Sudan University of Science and Technology

College of Graduate Studies

Extraction of Natural Flavours from Mint and Orange Peel and Their Use as Flavouring Agents for Biscuit.

إستخلاص نكهات طبيعية من النعناع وقشرالبرتقال وإستخدامهما عواملاً لنكهة البسكويت

Dissertation Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of the Degree of Master of Science in Food Science and Technology

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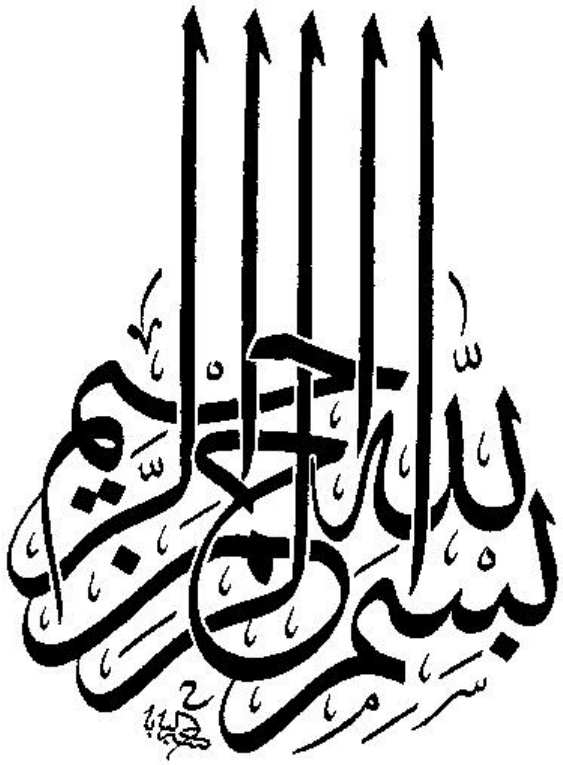
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الآية

قال تعالي:

(وَأَمْدَدْنَاهُمْ بِفَاكِهَةٍ وَلَحْمٍ مِمَّا يَشْتَهُونَ)

صدق الله العظيم

سورة الطور الآية(22)

Dedication

To my Father and Mother

To my sisters and brothers

To all my teachers and colleague

And finally to all my friends.

Somia.

Acknowledgment

First, Iam grateful to Allah for giving me health, patience, and assistance to complete this work.

Thanks and all thanks to my supervisor, **Prof.Dr. Mahdi Abbas** for his supervising and nice cooperation and help during this work.

Specially thanks to my brother Abdelrhman in Qatar and all staff at Industrial Research and Consultancy Centre especially food Department for their great of kind help and support.

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Abstract

The main goal of this study is to extract natural flavour from mint and orange peels by distillation production in sudan and its utilization in improving the biscuit product flavour. The flavours were extracted by steam and water distillation. It was conducted to determine the physico-chemical propereties of flavour and it's effect on different added levels (1,2,and 3 ml) on the sensory evaluation of the final product.

The proximate analysis for both flavoured biscuits ,with orange and mint (moisture, protein, fat, ash, fiber and carbohydrate) were compared with control

Results obtained showed significant difference ($P < 0.05$) in moisture (2.14%-3.56%), carbohydrate(77.6%-79.48%) and no significant differencein ($P > 0.05$) protein, fat, ash and fiber respectively. The physical characteristics of biscuit include thickness, wiedth, and spread ratio . showed significant difference

($P < 0.05$) . Mineral analysis showed significant difference ($P < 0.05$) in K,P and Zn. No significant difference($P > 0.05$) in(Fe ,Mg. Ca expect D1 with biscuit orange peel flavor. Moreover, Microbial analysis indicated that, all biscuit samples were free from contamination.

Finally, the sensory evaluation for the added mint flavoured biscuit compare showed no significant difference ($P > 0.05$) in their color, flavour, taste, texture, and over all acceptability. The orange flavoured biscuit showed significant difference ($P < 0.05$) in their color, flavour, taste, texture, and over all acceptability.

الملخص

الهدف الاساسي من هذه الدراسة استخلاص نكهات طبيعية من قشر البرتقال والنعناع المنتج محليا في السودان عن طريق التقطير بالماء والبخار واضافتها لتحسين نكهة البسكويت . تم إجراء اختبارات فيزيائية وكيميائية علي النكهات كما تم إجراء اختبارات فيزيائية وكيمائية وميكروبية وحسية علي المنتج النهائي من البسكويت بعد اضافة النكهات بأوزان مختلفة (1,2,3 مل) في نتايج التحليل التقريبي اظهرت النتايج في البسكويت المصنوع بنكهة قشر البرتقال, والنعناع لكل من (الرطوبه، البروتين، الدهون، الرماد،الالياف والكربوهيدرات) مقارنه مع الكنترول كالاتي توجد فروق معنوية($P<0.05$) في الرطوبة (2.14%-3.56%) والكاربوهيدرات (77.6%- 79.48%)بينما لاتوجد فروق معنوية ($P > 0.05$)في كل من البروتين والرماد والدهون والألياف. كذلك لاتوجد فروق معنوية ($P>0.05$) في التحليل التقريبي للبسكويت المضاف اليها النعناع . بالنسبة للتحليل الفيزيائي للبسكويت مقارنه مع الكنترول يوجد اختلاف معنوي ($P<0.05$) كذلك يوجد اختلاف معنوي ($P<0.05$) لكل البسكويت في تحليل المعادن لكل من (البوتاسيوم ، الفسفور ، والزنك) بينما لاتوجد فروق معنوية ($P > 0.05$) لكل من (الحديد، المغنسيوم ،الكالسيوم) ماعدا البسكويت المضاف اليها 3 مل من نكهة قشر البرتقال . بالنسبة للتحليل السلامة للبسكويت لاتوجد فروق معنوية

($P > 0.05$)

في التقييم الحسي في درجات (الرائحة ، الطعم، القوام ،اللون ، والقبول العام)أن هناك فروق معنوية

($P <0.05$) بين عينات البسكويت المنتجة بإضافة نكهة البرتقالبينما لاتوجد فروقات معنوية

($P> 0.05$) بين منتجات البسكويت المنتجة بإضافة النعناع .

CHAPTER ONE

INTRODUCTION

Flavor (American English) or flavour (British English; see spelling differences) is the sensory impression of food or other substance, and is determined primarily by the chemical senses of taste and smell. The "trigeminal senses", which detect chemical irritants in the mouth and throat, as well as temperature and texture, are also important to the overall gestalt of flavour perception. The flavour of the food, as such, can be altered with natural or artificial flavourants which affect these senses. More over "aflavourant" is defined as a substance that gives another substance flavour, altering the characteristics of the solute, causing it to become sweet, sour, tangy, etc. A flavour is a quality of something that affects the sense of taste (Iewellyn,2002**)**. Of the three chemical senses, smell is the main determinant of a food item's flavour. Five basic tastes – sweet, sour, bitter, salty and umami (savory) are universally recognized, although some cultures also include pungency (Iewellyn,2002**)** and oleogustus ("fattiness") (Oaklander, 2015). The number of food smells is unbounded; food's flavour, therefore, can be easily altered by changing its smell while keeping its taste similar. This is exemplified in artificially flavoured jellies, soft drinks and candies, which, made of bases with a similar taste, have dramatically different flavours due to the use of different scents or fragrances. The flavourings of commercially produced food products are typically created by flavoruists(Iewellyn,2002**)**.

Although the terms flavouring and flavourant in common language denote the combined chemical sensations of taste and smell, the same terms are used in the fragrance and flavours industry to refer to edible chemicals and extracts that alter the flavour of food and food products through the sense

of smell. Due to the high cost or unavailability of natural flavour extracts, most commercial flavourants are "nature-identical", that means they are the chemical equivalent of natural flavours, but chemically synthesized rather than being extracted from source materials. Identification of components of natural foods, for example a raspberry, may be done using technology such as headspace techniques, so the flavourist can imitate the flavour by using a few of the same chemicals present(Iewellyn,2002**)**

Mentha spear mint is a herbaceous, rhizomatous, perennial plant growing 30–100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading fleshy underground rhizome. The leaves are 5–9 cm long and 1.5–3 cm broad, with a serrated margin. The stem is square-shaped, a trademark of the mint family of herbs. Spearmint produces flowers in slender spikes, each flower pink or white, 2.5–3 mm long, and broad(Huxley, 1992).

