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Effect of Chamomile, Marjoram and their Oils Incorporation on Properties of Oat Biscuits

El-Zainy, A.R.M., Shalaby, A.O., El-Zamzamy, F.M. and Mostafa, M.Y.A.

Home Economics Dept., Faculty of Specific Education, Mansoura University, Egypt.

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ABSTRACT

Biscuits are convenient food products, becoming very popular among Egyptians. A great deal of effort has therefore focused on supplementing products with medicinal and herbal plants to enrich the functional value. This study was designed to evaluate the effects of supplementing of wheat biscuits with oat flour, herbs and essential oils on chemical, sensory, physical and microbiological characteristics. Hence, standard formulation based on wheat flour was substituted with oat *(Avena sativa* L.*)* flour at 20%. Then, 20% oat biscuit was flavored by 3% chamomile *(Matricaria chamomilla* L.), 3% marjoram (*Origanum majorana* L.) and the essential oils (EOs) of the same herbs respectively by 0.25% and 0.1%. Biscuits supplemented with herbs and its essential oils were subjected to sensory studies, chemical analysis and microbiological evaluation in comparison with wheat biscuits. Results revealed that the addition of examined herbs and their essential oils (EOs) enhanced the oat biscuit taste. Meanwhile, it caused a small decrease in all physical parameters except specific volume. The addition of oat, herbs or its essential oils increased the crispiness during fridge storage comparing to the wheat biscuit control. Also, it was observed that crispiness scores were more stable during fridge storage period as it increased slightly comparing to the room temperature storage. The changes occurred in acid value (AV), Peroxide value (PV) and Thiobarbituric acid (TBA) in oat biscuit control sample were lower than changes occurred in values of WF biscuit "control sample". Results indicated that the increments in AV, PV and TBA value for samples stored at low temperature (3°C) were less than that occurred for the samples stored at room temperature (25°C). Adding examined herbs essential oils to oat biscuits prevented any bacterial growth over storage period for six month in both room temperature and fridge temperature. Meanwhile, the addition of herbs controlled the bacterial growth in prepared biscuits among storage period in both room temperature and fridge temperature. It can be concluded that incorporation of oat and herbal powder or oils in bakery products improve the sensory properties, chemical, physical composition and prolong the shelf-life of prepared biscuits.

Key words: Biscuit, Oat, Chamomile, Marjoram, Essential oils, Sensory evaluation, Yeasts and molds.

Introduction

Biscuits are ready-to-eat, cheap and conveniently eating food among all age groups in many countries of the world (Hussein *et al*., 2006; Iwegbue, 2012). Plants contain a variety of substances called ‘‘Phytochemicals’’, that owe to naturally occurring components present in plants. The phytochemical preparations with dual functionalities in preventing lipid oxidation and antimicrobial properties have tremendous potential for extending shelf life of food products (Caragay, 1992).

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them desired shelf life. Because food products are now often sold in areas of the world far remote from their production sites, the need for extended safe shelf-life for these products has also expanded. Improvements in the cold distribution chain have made international trade of perish able foods possible, but refrigeration alone cannot assure the quality and safety of all perishes able foods. Although the value of traditional food preservatives has been recognized, their safety has been questioned (Branen, 1983).

Originally added to change or improve taste, spices and herbs can also enhance shelf-life because of their antimicrobial nature. Some of these substances are also known to contribute to the self-defense of plants against infectious organisms (Kim *et al*., 2001).

Oat (*Avena sativa* L.) is an important crop produced in many regions of Europe and North America. It has a well-balanced distribution of proteins and several vitamins and minerals essential for human health (Sadiq *et al*., 2008) as well as being a rich source of soluble fiber (predominantly β-glucan). Whole-grain oats have the greatest percentage of fat among the major cereals with a good balance of the essential fatty acids, which are primarily unsaturated. The high content of oleic and linoleic acid, results in a favorable polyunsaturated to saturated fatty acid ratio of 2:2 (Salehifar and Shahedi, 2007). Amongst the cereals, oat (*Avena sativa* L.) can have one of the greatest levels of lipids, in the range of 3.1–11.6% (Leonova *et al*., 2008). Piñero *et al*. (2008)

Corresponding Author: El-Zainy, A.R.M., Home Economics Dept., Faculty of Specific Education, Mansoura University, Egypt.
E-mail: monayaser86@yahoo.com

evaluated the effect of adding oat (*Avena sativa* L.) fiber source of β-glucan (13.45%) on physical, chemical, microbiological and sensory traits of low-fat (<10%) beef patties as compared to 20% fat control patties. Significant ($p < 0.05$) improvements in cooking yield (74.19%), and retentions of fat (79.74%) and moisture (48.41%) of low-fat patties were attributed to the water binding ability of β-glucan. Besides appearance, tenderness and color were not affected by the addition of oat's soluble fiber. Oat fiber can be used successfully as a fat substitute in low-fat beef patties. The crude fat level of naked grain oats was (8.4% *DM*) (Biel *et al*., 2009). Meanwhile Aro *et al*. (2007) mentioned that naked grain oats contain exceptional quantities in comparison to other feed cereals (on average about 7.9% more) and it is a rich source of unsaturated fatty acids.

