	2.8	2.6	2.9	2.7	2.725
Day 56	2.2	2.5	2	2.3	2.1	1.9	2.4	2.2	2.2
Day 63	1.7	2.1	1.1	1.5	0.2	0.9	2	1.8	1.4125

Appendix C11: Sensory evaluation data for the sensory parameters

	Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6
Run	A:Products	B:Panelist	Colour	Taste	Aroma	Spreadability	Finger feel	Overall acceptability
1	M1(654)	P1	0.32	0.42	0.59	0.55	0.67	0.62
2	M2(732)	P1	0.31	0.44	0.75	0.55	0.73	0.6
3	M3(654)	P1	0.24	0.24	0.55	0.42	0.52	0.36
4	M4(296)	P1	0.24	0.23	0.26	0.49	0.66	0.31
5	M5(254)	P1	0.22	0.24	0.56	0.59	0.68	0.68
6	M6(336)	P1	0.49	0.6	0.29	0.71	0.84	0.9
7	M7(963)	P1	0.51	0.69	0.6	0.05	0.78	0.5
8	M8(657)	P1	0.41	0.5	0.36	0.61	0.84	0.9
9	M1(654)	P2	0.45	0.58	0.68	0.71	0.65	0.75
10	M2(732)	P2	0.67	0.62	0.57	0.62	0.63	0.52
11	M3(654)	P2	0.41	0.59	0.67	0.81	0.88	0.69
12	M4(296)	P2	0.3	0.69	0.64	0.7	0.68	0.71
13	M5(254)	P2	0.3	0.59	0.39	0.69	0.7	0.55
14	M6(336)	P2	0.56	0.6	0.43	0.69	0.74	0.44
15	M7(963)	P2	0.55	0.6	0.54	0.59	0.61	0.71
16	M8(657)	P2	0.48	0.61	0.52	0.61	0.67	0.7
17	M1(654)	P3	0.38	0.69	0.68	0.77	0.76	0.71
18	M2(732)	P3	0.34	0.31	0.63	0.8	0.79	0.41
19	M3(654)	P3	0.58	0.55	0.5	0.61	0.7	0.42

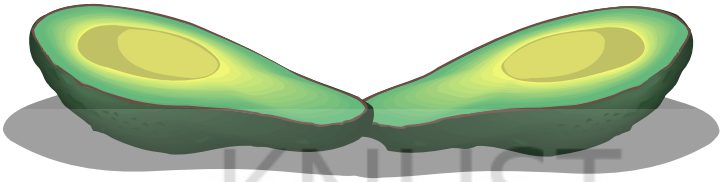
20	M4(296)	P3	0.45	0.67	0.43	0.7	0.66	0.34
21	M5(254)	P3	0.49	0.72	0.39	0.71	0.81	0.42
22	M6(336)	P3	0.47	0.66	0.49	0.78	0.8	0.45
23	M7(963)	P3	0.5	0.23	0.42	0.55	0.61	0.4
24	M8(657)	P3	0.6	0.26	0.34	0.68	0.66	0.48
25	M1(654)	P4	0.21	0.41	0.7	0.38	0.69	0.51
26	M2(732)	P4	0.38	0.28	0.5	0.23	0.6	0.48
27	M3(654)	P4	0.41	0.4	0.52	0.63	0.64	0.46
28	M4(296)	P4	0.45	0.56	0.49	0.35	0.68	0.33
29	M5(254)	P4	0.5	0.62	0.56	0.29	0.71	0.42
30	M6(336)	P4	0.45	0.55	0.49	0.76	0.7	0.53
31	M7(963)	P4	0.22	0.58	0.37	0.35	0.37	0.21
32	M8(657)	P4	0.31	0.29	0.5	0.71	0.77	0.53
33	M1(654)	P5	0.64	0.7	0.55	0.71	0.74	0.7
34	M2(732)	P5	0.6	0.67	0.67	0.74	0.75	0.59
35	M3(654)	P5	0.59	0.71	0.48	0.8	0.82	0.51
36	M4(296)	P5	0.62	0.65	0.58	0.6	0.78	0.63
37	M5(254)	P5	0.61	0.34	0.43	0.67	0.61	0.41
38	M6(336)	P5	0.61	0.6	0.62	0.7	0.69	0.7
39	M7(963)	P5	0.62	0.72	0.34	0.38	0.63	0.34
40	M8(657)	P5	0.67	0.68	0.18	0.43	0.5	0.42
41	M1(654)	P6	0.63	0.78	0.71	0.76	0.78	0.81
42	M2(732)	P6	0.72	0.56	0.57	0.62	0.69	0.65
43	M3(654)	P6	0.73	0.62	0.63	0.61	0.82	0.81
44	M4(296)	P6	0.75	0.74	0.64	0.73	0.82	0.84
45	M5(254)	P6	0.73	0.34	0.29	0.62	0.45	0.32
46	M6(336)	P6	0.63	0.61	0.55	0.31	0.59	0.73
47	M7(963)	P6	0.64	0.61	0.55	0.71	0.7	0.69
48	M8(657)	P6	0.82	0.72	0.72	0.72	0.76	0.83
49	M1(654)	P7	0.39	0.25	0.56	0.5	0.81	0.46
50	M2(732)	P7	0.27	0.8	0.71	0.65	0.76	0.69
51	M3(654)	P7	0.29	0.44	0.5	0.33	0.71	0.37
52	M4(296)	P7	0.71	0.58	0.47	0.37	0.66	0.27
53	M5(254)	P7	0.66	0.73	0.44	0.42	0.77	0.33
54	M6(336)	P7	0.57	0.47	0.24	0.2	0.24	0.3
55	M7(963)	P7	0.21	0.62	0.49	0.71	0.72	0.67
56	M8(657)	P7	0.5	0.69	0.69	0.74	0.75	0.66
57	M1(654)	P8	0.44	0.53	0.6	0.46	0.84	0.58

58	M2(732)	P8	0.5	0.67	0.69	0.83	0.81	0.76
59	M3(654)	P8	0.48	0.79	0.73	0.78	0.76	0.7
60	M4(296)	P8	0.62	0.7	0.67	0.71	0.72	0.74
61	M5(254)	P8	0.49	0.67	0.58	0.66	0.79	0.7
62	M6(336)	P8	0.6	0.58	0.51	0.65	0.77	0.68
63	M7(963)	P8	0.57	0.55	0.52	0.76	0.76	0.79
64	M8(657)	P8	0.66	0.76	0.49	0.61	0.83	0.51

Appendix D1: Product Label for Avocado Fruit Spread

BATCH NO. AFS 001
MFG DATE: .....
EXP. DATE: .....

WIGGLESWADE AVOCADO FRUIT SPREAD



Proudly made in Ghana by
MORE MONEY VENTURES
P. O Box K J 570, Kumasi
Plt A Blk 3 Maampehia,
Sunyani -Techiman Road.
Brong Ahafo - Region

FDB Reg. No: .....
Net wt. : 100g

To be Refrigerated

KEEP THE

CITY CLEAN

INGREDIENTS

Avocado Fruit, Sugar, Xanthan gum, Vinegar, Salt, Vanilla essence, Ascorbic Acid, Citric acid Potassium sorbate and Sodium benzoate.

Wiggleswade .....deliciously Nutritious!!!