Orange famed Creek plant acidic smell of the platoon, medium length evergreen summit round and thorns are thin, the aromatic bloom white spherical, native to South East Asia, Europe, America, and grown in Sudan and all parts of the world. Orange grow12-20 feet in width and height and can live for 50 year citrus are hardy to 23-26f (Morton,1987).

The history of planting Citrus in the Sudan was dated back to 1896 according to Mr. Wingates report on Dongla province. Citrus planting is known to be disseminated in the States of the North, Nile River, Khartoum, Kassala, Gazira, Blue Nile, Kordofan and Darfur. It would be recommended that to plant the highly demanded varieties of lime and grapefruit followed by oranges, mandarin and then Pummelo. More than 27000 hectare wereplanted with citrus trees. Mean production season is from October to March except for lime which produces all year round(Elhassan, 2010)

Objectives of the study:

1. To extract natural flavour from mint and orange peel and their use in Improving of biscuit flavour.
2. To determine physical and chemical properties of the natural essences obtained from orange and mint.
3. To determine the quality and safety of the produced flavoured biscuit .

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of flavour :

Extraction of natural assences from mint and orange and their use as flavouring(agents ascents) for Biscuit.

It stimulat salivary flow and acid digestion and can influence the metabolism.

The ability to perceive flavors and textures while eating and drinking serves not only a physiological but also a social function. These basic sensations are an interaction: direct, indirect and even through space time by triggering images and emotions from the depths of our memories. Who has not felt a certain when a familiar smell carries us back to the good or maybe bad experiences of our childhood.

No one disputes the fact that home-cooked food using fresh ingredients tastes better than industrially processed food. In addition, today fewer and fewer people have enough time for the regular meal preparation.

Therefore consumption of convince products is an option. Consumer demands is expected to remain consistent: food must always be available and be delicious. The home-cooked meal is consumed, in most cases, shortly after its preparation. On the contrary, industrially prepared food has a long way to go before being consumed by the final consumer. During storage of raw materials, cooking, transport, storage of the final product and sale time the original aromas are often lost or greatly altered. For this reason additional aromas take on an important role. The term “aroma” also designates food additives intended to provide specific flavours to foods. These products are devoloped by the flavour industry for the food industry.

Flavors are sold in high concentrations, cannot be used directly for human or animal consumption and are legally used as ingredients and regulated as additives. The few flavours that can be found in retail business have a very low concentration and are intended for domestic use.

Modern market trends and consumer expectations are moving towards products that are more natural, functional, healthy and easy to prepare. But not all flavours that the food industry uses can be natural or stem from natural sources or extracts due to the scarcity of the raw materials, their heterogeneous quality, or because of their intrinsic nature, leading to high prices. Thanks to modern biotechnological processes using microorganisms or enzymes it is, however, possible to obtain more natural aromatic substances from a wider range of natural raw materials (Xlmena,2017).

2.2 Flavourants or flavourings

Flavourings are focused on altering the flavours of natural food product such as meats and vegetables, or creating flavour for food products that do not have the desired flavours such as candies and other snacks. Most types of flavourings are working on scent and taste. Few commercial products exist to stimulate the trigeminal senses, since these are sharp, astringent, and typically unpleasant flavours .type natural flavoring substances ,description These flavouring substances are obtained from plant or animal raw materials, by physical, microbiological, or enzymatic processes. They can be either used in their natural state or processed for human consumption, but cannot contain any nature-identical or artificial flavoring substances (Smitha, 2005). Most artificial flavours are specific and often complex mixtures of singular naturally occurring flavour compounds combined together to either imitate or enhance a natural flavour. These mixtures are formulated by flavourists to give a food product a unique flavour and to maintain flavour consistency between different product

batches or after recipe changes. The list of known flavouring agents included thousands of molecular compounds, and the flavour chemist (flavourist) can often mix these together to produce many of the common flavours. Many flavourants consisted of esters, which are often described as being "sweet" or "fruity". Chemical Ethyl propionate ,odor fruity, chemical limonene, odor ,orange.etc .The compounds used to produce artificial flavours are almost identical to those that occur naturally. It has been suggested that artificial flavours may be safer to consume than natural flavours due to the standards of purity and mixture consistency that are enforced either by the company or by law Natural flavours, in contrast, may contain impurities from their sources, while artificial flavours are typically more pure and are required to undergo more testing before being sold for consumption (Smitha,2005). Flavours from food products are usually the result of a combination of natural flavours, which set up the basic smell profile of a food product, while artificial flavours modify the smell to accent it Unlike smelling, which occurs upon inhalation, the sensing of flavours in the mouth occurs in the exhalation phase of breathing and is perceived differently by an individual. In other words, the smell of food is different depending on whether one is smelling it before or after it has entered one's mouth(Amyfleming, 2015).

2.3 The fundamental differences between natural and artificial flavours

Natural flavours are typically complex mixtures of chemicals derived from plants or fruits. In many cases there will be one predominant flavour chemical, as well as dozens, or even hundreds of other components. It complex mixture that gives natural extracts a richer, more complex flavour. But it is usually the predominant flavour chemical that will be identified by someone’s sense of taste or smell. By contrast, an artificial flavour is

synthesized from other chemicals rather than being extracted from a natural source. Artificial flavours usually contain only a small number —often just one — of the same flavour chemicals found in the natural extract, but lack the others So, they can not precisely duplicate the flavour of the complex mixture. So, while some one tasting an artificially flavoured food will be able to identify the principal flavour, it may seem bland or taste like it is "missing something." Some are better than others (Bloom,2017).

2.4 Mentha

Mentha (also known as mint ,from Greek míntha,(Johannes, 2005). Mints are aromatic, almost exclusively perennial, rarely annual herbs. They have wide-spreading underground and over ground stolons [Biota of North America programe ,2013]and erect, square,(Huxley,1992) branched stems. The leaves are arranged in opposite pairs, from oblong to lanceolate, often downy, and with aserrated margin. Leaf colors range from dark green and gray-green to purple, blue, and sometimes pale yellow. The flowers are white to purple and produced in false whorls called verticillasters. The corolla is two-lipped with four subequal lobes, the upper lobe usually the largest. The fruit is a nutlet, containing one to four seeds. While the species that make up the Mentha genus are widely distributed and can be found in many environments, most grow best in wet environments and moist soils. Mints will grow 10–120 cm tall and can spread over an indeterminate area. Due to their tendency to spread unchecked, some mints are considered invasive.(Chritopher,2002).

2.4.1Species:

Spear mints it is found in most of sudan Scientific

Scientific Classification : Kingdom Plantae, Clade: Angiosperms, Clade Eudicots ,Clade Asterids, Order Lamiales, Family Lamiaceae, Genus

Mentha, Species M.spicata Binomial name Mentha spicata (binomial *Mentha spicata*, synonym *Mentha viridis*), also known as garden mint, *common mint*, *lamb mint* and *mackerel mint*,-(Johannes ,2005)Mentha spicata. Spearmint] is a species of mint native to much of Europe and AsiaMiddle East, Himalayas, China etc., and naturalized in parts of Northern and Western Africa, North America, and South America, as well as various oceanic islands.

2.4.2 Description

It is aherbaceous, rhizomatous, perennial plant growing 30–100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading fleshy underground rhizome. The leaves are 5–9 cm long and 1.5–3 cm broad, with a serrated margin. The stem is square-shaped, a trademark of the mint family of herbs. Spearmint produces flowers in slender spikes, each flower is pink or white, 2.5–3 mm long ,(Huxley, 1992).

2.4.3Cultivation

Spearmint grows well in nearly all temperate climates. Gardeners often grow it in pots or planters due to its invasive, spreading rhizomes. The plant prefers partial shade, but can flourish in full sun to mostly shade. Spearmint is best suited to loamy soils with abundant organic material.

Spearmint leaves can be used fresh, dried, or frozen. They can also be preserved in salt, sugar, sugar syrup, alcohol, or oil. The leaves lose their aromatic appeal after the plant flowers. It can be dried by cutting just before, or right (at peak) as the flowers open, about one-half to three-quarters the way down the stalk (leaving smaller shoots room to grow). Some dispute exists as to what drying method works best; some prefer different materials (such as plastic or cloth) and different lighting conditions (such as darkness or sunlight).