Chamomile is most popular used medicinal plant and extensively consumed as a tea or tisanes. Traditionally this plant was used for treatment of many ailments such as allergy disorders and inflammatory mediated diseases (Chandrashekhar *et al.*, 2011). Total of 21 compounds of *Matricaria chamomilla* L. flower essential oil were identified using gas chromatography/mass spectrometry (GC/MS) accounting for 92.86% of the oil composition. The main compounds identified were α-bisabolol (56.86%), trans-trans-farnesol (15.64%), cis-β-farnesene (7.12%), guaiazulene (4.24%), α-cubebene (2.69%), α-bisabolol oxide A (2.19%) and chamazulene (2.18%) (Tolouee *et al.*, 2010). Chemical profiling both by GC and GC–MS analyses of the hydrodistilled essential oil of *M. recutita* revealed α-bisabolol oxide A (28%), α-bisabolol oxide B (17.1%), (*Z*)-β-Farnesene (15.9%) and α-bisabolol (6.8%) as the main components (Can *et al.*, 2012). *Chamomilla nobile* proved to be an equilibrated valuable herb rich in carbohydrates and proteins, and poor in fat, providing tocopherols, carotenoids and essential fatty acids (C18:2n6 and C18:3n3). Moreover, the herb and its infusion are a source of phenolic compounds (flavonoids such as flavonols and flavones, phenolic acids and derivatives) and organic acids (oxalic, quinic, malic, citric and fumaric acids) that showed antioxidant and antitumour activities, without hepatotoxicity (Guimarães *et al.,* 2013). The potential of *M. chamomilla* L. essential oil in preventing fungal contamination and subsequent deterioration of stored food and other susceptible materials was indicated by (Tolouee *et al,* 2010). Essential oil and extracts of two Egyptian plants, fennel and chamomile were examined for their antioxidant and antimicrobial activities. The essential oil for fennel seeds and chamomile flowers were found to be 1.95 and 0.73%, respectively. Antioxidant activities of the extracts were evaluated using the DPPH radical scavenging. The statistical analysis showed that the highest antiradical power (ARP) was noticed for chamomile extracted by methanol, where is fennel extracted by hexane gave the least value which was 243 (Roby *et al.*, 2012). The results of the present investigation demonstrated significant variations in the antioxidant and antimicrobial activities of fennel and chamomile essential oil and extracts. The most abundant compounds in *Matricaria recutita* L. (German chamomile) extract and infusion were 5-*O*-caffeoylquinic acid and an apigenin derivative. These, as well as other bioactive compounds, are affected in *C. nobile* decoction, leading to a lower antioxidant potential and absence of antitumour potential (Guimarães *et al.*, 2013).

Commercial *O. majorana L.* oil is used as a spice and condiment. The fresh or dried highly aromatic leaves and flowering tops of marjoram (*O. majorana L.*) are widely used to flavour many foods. The volatile aromatic compounds are employed in the food industry as flavouring in foods and beverages. The oil is used in perfumery for its spicy herbaceous notes (Vera and Chane-Ming, 1999). Biologically active ingredients from different mixtures of water and organic solvents of dried aerial parts of *O. majorana* L. are phenolic acids (gallic, caffeic, p coumaric and ferulic) and flavonoids (rutin, apigenin and eriodyctiol) (Proestos and Komaitis, 2008). Marjoram contains phenolic terpenoids (thymol and carvacrol), flavonoids (diosmetin, luteolin, and apigenin), tannins, hydroquinone, phenolic glycosides (arbutin, methyl arbutin, vitexin, orientin, and thymonin) and triterpenoids (ursolic acid and oleanolic acid) (El-Ashmawy *et al*., 2005). The essential oil of *O. majorana* is known for its strong antimicrobial activity, so it could be used by food industries as natural preservatives (Sellamia *et al*., 2009). EOs might be more effective against food-borne pathogens and spoilage bacteria when applied to ready to use foods containing a high protein level at acidic pH, as well as lower levels of fats or carbohydrates (Gutierrez *et al*., 2008). Among several essential oils marjoram (*O. majorana* L.) may be useful as antimicrobial agents, marjoram oil possesses antimicrobial properties against food borne bacteria and mycotoxigenic fungi and therefore it may have the greatest potential for use in industrial applications (Ezzeddine *et al*., 2001). Significant linear correlation were observed between the total phenolics concentration and antioxidant values. Marjoram which is rich in phenolic content (40 GAE) had a strong antioxidant activity (Karakaya *et al*., 2001). Oils marjoram (*O. majorana* L.) has strong antioxidant activity, mainly because of its high content of phenolic acids and flavonoids, which is useful in health supplements and food preservation (Vagi *et al*., 2005). Several studies reported that methanolic extracts of marjoram (*Origanum majorana* L.) had high antioxidant capacity (Hossain *et al*., 2008).

Aim of the work:

The current study was designed to evaluate the effects of supplementing of wheat biscuits with oat flour, herbs and essential oils on chemical, sensory, physical and microbiological characteristics.

Materials and Methods

Materials:

Biscuits ingredients:

Chamomile (*Matricaria recutita* L.), marjoram (*Origanum marjorana*) and their essential oils were obtained from National Research Center, Giza, Egypt.

Green′s white oats (*Avena sativa* L.), American wheat flour (72% extraction), sunflower oil, full cream milk, sugar, backing powder, vanilla, salt and eggs were purchased from local market of different areas in Mansoura city, Egypt.