2.4.4Oil uses

Spearmint is used for its aromatic oil, referred to as oil of spearmint. The most abundant compound in spearmint oil is R-(–)-carvone, which gives spearmint its distinctive smell. Spearmint oil also contains significant amounts of limonene, dihydrocarvone, and 1,8-cineol. (Anwar**,**andNigam**,** 2010**)** Unlike oil of peppermint, oil of spearmint contains minimal amounts of menthol and menthone. It is used as a flavoring for toothpaste and confectionery, and is sometimes added to shampoos and soaps. It is also used as fumigant, spearmint essential oil is an effective insecticide against adult moths**(** Hassiotis**,** 2015**).**

2.4.5 Beverages

Spearmint is an essential ingredient of Maghrebi mint tea, grown in the mountainous regions of Morocco and known locally as na'na' (Arabic: نعناع. This variety of mint possesses a clear, pungent, but mild aroma Spearmint is an ingredient in several mixed drinks, such as the mojito and mint julep. Sweet tea, iced and flavored with spearmint, is a summer tradition in the Southern United States (Lisa Boalt, 2014).

2.4.6 The benefits of mint

Mint smell of taste delicious, fresh and beautiful (Axe , 2018). It is playing a role in the treatment of various health problems, relieve muscle and joint pain, seasonal allergy relief, increase energy and improve exercise performance, alleviate headaches, freshen breath and support oral health, promote hair growth and reduce dandruff, boost skin health and other benefits (Axe,2018).

2.5 Orange peel

Peel, also known as rind or skin, is the outer protective layer of fruit or vegetable which can be peeled off. The rind is usually the botanicalexocarp, but the term exocarp also includes the hard cases of nuts, which are not named peels. They are not peeled off by hand or peeler, but rather shells because of their hardness.

A fruit with a thick peel, such as a citrus fruit, is called a hesperidium. In hesperidiums, the inner layer (also called *albedo* or, among non-botanists, is peeled off together with the outer layer (called flavedo), and together they are called the peel. The flavedo and albedo, respectively, are the exocarp and the mesocarp. The juicy layer inside the peel, containing the seeds,is the endocarp.

2.5.1 Orange peel uses

Depending on thickness and taste, fruit peel is sometimes eaten as part of the fruit, such as with apples. In some cases the peel is unpleasant or inedible. In this case, it is usually removed and discarded, such as with bananas or grapefruits.

The peel of some fruits ,for example, pomegranates ,is high in tannins and other poly phenols, and is employed in the production of dyes.

The peel of citrus fruits is bitter and generally not eaten raw, but may be used in cooking, e.g. In gastronomy, the outermost, colored part of the peel is called the zest, which can be scraped off and used for its tangy flavor. The fleshy white part of the peel, bitter when raw in most species, is used as succade or is prepared with sugar to make marmalade or fruit soup. The peel can also be used in candy making.

Nutritional value per 100 grams of orange peel could be explained as follows :

The diet and nutritional value for example: calories 97 thermal price, starches 25grams, the protein 1.50grams, total fat 0.20 grams, food fiber 10.6 grams, Vitamin B1 0.0370.04 milligrams, Vitamin B2 0.04milli grams/proguard Acquisition corp, folicacid 30micro grams, vitamin B3 0.3340.04 milligrams, vitamin B6 Is 0.1760.04milli grams , the vitamin B12 0.00ҷg, vitamin A 420International unit, vitamin C 0.04 milligrams/136.0, vitamin E 0.25micro gram, Soduim 30.04 milligrams, potassium 212.04 milligrams, calcium 161 mg, Iron 80. Mg , magnesium 22mg, white phosphorus 21 mg, zinc 25. Mg

(Allseed and Huseen , 2010**).**

5.5.2 The benefits of orange peel :

Vitamins and minerals found in oranges include vitamin C it is vital in strengthening the immune system and protecting the body against common cold and flu. It is also a potent antioxidant that can help with cancer prevention, vision protection and cardiovascular health maintenance. Vitamin A is typically associated with carrots. and is known to maintain healthy vision, Vitamin B1is also known as thiamin, acts as a protector of the heart, muscles and nervous system. Like niacin, it also helps to convert food into energy. Vitamin B2is also known as riboflavin, can metabolize fats, carbohydrates and proteins. Vitamin B3is known as niacin. This vitamin is vital in the skin, digestive and nervous system. Research has shown it may help prevent Alzheimer's disease, help convert food to energy, and promote skin repair. Vitamin B5is also known as panto thenic acid. important in the metabolism of carbohydrates, proteins and fats. Vitamin B6it is helps with the metabolism of protein and red blood cells,

and may prevent heart disease. Folate is a very important nutrient for pregnancy and infancy, since it helps in cell production and maintenance. Since doctors often recommend pregnant women to increase their folate intake, oranges can be an excellent source, and other minerals such as calcium, potassium and magnesium(Tseng, 2016)

2.6 Distillation

It is the process of separating the components or substances from a liquid mixture by selective boiling and condensation. Distillation may result in essentially complete separation (nearly pure components), or it may be a partial separation that increases the concentration of selected components of the mixture. In either case, the process exploits differences in the volatility of the mixture's components. In industrial chemistry, distillation is a unit operation of practically universal importance, but it is a physical separation process and not a chemical reaction (Laurence and Christopher ,1989).

2.6.1 Applications of distillation

The application of distillation can roughly be divided in four groups: laboratory scale, industrial distillation, distillation of herbs for perfumery and medicinal (herbal distillate), and food processing. The latter two are distinctively different from the former two in that in the processing of beverages and herbs, the distillation is not used as a true purification method but more to transfer all volatiles from the source materials to the distillate (Laurence and Christopher,1989)

The main difference between laboratory scale distillation and industrial distillation is that laboratory scale distillation is often performed batch-wise, whereas industrial distillation often occurs continuously. In batch distillation, the composition of the source material, the vapors of the distilling compounds and the distillate change during the distillation. In

batch distillation, a still is charged (supplied) with a batch of feed mixture, which is then separated into its component fractions which are collected sequentially from most volatile to less volatile, with the bottoms (remaining least or non-volatile fraction) removed at the end. The still can then be recharged and the process repeated.In continuous

distillation, the source materials, vapors, and distillate are kept at a constant composition by carefully replenishing the source material and removing fractions from both vapor and liquid in the system. This results in a better control of the separation process *(*Laurence and Christopher ,1989).

2.6.2 Steam distillation

Like vacuum distillation, steam distillation is a method for distilling compounds which are heat-sensitive. The temperature of the steam is easier to control than the surface of a heating element, and allows a high rate of heat transfer without heating at a very high temperature. This process involves bubbling steam through a heated mixture of the raw material. By Raoult's law, some of the target compound will vaporize (in accordance with its partial pressure). The vapor mixture is cooled and condensed, usually yielding a layer of oil and a layer of water. Steam distillation of various aromatic herbs and flowers can result in two products; an essential oil as well as a watery herbal distillate. The essential oils are often used in perfumery and aromatherapy while the watery distillates have many applications in aromatherapy, food processing and skin care chemical reaction (Laurence and Christopher ,1989).

2.6.3Advantages of using Steam Distillation:

The advantage of steam distillation is that it is a relatively cheap process to operate at a basic level, and the properties of oils produced by this method are not altered. As steam reduces the boiling point of a particular

component of the oil, it never decomposes in this method. This method apart from being economical, it is also relatively faster than other methods (Sahoo,2010)

2.7 Biscuit

Biscuit is consumed extensively in large scale in developing countries where protein and caloric malnutrition are prevalent particularly among women and children . It can serve as a vehicle for important nutrients if made readily available to the population .

The increasing importance of snack foods such as cookies in to days eating habits has not been fully exploited. This could be due to the high cost of wheat in tropical areas leading to importation with economic drain and increased prices of baked goods in these countries.

The main problem facing the bakery industry in Sudan is the scarcity in available of wheat . flour So any effort made to substitute part of the wheat by other kind of available flours. (Algach, 2004)

CHAPTER THREE

MATERIALS AND METHODS

3.1Materials:

3.1.1Mentha:

The mentha was obtained from shambat farm near the River Nile , in Bahree City, Khartoum State. Extraction six kg of mint was processed by steam and water distillation. the obtained yield was 35 ml mint flavour .The Percentage was 35/6000g*100=,58%.

3.1.2 Orange peel:

Orange peel was obtained from Northen Sudan and dried in shadow in the Laboratory. On the other hand, the orange procured extraction was carried out for three kg of orange peel. The obtained yield was 25 ml orange peel flavour. the percentage 25/3000g*100=0.83%.