Growth media:

Potato Dextrose Agar REF 4019352 (Biolife) produced by Becton, Dickinson and Company sparks, France and Nutrient agar produced by El Nasr pharmaceutical chemicals, Egypt were purchased from El-Gomhoria company for chemical and drugs, Cairo, Egypt.

Methods:

Preparation of biscuits:

The oat grains were grinded well until become soft powder. Each of chamomile (*Matricaria recutita L.*) and marjoram (*Origanum marjorana*) was grinded well until become fine powder then sieved. Six formulas of biscuits were given in Table A; Wheat Flour (WF) biscuit, Oat Flour (OF) biscuit, Chamomile Powder Oat (ChPO) biscuit, Marjoram Powder Oat (MPO) biscuit, Chamomile Oil Oat (ChOO) biscuit and Marjoram Oil Oat (MOO) biscuit. For making biscuit, sugar, oil and salt were creamed together. Egg, milk, vanilla and backing powder were added to the creamed ingredients and then the flour was blended with a minimum of mixing. The molded biscuit dough was baked at about 230°C (El-Nemr and Fahmy,1979). Biscuits were packed in poly ethylene bags. A half of the quantity was stored at room temperature (25±2°C) and the other part was stored at fridge (3±1°C) at the beginning of October for six months.

Table A: Formulas used for making biscuits (g/100 g)

Ingredients	Biscuits					
(gm)	WF	OF	ChPO	MPO	ChOO	MOO
Wheat flour	100	80	77	77	80	80
Sucrose	40	40	40	40	40	40
Sunflower oil	16	16	16	16	15.75	15.9
Backing powder	1.6	1.6	1.6	1.6	1.6	1.6
Vanilla	1.0	1.0	1.0	1.0	1.0	1.0
Full cream milk	2.6	2.6	2.6	2.6	2.6	2.6
Salt	0.36	0.36	0.36	0.36	0.36	0.36
Egg	20	20	20	20	20	20
Oat	-	20	20	20	20	20
ChP	-	-	3	-		-
MP	-	-	-	3	-	
ChO					0.25	
MO						0.1

WF: Wheat Flour; OF: Oat Flour; ChP: Chamomile Powder; MP: Marjoram powder; ChO: Chamomile Oil; MO: Marjoram Oil; ChPO: Chamomile Powder Oat; MPO: Marjoram powder Oat; ChOO: Chamomile Oil Oat and MOO: Marjoram Oil Oat.

Chemical analysis:

Moisture, crude protein and crude fat were carried out according to the methods of A.O.A.C. (1995); Ash contents were carried out according to the method of A.O.A.C. (2000); Carbohydrates was calculated by the following equation: Carbohydrates = 100 – (% moisture + % protein + % fat + % ash).

Gas chromatographic (GC) analysis of essential oils:

The volatile oils were analyzed according to Singh *et al*. (2007) using Ds chrom 6200 Gas Chromatograph equipped with a flame ionization detector for separation of volatile oils. The analysis conditions were as follows: The chromatograph apparatus was filled with capillary column BPX-5.5% phenyl (equiv.) polysillphenylene-siloxane 30m × 0.25µm film. Temperature program increases with rate of 10°C/min from 70°C to 190°C. Flow rates of nitrogen gas were at 1 ml/min and air at 330 ml/min. Detector and injector temperatures were 300°C and 250°C, respectively. The obtained chromatogram and report of GC analysis for each sample was analyzed to calculate the percentage of main components of volatile oils.

Extraction of fat from prepared biscuits:

One hundred gram of crushed biscuit was placed in a closed stopper flask (500ml). The flask was shaken for 30 min using horizontal shaker and left for 24 hr at room temperature. The homogenized mixture was filtered with suction and the residue was re-extracted as mention above. The combined filtrates were evaporated under reduced pressure according to A O A C (2005).

Sensory evaluation of biscuits:

Sensory evaluation of biscuits were carried out to determine their sensory characteristics according to Moor (1970) which expressed as (10) excellent, (9) very good, (8) good, (7) medium, (6) fair, (5) poor, (4) very poor and (3) extremely poor.

Physical analysis:

Physical analysis of biscuits was carried out in the National Research Center, Giza, Egypt according to AACC, (2000) method as following: Thickness of biscuits was measured by stacking six biscuits on top of one another and taking average thickness of six biscuits in *cm*. Percent spread ratio was calculated by dividing the average value of diameter by average value of thickness of biscuits. Diameter was measured by Boclase (HL 474938, STECO, Germany). Also, volume and weight of biscuits were determined according to standard methods (AACC, 2000).

Color determinations:

Objective evaluation of biscuit surface color of biscuits samples was measured in the National Research Center, Giza, Egypt. Hunter L*(luminosity) ,a*(red intensity), and b*(yellow intensity) parameters were measured with a color difference meter using a spectrocolorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode. The instrument was standardized with white tile of Hunter Lab Color Standard (LX No.16379): X= 72.26, Y= 81.94 and Z= 88.14 (L*= 92.46; a*= -0.86; b*= -0.16) (Sapers & Douglas, 1987). The Hue (H)* and Chroma (C)* were calculated according to the method of (Palou et al., 1999).

Rheological properties:

Dough characteristics (water absorption, dough development time, dough stability, weakening and mixing tolerance index) were carried out in the National Research Center, Giza, Egypt according to A. A. C. C. (2000) using Farinograph (model No: 81010, Duisburg, Germany). Fifty grams of flour were mixed at the optimum water absorpition level and the farinograph curve was centered on the 500 BU(Branbender Unit) line.