3.1.3 Biscuit

Flour, vegetable oil,sugar, milk powder ,baking powder , few salt, water and glucose were obtained from the market. Menthaflavour and orange peel flavour were extracted from mentha and orange peel.

Laboratory Biscuit recipe :

Flour (200g)

+

Powder sugar (60g)

+

Vegatable ghee(saminoil) (60ml)

+

Milk powder (60g)

+ salt (2g)

Sodium bicarbonate (,8g)

+

Ammonia(NH3) (3g)

+

Glucose (4g)

+

Water (30ml)

Extraction procedure

Extracted flavour from mint and orange peel were added in different volumes ,(1ml, 2ml,3ml) and beside acontrol sample for recipe formulation .

A= Control, B1= Biscuit treated with orange peel (1ml), C1= Biscuit treated with orange peel (2ml) ,D1= Biscuit treated with orange peel (3ml), B2=Biscuit treated with mint (1ml), C2= Biscuit treated with mint (2ml), D2= Biscuit treated with mint (3ml).

Biscuit Laboratory prepration :

- The wheat flour and the flavour were added to the above materials (powder sugar+ghee+milkpowder+salt+ sodium bicarbonate +NH3+glucose+ water).
- These following materials were mixed for(3-8 minutes).
- The quantities mentioned above forming a dough .
- Divided it in small pieces and placed in to thermal oven at 250°C

  For (20-25minutes).

All experiment processes are carried in the Food Research Center.

Two hundred gram of wheat flour to sample A, B1, C1, D1 ,B2,C2,D2, sugar powder, skim milk and glucose were added each recipe in Hobart N-50 mixer with a flat beaker for 3 min at 61 rpm.

Salt, ammonium, bicarbonate were dissolved separately in part of the required water and added to the dough.

Mixing was done for 8 min at 125 rpm to obtain homogenous dough.

- Finally, flour was added and mixed for 3 min at 61 rpm .
- The dough was shaped or flattened to a thickness of 4 mm with the help of two roller placed at two sides of the dough.
- The formed dough was cut into round shape using 4.985 mm diameter cutter.
- The cut dough was transferred to an aluminum tray.
- The biscuits were baked in an electric oven maintained at 250°C

for (20-25 min).

The baked biscuits were cooled for about (20 min), packed in plastic bags and stored at room temperature for further analysis.

- Orange peel was washed and dried in the laboratory. Mint leaves were cleaned , washed and put under fan for 2 hrs before distillation.

3.2.1 Physical analysis

3.2.1.1Viscosity

Viscosity of the volatile extracted oil was determined by using an Ostwald U-Tube viscometer .

The viscometer was suspended in a constant temperature bath (32+2°C) so that the capillary was vertical. The tube was filled to the mark at the top of the lower reservoir with the oil by means of pipette inserted side arm. so that the tube above the mark was not wetted .The instrument was then left to stand for few minutes before reading in order to equilibrate the sample temperature with that of the instrument (32+2°C) . By means of the pressure on the respective sidearm of the tube, the oil moved into the other arm so that the meniscus was 1cm above the mark at the top of the upper reservoir. The liquid was then allowed to flow freely through the tube and the time required for the meniscus to pass from the mark above the upper reservoir to that at the bottom of the upper reservoir recorded. measure by viscosity Pascal-second.

Calculation

$$\text{Relative viscosity of the fat} = \frac{T - T0}{T}$$

Where:

T is the flow-time of the fat and T_0 is the flow-time of the distilled water**.**

3.2.1.2 Refractive index

The refractive index measured by bench refracto meter. Refractive index of the extracted oil was measured according to the(AOCS ,1993). Several drops of the oil were placed on the lower prism of an Abbe refracto meter which was also adjusted to the same temperature as that of the sample. The prisms were closed and tightened firmly with the screw head, ensuring that the sample reached the same temperature of the instrument. The instrument

was adjusted until the most distinct reading possible was obtained and the refractive index was read.

The value was given in figure.

3.2.1.3 Colour

The color intensity of the oil was recorded using a Lovibondtintometer as units of red, yellow and blue in the manner described by (Balla,2000).

Samples of the oil were filtered through filter paper grade immediately before testing. An appropriate 5.25 inches was filled with extracted oil and placed in the tintometer in specific place .The instrument was switched on and looked through the eyepiece and then slides were adjusted until a color match was

obtained .The values obtained by matching were recorded as red, yellow and blue,reflecting the wave length range of the instrument (Lovibond).

3.2.1.4 Density

Density determination was caried by pycnometer in a very precise method. It uses a working liquid with well-known density, such as water. Distilled water was added, for which temperature dependent values of density of H_2O. The pycnometer was a glass flask with a closed-fitting ground glass stopper with a capillary hole. The fine hole releasd a spare liquid after closing a top-filled pycnometer and allowed for obtaining a given volume of measured and/or working liquid with a high accuracy.

The density was calculated as follows:

$$\text{The density at } 25°C = W_1 / W_2$$

Where:

W_1= Weight of oil at 25°C

W_2 = weight of water at 25°C

The value was recorded as gram per cubic centimeter.

3.2.2 Chemical analysis

3.2.2.1Acid value

Five g of the cooled oil sample was weighed in a 250 ml conical flask , with (50) ml of freshly neutralized ethyl alcohol, 1.0 ml of phenolphthalein indicator solution added. The mixture was boiled for 5 minutes, titrated while hot against standard alkali solution(KOH) and shake vigorously during the titration.

$$\text{Acid Value} = \frac{(56.1)VxN}{W}$$

Where:

V= volume in ml of standard potassium hydroxide.

N= normality of the potassium hydroxide solution.

W =weight of the sample in gm.

Acid value was recorded by KOH in mg needed to neutralize the organicacids present in 1g of fat and it is ameasure of the (FFA) present in the fat.

3.2.2.2 Free Fatty Acids (FFA)

Percentage free fatty acid was determined using the recommended method of the American Oil Chemists' Society (AOCS, 1993). One point four grams (1.4 g) of fat was weighed into a flask containing 15 ml of hot neutralized alcohol, and 0.4 ml of phenolphthalein indicator was then added. The content was titrated with 0.5 N NaOH. Percentage free fatty acid value was calculated (as oleic acid) using the formula

$$\textbf{F.F.A} = \frac{\textbf{a (ml)} \times \textbf{N} \times \textbf{28}}{\textbf{S}}$$

Where:

a: reading of fat sample (ml)

S: Original weight of sample

3.2.23 Peroxide value:

The peroxide value of the oil sample was determined according to the (AOAC ,1994)methods . One g of the oil was accurately weighed into 250 ml conical flask. Thirty ml of glacial acetic acid and chloroform (3:2) were added and the solution was swirled gently to dissolve the oil. A 0.5 ml of 0.1N KI was added to the flask, and the content of the flask were left to the stand for one minute before adding 30 ml of distilled water. After a while, the contents were titrated with 0.01 N sodium thiosulphate until the yellow colour almost disappeared. The number of ml 0.01 N sodium thiosulphate required (a) were recorded. The same process was repeated for blank. The number of ml of 0.01 sodium thiosulphate required by the blank (b) was recorded.

Calculation

$$\text{Peroxide value} = \frac{(b-a)\ X\ N\ X1000}{S}$$

Where:

b: reading of blank (ml)

a: reading of fat sample (ml)

s: original weight of fat sample (g)

1000 to convert millimeter to gram

The value was recorded as milli equivalents (meq) O_2 per 1kg of fat .

Determination of Iodine value:

The iodine value of the oil was determined according to procedure of the British Standard Institution (1958). Approximately, 0.2 g of fat was accurately weighed and placed in a dry and clean flask especially offered

for the test. Ten ml of chloroform was used for dissolving the fat, 25ml of pyridine sulphatedibromide solution was added and finally 20 ml of KI (0.1N) were added to the contents of the flask. The flask was then stoppered and the mixture was allowed to stand for 10 minutes in a dark place. The stopper and the side of the flask were rinsed with enough amount of distilled water, and the content of the flask were then shaken and titrated against 0.1N sodium thiosulphate solution using starch liquid as an indicator. A blank determination was carried out simultaneously.