Crispiness determinations:

Crispiness was calculated by the following equation:

$$\text{Ratio of crispiness} = \frac{\%\ \text{moisture}}{\%\ \text{fat}}$$

Peroxide value (PV):

Peroxide value (PV) of stored oil was determined as described by Leonard *et al*., (1987) by dissolving one g of oil sample in 30 ml glacial acetic acid/chloroform solution (60/40 v/v), then adding of 1 ml potassium iodide (15%) and titrating the iodide liberated with 0.1N sodium thiosilfate solution. The peroxide value was expressed as milli-equivalent of oxygen per 1 kg of sample.

Titrable acidity (AV):

Acid value (AV) was determined according to the method of A.O.A.C. (2005). A known weight (5-10 g) of oil was dissolved in 25 ml of ethanol. The solution was then warmed and directly titrated using 0.01N potassium hydroxide solution using phenolphthalein as an indicator until appearance the pink colour. A blank was carried out by the same manner using distilled water.

Calculation: $$AV = \frac{(Vs - Vb) \times N \times 56.1}{W}$$

Where:

Vs = Volume of KOH required for sample titration.
Vb = Volume of KOH required for blank titration.
N = Normality of KOH.
W = Weight of oil (g).

Thiobarbituric acid (TBA):

Thiobarbituric acid value was determined according to the method described by Ottolenghi (1959). Known weight of oil (0.5 g) was put in test tube and thoroughly mixed with TBA reagent (6 ml of 0.3% TBA and 3 ml of 20% Trichloroacetic acid). After closing the tube and shaking the mixture was heated in boiling water for 30 min and then cooled under running tap water. The interfering substances were extracted 3 times with ether (3 ml each) and evaporation loss was replaced by adding distilled water. The aqueous phase was measured at 538nm using a spectrophotometer model Perkin-Elmer 35 spectrophotometer, for in vitro Diagnostic, USA.

TBA value (mg malondialdehyde/kg oil) = D

Where: D is absorbance at 538 nm.

Microbiological analysis:

Microbiological count data are expressed as colony forming units (cfu) per 1 ml. eight dilutions were carried out to determine the number of bacteria during storage. One ml of each dilution was aseptically plated in the media plate.

Enumeration of total bacterial count (TBC):

Plate count agar medium (A P H A; 1971) was used for enumeration the total microbiological count. A known volume of sterile sample 1.00 ml was added to Petri-plates containing agar medium, then the Petri-plates was incubated at 37°C for 48 hours. The total bacterial count was recorded as colony numbers per 1.00 ml of sample.

Enumeration of yeast and moulds counts:

Potato dextrose agar medium (Difico) was used for moulds and yeasts count by poured plates method according to A P H A (1992). The plates were incubated at 30 °C for 3.5 days.

Statistical analysis:

All the obtained data were statistically analyzed by SPSS computer software. The calculated data occurred by analysis of variance ANOVA and follow up test LSD by SPSS ver.11 according to Abo-Allam (2003).

Results and Discussion

Chemical composition of marjoram and chamomile:

As shown in Table 1, chamomile recorded 9.970±0.22, 18.340±0.33, 8.568±0.09, 3.716±0.03 and 59.405±0.29 *g/100g* wet weight for moisture, protein, ash, fat and carbohydrates respectively. On the other hand, the marjoram recorded 8.940±0.10, 14.076±0.05, 13.263±0.18, 5.21±0.22 and 58.509±0.22 *g/100g* wet weight for moisture, protein, ash, fat and carbohydrates respectively. It could be observed from these results that chamomile was richer in moisture, protein and carbohydrates than marjoram which recorded higher contents of ash and fat than chamomile.

According to National Nutrient Database (2002), every 100 *g* of marjoram *(Origanum marjorana)* contains moisture (7.64 *g*), protein (12.66 *g*), fat (7.04 *g*), carbohydrates (60.56 *g*) and energy (271*kcal*).

Table 1: Chemical composition of tested herbs (marjoram and chamomile)

Samples		Parameters					
		Moisture%	Total protein%	Ash%	Fat%	Carbohydrate%	Energy (*kc*)
Chamomile	Mean	9.970^{a}	18.340^{a}	8.568^{b}	3.716^{b}	59.405^{a}	343.866^{a}
	SD	±0.22	±0.33	±0.09	±0.03	±0.29	±1.84
Marjoram	Mean	8.940^{b}	14.076^{b}	13.263^{a}	5.21^{a}	58.509^{b}	337.232^{b}
	SD	±0.10	±0.05	±0.18	±0.04	±0.22	±1.12

SD: Standard Division. a, b, c, d,. . . :Different superscripts within the same column represent significant differences between the results (p ≤ 0.05).