Calculation

$$\text{Iodine value} = \frac{(b-a)\ X\ 0.01269\ X100}{S}$$

Where

b: Volume (ml) sodium thiosulphate in blank solution.

a: Volume (ml) sodium thiosulphate in active test solution.

S: weight (g) of the fat sample.

0.01269: Iodine factor.

The value was recorded as (the amount of iodine in grams , that is taken up by 100 grams of the oil .

3.2.2.4 Saponification:

Saponification value was determined according to the method of (AOCS ,1993). Two grams of the fat was weighed into a flask. Twenty five milliliters (25 ml) of alcoholic KOH was pipetted and allowed to drain for about 1 min into the mixture. A blank determination with water was prepared and determined simultaneously with the sample. A condenser was connected to the flask and the mixed sample was allowed to boil gently and steadily for 45 min for complete saponification. The flask and condenser were then cooled but not sufficient to form a gel. The condenser was disconnected and 1 ml of phenolphthalein indicator was added to the content of the flask. The solution was titrated with 0.5 N HCl until the pink

color just disappeared. The saponification value was calculated using the formula:

$$\text{Saponification value} = \frac{(b-a)\ X0.5X56.5}{S}$$

Where
a : ml of HCL for sample
b: ml of HCL for blank
s: Weight of fat (g)

Saponification value was recorded as the number of the milligrams of KOH required to saponify 1 g of fat under the conditions specified.

3.3 Proximate composition

3.3.1Determination of moisture content

The determination of moisture content were carried out on the samples according to the(AACC ,2000) methods. Two grams of well mixed samples were weighed accurately in aclean preheated moisture dish of known weight by using asensitive balance. The uncovered sample and dish were kept in an oven provided with a fan at 105˚C and left to stay overnight. The dish was covered and transferred to a desiccator, and weighed after reaching room temperature.

The loss of weight was calculated as moisture expressed as moisture%

$$\text{Moisture content (\%)} = \frac{\text{W1}-\text{W2}}{\text{Sample weight}} \times 100$$

Where:

W1 = Weight of sample + dish before oven drying.

W2 = Weight of sample + dish after oven drying.

3.3.2Determination of crude protein

The determination of crude protein was carried out on the samples according to the (AOAC ,1990) methods. A 0.2 gram of sample, plus 0.4 gram catalyst mixture (potassium sulfate + cupric sulfate 10:1 by wt), and 7 ml concentrated nitrogen free sulfuric acid, were mixed in a small Kjeldahl flask (100 ml). The mixture was digested for two hours, then cooled, diluted, and placed in the distillation apparatus. Fifteen milliliters of 40% NaOH solution were added and the mixture was heated and distilled until 50 ml were collected in a 100 ml conical flask. The ammonia evolved was received in 10 ml of 2% boric acid solution plus 3-4 drops of universal indicators (methyl red and bromo cresol green). The trapped ammonia was titrated against 0.02N HCL.

The percentage (g/100) of protein was calculated by using an empirical factor to convert nitrogen into protein as follows:

$$Nitrogen\ content\ \% = \frac{TV \times N \times 14.00 \times 100}{1000 \times Wt.of\ sample}$$

Protein content % = (nitrogen content %) X PF

Where:

TV = Actual volume of HCL used for titration (ml HCL – ml blank).

N = Normality of HCL.

14.00 = each ml of HCL is equivalent to 14 mg nitrogen.

1000 = to convert from mg to gm.

6.25 = Constant factor for other grains.

5.7 = constant factor for wheat flour.

3.3.3 Determination of crude fat

Crude fat was determined according to the standard method of (Association of Official Analytical Chemists (AOAC, 1990). A sample of 3 g was

weighed into an extraction thimble and covered with cotton that was previously extracted with hexane (boiling point 60-70°C), and then the sample and a pre-dried and weighed Erlenmeyer flask containing about 50 ml of solvent were attached to extraction unit for 45 minutes. At the end of distillation period, the solvent was recovered from the oil. Later, the flask with the remaining crude hexane extract was put in an oven at 105 °C for about an hour, cooled in a desiccators, reweighed and dried extract was recorded as crude fat% (DM) according to the following formula:

$$\textit{Crude oil content \%} = \frac{\textit{Weight of extracted oil}}{\textit{Weight of dry sample}} \times 100$$

3.3.4 Determination of crude fiber

Two grams of an air dried fat-free sample were transferred to a dry 600 ml beaker. The sample was digested with 200 ml of 1.25% (0.26N) H_2SO_4 for 30 minutes, and the beaker was periodically swirled. The contents were removed and filtered through Buchner funnel, and washed with boiling water. The digestion was repeated using 200 ml of 1.25% (0.23N) NaOH for 30 minutes, and treated similarly as above. After the last washing ,the residue was transferred to ashing dish, and dried in an oven at 105°C over night, then cooled and weighed. The dried residue was ignited in a muffle furnace at 550°C to constant weight, and allowed to cool, then weighed.

The fiber percentage was calculated as follows:

$$\textit{Crude fibre \%} = \frac{W1-W2}{Drysampleweight} \times 100$$

Where:

W1 = The weight of oven dry sample after treatment by H2SO4 and KOH

W2 = The weight of the treated sample after ashing.

3.3.5 Determination of ash content

Crucibles weighed empty, two grams of sample**s** were placed in a muffle furnace at 550˚C for 3 hr until white grey or reddish ash was obtained. The crucible was removed from furnace and placed in a desicator to cool, then reweighed. The process was repeated until constant weight was obtained.

$$\text{Ash content (\%)} = \frac{(W2-W1)\times 100}{Ws}$$

Where: W1 = weight of empty crucible

W2 = weight of crucible + sample after ashing

Ws = weight of dry sample

Value were recorded as percentage (%).

3.3.6 Determination of carbohydrates

The carbohydrates were calculated by difference. The sum of moisture, fat, protein and ash contents was subtracted from 100 as described by (Wade *et al.* 1988).

3.4 Determination of minerals content

Minerals of sample were extracted according to (Pearson's ,1981). The sample was burned in a muffle furnace at 550˚c. The method of the sample was placed in a sand bath for 10 min after addition of 10 ml of 5 N HCL, then the solution was carefully filtered in a 100 ml volumetric flask and finally distilled water was added to make up to mark. The extracts were stored in bottles for further analysis. (Minerals, Ca, Mg Fe, were determined using Atomic Absorption Spectrophotometer (AA 6800) Shimadzu, Japan).

3.4.1 Potassium and sodium contents

Potassium and sodium contents of extracted sample were determined according to AOAC (1984) (Association of Official Analytical Chemists) method using flame photometer. One milliliter of the extract was taken and diluted in a 50 ml conical flask with distilled water. The standard solutions of the KCL and NaCL were prepared by dissolving 2.54, 3.33 g of KCL and NaCL. Respectively, each in 1000 ml distilled water. Ten ml of this solution were taken and diluted to one liter to give a 10 ppm concentration. The flame photometer was adjusted to zero using distilled water as a blank and to 100 degree using standard solution.

Calculation:

$$\text{K or Na (mg/100g)} = \frac{\text{F.R}\times\text{D.F}}{103\times\text{S}\times 10}\times 100$$

Where:
F.R = Flame photometer reading
D.F = Dilution factor
S = Sample weight.
Result were expressed as mg/100g of sample.

3.4.2 Phosphorous content

The determination of phosphorous content was carried according to the method of Chapman and Pratt (1982). Two ml of the extracted samples were pippetted into a 50 ml volumetric flask. Ten ml of ammonium molybdate- ammonium vanadate reagent [(22.5 g of WH_4) 6 MO_7 O_{24} $4H_2O$ in 400 ml distilled water + 1.25 g ammonium vandate in 300 ml boiling water + 250 ml conc. HNO_3, then diluted to 1 liter] were added. The content of the flask were mixed and diluted to volume. The density of the color was read after 30 minutes at 470 nm using using colorimeter (Lab System Analysis-9 filters, (J. Mitra and Bros Pvt. Ltd). A standard curve of different KH2 PO4 concentration was plotted to calculate the ion phosphorous concentration in mg/100g of sample.

Calculation:

$$\text{Phosphorous (mg/100g)} = \frac{\text{Curve reading} \times \text{ash dilution}}{103 \times \text{oven dry weight of sample}} \times 1000$$

3.5 Microbiological methods

3.5.1 Sterilization of Glassware

Glass type (Petri dishes, test tube, flask, pipettes…etc)., were sterilized in hot air oven at 160 – 180˚c for 3 hours .They were washed and packed in stainless steel cans or sometimes in aluminum foil till used.

3.5.2 Sterilization of media

Culture media were prepared following manufacturing instructions then sterilization was achieved by autoclaving at 121˚c for 15 minutes .

3.5.3 Preparation of serial dilutions

Aseptically 10 grams of the sample were homogenized in 90 ml of sterile diluents (0.1 Peptone water). It was mixed well to give dilution (10^{-1}) by using sterile pipette 1ml sample was transferred aseptically from dilution (10^{-1}) to a test tube containing 1ml of sterile diluents (10^{-2}). In the same away the preparation of serial dilution was continued until the dilution(10^{6}).

3.5.4 Total Viable Count of Bacteria

It was carried out by using the pour plate count method as described by (Harrigan and Macance. 1998). One ml of each dilution was transferred into sterile petri dish, and then 15 ml of sterile melted Plate Count Agar medium were added to each plate. The inoculums was mixed medium and allowed to solidify.

The plates were incubated at 37 C° for 48 hours. A colony counter was used to count the viable bacterial colonies after incubation and the results were reported as colony- forming units (CFU) per gram.

3.5.5 Determination of Coliform Bacteria

It was carried out by using the most Probable Number (MPN) technique as described by (Harrigan and Mccance,1998).

3.5.6 Presumptive Coliform test

Values of 10, 1.0 and 0.1 ml prepared samples were inoculated in triplicate test tubes containing Macconkey. Borth with tube containing Durham vials. All sample were were incubated at 37 C° for 48 hours. The production of acid together with sufficient gas to fill the concave of the Durham vials was recorded as positive presumptive test.

3.5.7 Confirmed test for total coliforms

From tubes that showed positive results asample was picked, using a sterile loop in to tubes of Brilliant Green 2% bile Broth and inoculated. The tubes were inoculated at 37 C° for 48 hours. Tubes showing positive and negative result were recorded. The Most Probable Number (MPN) of total colliform was found out by using the Most Probable Number (MPN) tubes.

3.5.8 Confirmed *E.coli* test

Medium used was EC Broth. From every tube showing positive result in the presumptive test, a small amount was used to inoculate a tube of EC Broth containing Durham vial incubated at 44.5˚C for 24 hours. Tubes showing any amount of gas were considered positive. For further confirmation of *E. coli* tubes of EC Broth showing positive results at 44.5C° for 24 hours were streaked on Eosin Methylene Blue Agar (EMB)

plates. The plates incubated at 37C° for 48 hours. Colonies of *E. coli* are usually small with metallic green sheen on EMB Agar.

3.5.9 Staphylococcus aureus

Medium used was Baird-Parker Agar. The amount 0.1 ml from every dilution was transferred onto the surface of each well dried Baird-Parker Agar medium plates. The inoculum was spreaded all over the plate using sterile bent glass rod. The plates were incubated at 37C° for 24 hours. After incubation, the plates were examined for *Staphylococcus aureus*, which appears as black shiny convex surrounded by a zone clearing 2-5 mm in width of colony.

3.5.10 Yeast and Moulds

From suitable dilution of sample, 0.1 ml was aseptically transferred onto solidified Potato-Dextrose Agar containing 0.1 gram chloramphenicol per one liter of medium to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod. Plates were incubated at 28C° for 72 hours. Colonies were counted using a colony counter and the result were presented as CFU/ml.

3.5.11 Detection of *Salmonella*

Ten gram of the sample were added to a conical flask containing 90 ml of sterile Nutrient Broth and incubated at 37C° for 24 hours. A loopfull of 24 hours incubated Nutrient Broth was transferred aseptically to sterilized Selenite Cysteine Broth and incubated at 37 C° for 24 hours. A loopfull of 24 hours inoculums of Selenite Cysteine Broth was streak on Bismuth Sulphite Agar surface and incubated at 37 C° for 24-72 hours. Black metallic sheen discrete colonies indicates the presence of *Salmonella.*

3.6 Sensory evaluation of Biscuit

The Biscuit were sliced with knife and prepared for sensory evaluation on the same day. The sensory evaluation of biscuit samples (flavour, taste, texture, colour, , general acceptability) was carried out by 20 semi trained panelists. The surrounding conditions were kept the same all through the panel test.

3.7 Statistical analysis

The analysis of variance was performed to examine the level of significance in all parameters measured. Duncan Multiple Range Test was used to separate the means (SAS, 2002).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physico- Chemical analysis.

Table (1): Colour measurement of extracts

Sample	**Colour**
Orange peel	Blue =zero
	Yellow=7.4 – 7.4
	Red= ,7-.7 -.7 Yellow and Red
Mint	Blue= nil
	Red= 2.4 Constance
	Yellow=7.5-7.6-7.5 Yellow and Red

Sample	**Viscosity**	**Refractive index**	**Density**
Mint	1.75±0.05[a]	1.54±0.01[a]	0.94±0.001[a]
Orange	1.05 ±0.01[b]	1.51±0.00[b]	0.87±0.03[ab]

Mean values were ±SD of triplicate independent analysis.

Values in the same column carrying different superscript letter were significantly different at ($P<0.05$).

Table (2): Chemical composition of mint and orange oil .

Sample	Acid value	Free Faty Acid	Peroxide value	Iodine-value	Saponification
Mint	.93 ±0.19^{ab}	0.47 ±0.09^{a}	1.94 ±0.07^{a}	135.42 ±0.55^{ab}	252..28 ±3.01^{ab}
Orange	.72 ±0.00^{a}	0.36 ±0.00^{ab}	3.87 ±0.12^{b}	138.17 ±2.77^{a}	233.17 ±2,74^{a}

Mean values were ±SD of triplicate independent analysis (Minitab2017).

values in the same column carrying different superscript letter were significant different at ($P<0.05$).

Table (3): Physical characteristics of treated biscuit.

Samples	Thickness(cm)	Width(cm)	Spread ratio
A	1.17 ± 0.31^{ab}	6.57 ± 0.94^{ab}	5.98 ± 0.31^{ab}
B1	1.00 ± 0.00^{ab}	5.41 ± 0.27^{b}	5.41 ± 0.27^{b}
C1	1.14 ± 0.01^{ab}	5.65 ± 0.52^{b}	4.95 ± 0.47^{b}
D1	1.41 ± 0.11^{a}	6.22 ± 0.023^{ab}	4.43 ± 0.32^{b}
B2	$.83 \pm 0.04^{b}$	6.90 ± 0.012^{a}	8.33 ± 0.37^{a}
C2	1.41 ± 0.18^{a}	6.28 ± 0.58^{ab}	4.52 ± 0.66^{b}
D2	1.23 ± 0.12^{ab}	6.18 ± 0.061^{ab}	5.04 ± 0.44^{b}

Mean values were ±SD of triplicate independent analysis.

values in the same column carrying different superscript litter are significant different at ($P<0.05$).

Where:

A : Control biscuit sample.

B1: Biscuit with 1 ml orange peel flavour.

C1: Biscuit with 2 ml orange peel flavour.

D1: Biscuit with 3ml orange peel flavour .

B2: Biscuit with 1ml mint flavour .

C2: Biscuit with 2 ml mint flavour .

D2: Biscuit with Values were mean ±SD of triplicate independent analysis.

3 ml mint flavour .

4.2 physical characteristics of treated Biscuit with different ratio of orange-peel and mint oil

Table(3)shows physical characteristics of treated biscuit with different added ratio of natural orange peel oil with biscuit (A=control, B1= 1ml, C1=2ml, D1=3ml) and mint oil(B2=1ml, C2=2m, D2=3ml).

For thickness parameter, there was no significant difference ($p \leq 0.05$) between A the control(1.17cm) and B1, C1 (1.00cm, 1.14cm) when compared with Hanan and Rasha (2012) . They added 10% of Baladi orange peel powder with biscuit (1.18cm) different with D1 (1.41cm) .

While B2, C2and D2 sample showed significant difference ($P<0.05$)against the control for the same parameter which was biscuit thickness. Their reading were (.83 , 1.41, 1.23 cm) ,respectively.