Volatile components of chamomile (*Matricaria recutita* L.) essential oil by Gas Chromatographic (GC) analysis:

The chamomile essential oils were fractionated into 21 peaks as shown in Table 2. These peaks were identified and quantified as follows: α-pinene (1.4%), Sabinene (0.3%), β-pinene (0.1%), α-phellandrene (0.4%), α-terpinene (0.1%). Γ-terpinene (0.3%), Terpinen-4-ol (22.1%), Methyl acetate (0.3%), α-cubebene (1.9%), Cis-β-farnesene (0.3%), β-bisabolene (2.1%), Trans-nerolidol (1%), Spathulenol (0.5%), Caryophyllene oxide (1.2%), Viridiflorene (6.6%), β -bisabolol (7.3%), α-bisabolol oxide A (1.4%), α-bisabolol (46.4%), Chamazulene (0.3%), Trans-trans-farnesol (6.1%) and Guaiazulene (0.6%). Results also show that α-bisabolol is the major constituent followed by Terpinen-4-ol, β -bisabolol then Viridiflorene. These results are in agreement with those obtained by Afify *et al.* (2012) as they mentioned that the major essential oil contents of chamomile are α-bisabolol oxide A (35.251%) and trans- α -farersene (7.758%). Also, Orav *et al.* (2010) stated that the main biologically active compounds in chamomile oil have been found to be bisabolol oxides, bisabolone oxide, a-bisabolol, spathulenol, enyne-dicycloethers, and chamazulene. Meanwhile, Raal *et al.* (2012) found that the major phenolic compounds in the chamomile infusions were chlorogenic acids, ferulic acid glycosides, dicaffeoyl quinic acids and apigenin glycosides.

Table 2: Volatile components of Chamomile essential oil by Gas Chromatographic (GC) analysis

Peak no.	Identification	KI	area %
1	α-pinene	930	1.4
2	Sabinene	962	0.3
3	β-pinene	965	0.1
4	α-phellandrene	985	0.4
5	α-terpinene	1012	0.1
6	Γ-terpinene	1043	0.3
7	Terpinen-4-ol	1150	22.1
8	Methyl acetate	1278	0.3
9	α-cubebene	1356	1.9
10	Cis-β-farnesene	1447	0.3
11	β-bisabolene	1480	2.1
12	Trans-nerolidol	1494	1
13	Spathulenol	1549	0.5
14	Caryophyllene oxide	1553	1.2
15	Viridiflorene	1600	6.6
16	β -bisabolol	1616	7.3
17	A-bisabolol oxide A	1647	1.4
18	α-bisabolol	1680	46.4
19	Chamazulene	1720	0.3
20	Trans-trans-farnesol	1739	6.1
21	Guaiazulene	1840	0.6

Volatile components of marjoram (*Origanum marjorana*) essential oil by Gas Chromatographic (GC) analysis:

The volatile components of marjoram essential oils which were determined by Gc-Ms are shown in Table 3 as follows:

Table 3: Volatile components of marjoram essential oil by Gas Chromatographic (GC) analysis:

Peak no.	Identification	KI	Area %
1	α –Thujene	925	0.7
2	α -Pinene	934	0.8
3	Sabinene	978	6.2
4	Myrcene	985	0.5
5	α -Phellandrene	1002	2
6	p-Cymene	1019	0.4
7	Limonene	1028	9.4
8	Ɣ-terpinene	1053	2.2
9	trans-sabinene hydrate	1084	4.6
10	Linalool	1085	16
11	cis-Sabinene	1090	4.7
12	cis-Sabinene hydrate	1090	3.7
13	trans-para-Menth-2en-1-ol	1117	10.6
14	Camphor	1139	1.3
15	Terpinen-4-ol	1175	27.6
16	α -Terpineol	1182	3.7
17	Thymol	1286	0.6
18	Geranyl acetate	1360	2
19	α –Caryophyllene	1426	2.1
20	α -Humulene	1460	0.9

α –Thujene (0.7%), α -Pinene (0.8%), Sabinene (6.2%), Myrcene (0.5%), α -Phellandrene (2%), p-Cymene (0.4%), Limonene (9.4%) Ɣ-terpinene (2.2%), trans-sabinene hydrate (4.6%), Linalool (16%), cis-Sabinene (4.7%), cis-Sabinene hydrate (3.7%), trans-para-Menth-2en-1-ol (10.6%), Camphor (1.3%), Terpinen-4-ol (27.6%), α -Terpineol (3.7%), Thymol (0.6%), Geranyl acetate (2%), α -Caryophyllene (2.1) and α -Humulene (0.9%). Terpinen-4-ol was the main constituent followed by trans-para-Menth-2en-1-ol, lumonene then sabinene.

These results were in agreement with Afify *et al.* (2012) who found that the main components of marjoram are terpinen-4-ol (23.860%), p-cymene (23.404%) and sabinene (10.904%). Meanwhile, the main constituents found by Ramos *et al*. (2011) in *Origanum majorana* L. (Lamiaceae) were: *cis*-sabinene hydrate (30.2%), terpinen-4-ol (28.8%), γ-terpinene (7.2%), α-terpineol (6.9%), *trans*-sabinene hydrate (4.4%), linalyl acetate (3.8%), and α-terpinene (3.6%). Also our results were in the same trend as those of Vera and Chane-Ming

(1999) who stated that the essential oil of the water-distilled marjoram (*Origanum majorana L.*) was found to be rich in terpinen-4-ol (38.4%), cis-sabinene hydrate (15.0%), p-cymene (7.0%) and γ-terpinene (6.9%).