Another characteristics physical property measured for treated biscuit with natural oil extracted from orange peel and mint for flavour was the width of the biscuit. It showed significant difference ($P<0.05$) between the control A, and B1. However C1 was similar to D1. Their reading were (6.57,5.41, 5.65and 6.22cm) These results were higher than those obtained by Hanan and Rasha (2012) who used 10% of Baladi orange peel powder to biscuit to enhance flavour, which was (3.38cm). While Awas similar to C2 , D2 biscuits with added mint oil that showed no significant difference ($p \leq 0.05$) but different from B2.

The third physical – property of treated biscuit in the same table (3) was spread ratio . It showed significant difference ($P<0.05$) between control A and all other mixture of B1,C1, D1, B2,C2 and D2 Their reading were (5.98, 5.41, 4.95, 4.43, 8.33, 4.52 and 5.04) , respectively. These results of spread ratio proparty were lower when comared with Hanan and Rasha (2012) findind for the same parameter (2.89 cm) .

Table (2): Chemical composition of biscuit

Sample	Moisture	Protein	Fat	Fiber	Ash	Carbohydrate
A	3.11 ±0.17 ^{ab}	10.83 ±0.15^{a}	3.58 ±0.19^{a}	1.73 ±0.18^{a}	2.23 ±0.06^{a}	78.72 ±0.25^{ab}
B1	3.56 ±0.07α	11.23 ±0.32^{a}	3.76 ±0.21^{a}	1.88 ±0.09^{a}	2.62 ±0.63^{a}	77.61 ±0.28^{cde}
C1	2.82 ±0.07^{b}	10.80 ±0.14^{a}	3.84 ±0.11^{a}	1.57 ±0.15^{a}	2.55 ±0.17^{a}	78.42 ±0.40^{bc}
D1	3.39 ±0.33^{a}	11.08 ±0.42^{a}	3.86 ±0.24^{a}	1.66 ±0.19^{a}	2.48 ±0.04^{a}	77.46 ±0.59^{de}
B2	2.14 ±0.03^{c}	10.61 ±0.03^{a}	3.82 ±0.14^{a}	1.74 ±0.05^{a}	2.21 ±0.02^{a}	79.48 ±0.11^{a}
C2	3.35 ±0.12^{a}	10.75 ±0.34^{a}	3.92 ±0.16^{a}	1.65 ±0.14^{a}	2.17 ±0.11^{a}	78.17 ±0.30^{bcd}
D2	3.57 ±0.17^{a}	11.20 ±0.02^{a}	3.63 ±0.12^{a}	1.79 ±0.06^{a}	2.63 ±0.15^{a}	77.14 ±0.17^{e}

Mean values were ±SD of triplicate independent analysis.

Values in the same column carrying different superscript letter were significantly different ($P<0.05$).

Where:

A : Control biscuit sample.

B1 : Biscuit with 1 ml orange peel flavour.

C1 : Biscuit with 2 ml orange peel flavour.

D1: Biscuit with 3ml orange peel flavour .

B2 : Biscuit with 1ml mint flavour .

C2 : Biscuit with 2 ml mint flavour .

D2: Biscuit with 3 ml mint flavour

4.3 Chemical composition of biscuit:

Table (4) shows the chemical composition of biscuit treated with orange peel flavour. The moisture content of Biscuit (B1, C1 and D1) were (3.56, 2.82 and 3.39%) , respectively. These results were lower values in comporasion to that obtained by (Hanan and Rasha ,2012) who reported (6.40%) as moisture content for Biscuit made from 90% wheat and 10% orange peel powder. This variation might be due to different oven degree.

Moreover, as presented in Table (4), the protein content of biscuits treated with orange peel flavor for (B1, C1and D1) were (11.23, 10.80 and 11.08%). These results were extremly higher than that reported by Hanan and Rasha (2012), who stated protein content of (7.44%). for biscuit made from 90% wheat and 10% orange peel powder, this variation might be due to quantity and type of wheat.

Table (4) indicated the fat content values of the biscuit treated with orange peel flavor for sample (B1, C1 and D1), were (3.76, 3.84, and 3.86%), respectively. These results were extremly lower than that reported by (Hanan and Rasha, 2012). who stated the fat content was (15.70 %). for biscuit made from 90% wheat and 10% orange peel powder. This variation might be due to added vegetable ghee(oil).

Table (4) also presented the ash percent of the biscuit treated with orange peel flavor for sample (B1, C1and D1) which were (2.62, 2.55 and 2.48%), respectively. The obtained results, compared with that stated by Hanan and Rasha (2012), which was (1.00%), were significantly different.

Table (4) showed the results of fiber content of Biscuit treated with orange peel flavor for sample (B1, C1, and D1) which were (1.88, 1.57 and 1.66%), respectively. These results were higher than that stated by (Hanan and Rasha, 2012). which was (1.21%).

Finally, Table (4) illustrated the results of the carbohydrates of the biscuit treated with orange peel flavor for samples (B1, C1, and D1) which were (77.61, 78.42 and 77.46%), respectively. These results were not matching that obtained by (Hanan and Rasha ,2012) which was (74.65%).

The orange peel flavoured biscuits with different added levels (B1, C1, D1) in comparssion to the control sample (A), showed a significant difference ($P<0.05$) in their moisture and carbohydrate.

On the other hand , The mint flavoured biscuits with different added levels (B2, C2, D2) in comparssion to their control sample (A), showed no significant difference between them ($p >0.05$).

Table (5): Minerals content of treated biscuit.

Sample	Fe	Mg	Ca	P	K	Zn
A	1.49 ±0.20^{bc}	2.41 ±0.21^{a}	221.94 ±9.90^{ab}	217.30 ±6.04^{ab}	216.81 ±0.69^{a}	0.48 ±0.14^{b}
B1	1.46 ±0.09^{bc}	2.41 ±0.04^{a}	214.14 ±2.68^{ab}	219.84 ±1.94^{ab}	216.82 ±1.68^{a}	0.57 ±0.06^{ab}
C1	1.35 ±0.01^{f}	2.45 ±0.20^{a}	218.04 ±3.81^{ab}	212.11 ±1.88^{b}	215.34 ±0.98^{a}	0.44 ±0.04^{b}
D1	1.76 ±0.10^{ab}	1.61 ±0.31^{b}	226.72 ±4.98^{a}	221.60 ±0.16^{a}	217.21 ±1.73^{a}	0.73 ±0.11^{a}
B2	1.61 ±0.20^{bc}	2.28 ±0.06^{a}	218.13 ±4.58^{ab}	217.95 ±3.55^{ab}	217.08 ±1.20^{a}	0.51 ±0.03^{b}
C2	2.07 ±0.19^{a}	2.42 ±0.07^{a}	215.48 ±0.88^{ab}	210.30 ±6.04^{b}	221.17 ±0.71^{a}	0.54 ±0.10^{ab}
D2	1.70 ±0.06^{abc}	1.66 ±0.19^{b}	211.16 ±0.55^{b}	220.73 ±1.02^{a}	207.43 ±6.20^{b}	0.58 ±0.02^{ab}

Mean values were mean ±SD of triplicate independent analysis.

values in the same column carrying different superscript letter were significantly different at ($P<0.05$) . where:

A : Control biscuit sample.

B1 : Biscuit with 1 ml orange peel flavour.

C1 : Biscuit with 2 ml orange peel flavour.

D1: Biscuit with 3ml orange peel flavour .

B2 : Biscuit with 1ml mint flavour .

C2 : Biscuit with 2 ml mint flavour .

D2: Biscuit with 3 ml mint flavour.

4.4 Mineral content of treated biscuit

The minerals content of biscuits with added orange peel flavour of deferent levels (A=control, B1=1ML orange peel flavor, C1= 2ML orange peel flavour, D1= 3 ML orange peel flavour) are showed in table (5).

Results for ion (Fe) for biscuit treated with orange peel flavour of the sample (B1, C1, andD1) were (1.46, 1.35and 1.76 mg/100g). These results were not matching that of Hanan and Rasha (2012) which was (19.23mg/100g),

Results of the mineral (Mg) for biscuit treated with orang peel flavour of the sample (B1, C1, and D1) were (1.41, 2.45, and 1.61mg/100g) .These results were not matching that obtained by Hanan and Rasha (2012), which was (0.02 mg/100g),

The mineral (Ca) for biscuit treated with orange peel flavour of the sample (B1, C1, and D1) which were (226.72, 214.14, and 218.04mg/100g). These results were not matching that obtained by Hanan and Rasha (2012), which was (0.05 mg/100 g)

Results of phosphorus (P) for biscuit treated with orange peel flavour of the sample (B1, C1, and D1) which were (219.84, 212.11, and 221.60mg/100g) these results were not matching the result that obtained by Hanan and Rasha (2012), which was (0.17 mg/100g).