Comparison between organoleptic scores of oat biscuits flavored with herbs̉ powder and herbs̉ essential oils:

Table 4 represented the effect of flavoring OF biscuit with (3% chamomile & 3% marjoram) powder and (0.25% chamomile & 0.1% marjoram) essential oils on the orangoleptic parameters. Color scores ranged from 8.5± 0.22 to 6.8 ± 0.18. It could be observed that the addition of (chamomile and marjoram) EOs decreased the color scores as compared to the control sample which recorded the highest color score. Significant differences were observed between the WF biscuit and the other samples in taste. It could be noticed that the addition of oat, chamomile and its essential oil enhanced the biscuit taste. No significant (p>0.05) differences were found between control and samples in texture as it ranged from 9.2 ± 0.15 to 8.6 ± 0.13 while, the odor scores ranged from 8.7 ± 0.35 for OF biscuit to 7.5 ± 0.19 for the ChPO biscuit. The difference in texture is due to high protein content in oat as reported by Naseem *et al*. (2013).

Similar findings were obtained by Conforti *et al*. (1997). There were significant differences between the control and the biscuit samples in the crust appearance for the control. The lowest score of crust appearance was 7.1 ± 0.36 for the MOO biscuit. All samples recorded high scores of overall acceptability as it ranged from 9.4 ± 0.03 for the OF biscuit as control to 8.0 ± 0.26 for MOO biscuits.

Naseem *et al*. (2013) mentioned that data regarding overall acceptability vary significantly among different treatments. Overall acceptability is totally different in quality parameters and it was not affected by individual trend of color texture and flavor. Chakrabarti *et al*. (2001) also obtained results in conformity with the present study. The refreshing pleasant aroma, biting taste and carminative properties of ginger make it an indispensable ingredient of food processing throughout the world. In western countries ginger is used widely for culinary purpose such as in ginger bread, biscuits, cakes, puddings, soups, and pickles. In the East, fresh ginger chopped in small bites and in the ground form is used widely in vegetarian and nonvegetarian food preparations. It is also used in pickling, soft drink making, confectionery and curry powder preparations (Vasala, 2001).

Table 4: Organoleptic evaluation of herbal biscuits.

Samples	Parameters						
	Color (10)	Taste (10)	Odor (10)	Texture (10)	Appearance (10)	Overall acceptability (10)	Mean scores (10)
Control WF biscuit	8.5^a±0.22	8.4^a±0.16	8.5^a±0.22	9.0 ± 0.12	8. 5^a±0.18	9.3^a±0.07	8.7
OF biscuit	8.4^a±0.24	8.5^a±0.15	8.7^a±0.35	9.2 ± 0.15	8. 3^a±0.22	9.4^a±0.03	8.8
ChPO biscuit	8.4^a ±0.33	8.7^a±0.11	7.5^b±0.19	8.7 ± 0.15	8.1^a±0.15	8.1^b±0.13	8.2
MPO biscuit	8.5^a±0.28	8.1^b±0.32	8.0^{ab}±0.21	8.6 ± 0.13	7.8^{ab}±0.26	8.2^b±0.15	8.2
ChOO biscuit	7.8^a ±0.36	8.4^a±0.16	8.4^a ±0.30	8.7 ± 0.15	7.3^b±0.22	8.2^b±0.35	8.1
MOO biscuit	6.8^b±0.18	7.8^b±0.22	7.8^b ±0.18	8.9±0.22	7.1^b±0.36	8.0^b±0.26	7.7
LSD at 0.05	0.93	0.62	0.75	NS	0.82	1.03	-

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05.

Proximate chemical composition of herbal oat biscuits: Results in Table 5 shows that moisture content ranged from 3.6 to 4.2% the highest moisture percentage (4.2±0.05 %) was for ChPO biscuit and the lowest moisture content (3.6±0.02 %) was for OF biscuit. Significant differences were observed between samples and control in the protein content (g/100g ww) which ranged from 7.79 to 8.84%. MOO biscuit gained the highest protein content (8.84±0.07%), while the lowest protein content (7.79±0.05%) was for ChOO biscuit. MPO biscuit recorded the highest ash content (1.20±0.01%) followed by ChPO biscuit, (1.17±0.02%); however the lowest ash content (0.91±0.02%) was for OF biscuit. There were a significant difference between the control and other samples in the fat content which ranged from 14.8% to 15.6%.

The highest fat content 15.5% was for OF and MPO biscuits and the lowest fat value (15.0±0.11 %) was for MOO biscuit. On the other hand, the carbohydrate content ranged from 70.93% to 72.15%. Also the energy recorded (457.72±0.1, 459.43±0.2, 455.55±0.0, 457.07±0.1, 456.30±0.6 and 455.54±0.4 kcal/100g) for WF, OF, ChPO, MPO, ChOO and MOO biscuit, respectively. Results are in accordance with Moudr, (1992) who recorded that naked oat grains in comparison with the husked ones have not only less fiber but their chemical composition is also much more favorable. In the Czech Republic, naked oats are recommended as food for children, the youth, hardworking and ill or old people.

Considering a specific chemical composition of oat grains and their products, it is recommended to use them in the baking and confectionery industry to a larger extent (Flander *et al*., 2007). In Poland, there is still not enough interest in producing oat products using the oat wholemeal. Some authors reported that oats show the highest variability of chemical composition under the influence of environmental factors among cereals (Pettersson *et al*., 1996). This also resulted in the differentiation of its nutritive value.

Table 5: Chemical composition of herbal oat biscuits.

Samples	Parameters					
	Moisture%	Total protein%	Ash%	Fat%	Carbohydrates%	Energy (*kc*)
Control WF biscuit	$3.7^{d}\pm0.07$	$8.01^{d}\pm0.04$	$1.12^{c}\pm0.01$	$15.4^{ab}\pm0.09$	$71.77^{b}\pm0.19$	$457.72^{b}\pm0.1$
OF biscuit	$3.6^{e}\pm0.02$	$8.38^{b}\pm0.03$	$0.91^{d}\pm0.02$	$15.5^{a}\pm0.08$	$71.60^{bc}\pm0.15$	$459.43^{a}\pm0.2$
ChPO biscuit	$4.2^{a}\pm0.05$	$8.30^{bc}\pm0.09$	$1.17^{b}\pm0.02$	$15.4^{ab}\pm0.05$	$70.93^{d}\pm0.12$	$455.55^{e}\pm0.0$
MPO biscuit	$3.9^{b}\pm0.02$	$8.24^{c}\pm0.11$	$1.20^{a}\pm0.01$	$15.5^{a}\pm0.03$	$71.15^{cd}\pm0.15$	$457.07^{c}\pm0.1$
ChOO biscuit	$3.8^{c}\pm0.04$	$7.79^{e}\pm0.05$	$1.12^{c}\pm0.01$	$15.2^{b}\pm0.17$	$72.15^{a}\pm0.24$	$456.30^{d}\pm0.6$
MOO biscuit	$3.7^{d}\pm0.03$	$8.84^{a}\pm0.07$	$1.16^{bc}\pm0.01$	$15.0^{c}\pm0.11$	$71.29^{c}\pm0.20$	$455.54^{e}\pm0.4$

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05.

Physical characteristics (baking quality) of herbal oat biscuits:

Physical properties of biscuit samples are recorded in Table 6. Significant differences were observed between all samples in both of weight and volume in comparison with the control. It could be noticed that the addition of herbs and its EOs decreased weight and volume scores. Result show that there are significant differences between all samples in specific volume (*cc/g*). It is noticeable that the addition of herbs to oat biscuits decreased the specific volume; however the addition of the same herbs EOs increased the specific volume scores. Also significant differences were observed between samples in both diameter and thickness. Data indicated that the addition of herbs and its EOs decreased the diameter and thickness scores. Data also show that spread ratio ranged from 5.13 for WF and ChPO biscuits to 4.34 ± 0.07 for the ChOO biscuits. Finally, it could be observed from the previous table that the addition of tests herbs to the prepared oat biscuits caused a small decrease in all of physical parameter. Meanwhile, the addition of tests herbs essential oils to the prepared oat biscuits caused a small decreasing in all physical parameters except specific volume which recorded high scores in oat biscuits flavored by herbs essential oils when compared to control.

Results are in harmony with Chappalwar *et al*. (2013) as they reported that physical properties of the oat and finger millet and wheat flour cookies revealed that maximum spread factor was observed in sample containing 30 and 40% oat flour. Hence it could be concluded that oat and finger millet flour up to the level of 40% enhances physical properties of cookies. Cookies with 20% oat flour recorded 10.80, 4.29, 0.78 and 55.0 for weight, diameter, Thickness and Spread Factor, respectively. Oat flour is characterized by low baking performance, due to small amount of gluteins, which after hydratation are responsible for creation and maintaining of wheat bread proper structure. Moreover oat flour contains considerable greater amounts of dietary fiber, which also has detrimental effect of bread quality (Gambus *et al*., 2011).

Table 6: Physical characteristics (baking quality) of herbal oat biscuits.

Samples	Parameters					
	Weight (*g*)	Volume (*ml*)	Specific volume (*ml/g*)	Diameter (*cm*)	Thickness (*cm*)	Spread ratio (diameter/ Thickness)
Control WF biscuit	$14.28^{a}\pm0.30$	$30.0^{b}\pm0.1$	$2.10^{d}\pm0.09$	$6.73^{a}\pm0.05$	$1.31^{a}\pm0.06$	$5.13^{a}\pm0.05$
OF biscuit	$12.86^{c}\pm0.47$	$31.5^{a}\pm0.2$	$2.45^{c}\pm0.01$	$6.02^{c}\pm0.06$	$1.18^{b}\pm0.08$	$5.10^{a}\pm0.06$
ChPO biscuit	$11.73^{d}\pm0.13$	$18.5^{f}\pm0.10$	$1.58^{f}\pm0.09$	$5.54^{d}\pm0.02$	$1.08^{c}\pm0.03$	$5.13^{a}\pm0.03$
MPO biscuit	$13.13^{b}\pm0.15$	$22.0^{e}\pm0.25$	$1.68^{e}\pm0.03$	$6.20^{b}\pm0.05$	$1.21^{b}\pm0.02$	$5.12^{a}\pm0.01$
ChOO biscuit	$10.13^{e}\pm0.25$	$27.5^{d}\pm0.1$	$2.71^{b}\pm0.07$	$4.77^{e}\pm0.06$	$1.10^{c}\pm0.02$	$4.34^{b}\pm0.07$
MOO biscuit	$9.64^{f}\pm0.03$	$28.5^{c}\pm0.2$	$2.96^{a}\pm0.04$	$4.55^{f}\pm0.04$	$0.90^{d}\pm0.02$	$5.05^{a}\pm0.09$
LSD at 0.05	0.22	0.48	0.056	0.534	0.048	0.542

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05.