Potassium (K) content for biscuit treated with orange peel flavour of the sample (B1, C1, and D1) which were (226.72, 214.14, and 218.04mg/100g) .These results were not matching the result that obtained by Hanan and Rasha (2012), which was (0.017 mg/100g)

Finally, zinc (zn) content for biscuit treated with orange peel flavour of the sample (B1, C1, and D1) which were (57 , 44 and 73 mg/100g) respectively. These results were not matching that obtained by Hanan and Rasha (2012), which was (0.17 mg/100g). This variation in all of these results might be due to the quantity and kind of wheat.

4.5 Microbial analysis of biscuits

Food is considered safe when it free of pathogenic microorganism (Total count bacteria, yeast and moulds, stafilo. cocus , E. coli and Salmonella) biscuits with different levels of orange peel and mint flavor showed no appearance of microorganisms . However, pathogenic flora did not exist in all types of biscuits. There fore this biscuits could be considered as safe for human consumption.

Table (3): Microbial analysis of biscuits

Sample	Total count bacteria	Yeast and moulds	Coli form	Stafilococus	Salmonella
A	10^{-2}	10^{-1}	Nill	Nill	Nill
B1	Nill	Nill	Nill	Nill	Nill
C1	Nill	Nill	Nill	Nill	Nill
D1	Nill	Nill	Nill	Nill	Nill
B2	Nill	Nill	Nill	Nill	Nill
C2	Nill	Nill	Nill	Nill	Nill
D2	Nill	Nill	Nill	Nill	Nill

Where:

A : Control biscuit sample.

B1 : Biscuit with 1 ml orange peel flavour.

C1 : Biscuit with 2 ml orange peel flavour.

D1: Biscuit with 3ml orange peel flavour .

B2 : Biscuit with 1ml mint flavour .

C2 : Biscuit with 2 ml mint flavour .

D2: Biscuit with 3 ml mint flavor

Table (7) Sensory evalution of biscuit with orange peel oil

Treatment–Sample	Colour	Odor	Taste	Crumb Texture	General Acceptability
A	2.00± 0,86^{bc}	2.45 ±1.23^{a}	2.70 ±1.1.03^{a}	2,10 ±0.91^{ab}	2.45 ±1.15^{a}
B1	2.90 ±1.17^{a}	2.25 ±0.91^{a}	2.30 ±0.98^{a}	2.60 ±1.14^{a}	2.55 ±1.19^{a}
C1	2.35 ±0.93^{ab}	2.35 ±0.88^{a}	3.30 ±4.50^{a}	2.40 ±0.82^{ab}	2.70 ±0.98^{a}
D1	1.45 ±0.76^{c}	2.25 ±0.97^{a}	2.30 ±1.22^{a}	1.70 ±0.87^{b}	2.35 ±1.14^{a}

Mean values were ±SD of triplicate independent analysis.

values in the same column carrying different superscript letter were significantly different at ($P<0.05$).

Where:

A : Control biscuit sample.

B1 : Biscuit with 1 ml orange peel flavour .

C1 : Biscuit with 2 ml orange peel flavour .

D1: Biscuit with 3 ml orange peel flavour.

The sensorey characteristic of orange peel flavour added to biscuit products in deferent level declared significant ($p<0.05$) differences in color, crumtexture, and similar in odor and taste, and of general acceptability.

Table **(7)** shows the results of the sensory evaluation of biscuit products of samples (B1, C1 and D1) beside their control sample (A) treated with orange peel flavour in different levels for their colour, odor, taste, crumbtexure and

general acceptability. Results showed no significant difference ($p > 0.05$) in their odor, taste and general acceptability, as well as that obtained results different in crumb texture between(A,C1)and between (B1,D1) these variation might be due to different added flavour.

Compared to Hanan and Rasha(2012) , who reported biscuit with 10% of the four studied citrus peels powders improved all studied sensory characteristics in all the studied biscuit samples. However, the best scores of all studied sensory evalution were recorded for 10% Baladi orange peel powder supplemented biscuit and (abosora) orange peel powder supplemented biscuit.

Table (8):. Sensory evolution of biscuit with Mentha oil

Treatment–sample	Color	Odor	Taste	Crumb texture	General acceptability
A	1.85 ±0.75ᵅ	2.50 ±1.15ᵅ	2.15 ±0.99ᵅ	2.15 ±0.81ᵅ	2.40 ±0.88ᵅ
B2	1.85 ±0.93ᵅ	2.05 ±1.05ᵅ	2.45 ±1.23ᵅ	2.55 ±1.19ᵅ	2.55 ±1.36ᵅ
C2	2.25 ±1.21ᵅ	2.40 ±1.19ᵅ	2.60 ±1.05ᵅ	2.40 ±1.19ᵅ	2.70 ±1.22ᵅ
D2	2.10 ±0.91ᵅ	2.25 ±1.16ᵅ	2.30 ±1.08ᵅ	2.15 ±1.14ᵅ	2.55 ±1.19ᵅ

Mean values were ±SD of triplicate independent analysis.

values in the same column carrying different superscript letter were significantly different at ($P<0.05$).

Where:

A : Control biscuit sample.

B2 : Biscuit with 1ml mint flavour .

C2 : Biscuit with 2 ml mint flavour.

D2: Biscuit with 3 ml mint flavour .

There were no significant ($p > 0.05$) differences in color, odor, taste, and crumb texture and general acceptability between A2, B2 ,C2, D2.

4.6 Sensory analysis

Biscuit with mint flavour similar in colour , odor, taste , crumb texture , and general acceptability were compared with control A . Mint (*MenthaSpicata* L.) was used as a source of natural antioxidant in the preparation of biscuits with optimized levels of different forms of mint (powder, extract and pure menthol). The biscuits were stored for five months packed in unit pouches of metalized polyester/poly laminated pouch at room temperature and tested periodically for sensory parameters. The stability of biscuit lipids were studied by determining free fatty acid, peroxide value, acid value and total antioxidant capacity. In sensory evaluation, pure menthol (MNT-M) scored higher ($p<0.05$) panel scored than mint powder (MNT-P) and mint extract (MNT-E) biscuit
(ShIv et al , 2016).

The overall acceptability of biscuits prepared with natural antioxidant in different forms of mint was acceptable up to a storage period of 5 months.

In terms of both chemical and sensory evaluation, MNT-P biscuits were comparable to that of BHA biscuits when compared to MNT–M and MNT-E variations. The study syndicated the antioxidant efficiency of different mint forms in preventing the onset of rancidity in biscuits during storage suggesting the retention of bioactive components of mint. With rapid growth and changing eating habits of people, bakery products have gained popularity among masses in India. The bakery products including bread and biscuit form the major baked foods of total bakery product produced in the country. The study emphasized the use of mint in baked products as potential substitute for synthetic antioxidants in food preservation.
(ShIv, et al 2016).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

1- The study concluded that local row material must be available while making biscuit at home and manufactory.

2-Also it is found that the biscuit treated with low level(1 ml) of orange peel flavour best than high and very acceptability in sensory evalution.

3- It was found no deference between biscuit treated with mint compared with control.

5.2 Recommendation

It is recommended that more investigation are needed to:-

1- Encourage use natural flavour in biscuit in low level of flavour (1ml) with biscuit treated orange peel .
2- More research is recommended on the use of natural flavour from local fruits for the production of biscuit.
3- Orange peel and mint flavour can be used in other food.

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Appendices

Sensory evaluation of biscuit samples

Please evaluate the following samples of Biscuit added orange peel and mintflavor according to their colour, Flavor, taste, texture, Overall quality,. The ranking scores are given below:

1= Excellent 2= Very good 3= Good 4= Acceptable 5= unacceptable

Samples	**Colour**	**Flavor**	**Taste**	**Texture**	**Overall Quality**
A					
B1					
C1					
D1					
B2					
C2					
D2					

A : Control biscuit sample.

B1 : Biscuit add orange peel flavour 1ml .

B2 : Biscuit add mint flavour 1ml.

C1 : Biscuit add orange peel flavour 2ml.

C2 : Biscuit add mint flavour 2ml .

D1: Biscuit add orange peel flavour 3 ml.

D2: Biscuit add mint flavour 3 ml .