Color characteristics of herbal oat biscuits:

The behavior of the hunter characteristics (*l* "luminosity", *a* "red intensity" and *b* "yellow intensity") of biscuit samples was studied and the obtained results are tabulated in Table 7. Results show that luminosity values ranged from 56.11 ± 0.06 to 44.90 ± 0.42. It could be noticed that OF biscuit without herbs powder or its EOs additions recorded the lightest color (56.11±0.06) as compared to the rest samples, It is noticeable also that the addition of herbs EOs made biscuits lighter than biscuits contained herbs powder. Data indicated that the intensity of red color (*a* value) ranged from 5.05 ± 0.02 for the control to 8.98 ± 0.06 for MOO biscuit.

It could be observed that the addition of herbs EOs to oat biscuit increased the red color of biscuit as compared to the biscuit containing herbs powder. Results obtained by Popov-Raljić *et al*. (2013) indicated that *a** values, with all measurements above zero, confirm that the red tone is dominating over the green in all fiber enriched dietary cookies. However, significantly more expressed red tone was detected for both surfaces of dietary cookies produced with the addition of wholemeal flour as fiber source, while dietary cookies produced with oat flakes had significantly less expressed red tone. The intensity of yellow color (*b* value) as an important color in bakery products especially biscuits, ranged from 16.93 ± 0.22 for MPO biscuit to 19.05 ± 0.35 for ChOO biscuit. It is noticeable that the addition of herbs EOs to the oat biscuit improved the yellow color.

Data obtained by Popov-Raljić *et al.* (2013) explained that the *b** values, with all measurements high above zero, confirm that the yellow tone is dominating over the blue in all fiber enriched dietary cookies, with the yellow tone being more expressed than the red tone. The intensity of the yellow tone for different dietary cookies compositions differed regarding the surface at which it was measured. The most expressed yellow tone of upper surface was detected in the case of addition of cookies with wholemeal wheat flour as fiber source. Finally, it could be observed from the previous table that hunter characteristic, improved by the addition of herbs and its EOs as compared to control, especially biscuit containing herbs EOs which gained higher color characteristics than biscuit containing herbs powder. Findings are in accordance with those obtained by Popov-Raljić *et al.* (2013) who stated that cookies produced with addition of oat flakes, wholemeal wheat flour and their mixtures as fiber sources appeared to show no difference concerning the lightness of upper surface. Meanwhile, significantly higher hue values for both surfaces of cookies produced with addition of oat flakes indicated that these products tend to have more expressed yellow tone while significantly lower hue values for cookies produced with addition of wholemeal wheat flour indicated that this product tends to have more expressed red tone.

Table 7: Color characteristics of herbal oat biscuits.

Samples	Parameters						
	L	a	B	a/b	Saturation	Hue	ΔE*
Control WF biscuit	53.40^{b}±0.18	5.05^{f}± 0.02	18.32±0.12	0.28^{d}± 0.002	19.0^{b}±0.22	74.6^{a}±0.35	56.96^{b}±0.2
OF biscuit	56.11^{a}±0.06	5.91^{d}±0.03	18.84±0.31	0.31^{c}±0.001	19.7^{ab}±0.42	72.6^{b}±0.56	59.48^{a}±0.2
ChPO biscuit	49.29^{d}±0.13	6.05^{c}± 0.05	18.25±0.45	0.33^{c}±0.007	19.2^{b}±0.22	71.7^{bc}±0.76	52.90^{d}±0.4
MPO biscuit	45.24^{e}±0.22	5.52^{e}±0.11	16.93±0.22	0.33^{c}±0.006	17.8^{c}±0.15	71.9^{c}±0.73	48.62^{f}±0.5
ChOO biscuit	51.86^{c}±0.16	6.63^{b}±0.08	19.05±0.35	0.35^{b}±0.002	20.2^{a}±0.19	70.8^{d}±0.48	55.64^{c}±0.1
MOO biscuit	44.90^{f}±0.42	8.98^{a}± 0.06	18.17±0.32	0.49^{a}±0.001	20.3^{a}±0.35	63.7^{e}±0.39	49.26^{e}±0.6
LSD at 0.05	0.178	0.073	NS	0.021	0.63	0.47	0.58

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat; Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05. l: luminosity, a: red intensity, and b: yellow intensity.

Farinograph parameters of wheat flour dough:

Rheological properties (farinograph parameters) of six different dough formula {wheat flour, wheat & oat flour, wheat & oat flour with herbs powder (3% ChP, 3% MP), wheat & oat flour with herbs essential oils (0.25% ChO and 0.1% MO)} were studied and the obtained data are shown in Table 8. Data show that all additions of herbs and their essential oils increased the water absorption expect in the case of oat flour which decreased the water absorption to 64.5%.

Earlier studies (Gibiński *et al.*, 2010) have shown that oat flour and wheat-oat flour blends were characterized by higher water absorption, which allows on the introduction of more water to the dough during mixing. Meanwhile, Rossel *et al*. (2001) proved that oat starch has higher water absorption capacity than other cereals due to the hydroxyl groups of the fiber structure which allows more water interaction through hydrogen bonding. Peymanpour *et al*. (2012) stated that water absorption capacity and the length of developmen time were both increased as the oat proportion was increased in the dough, which was consistent with the dough energy as obtained by use of the extensograph. Result in the same table revealed that adding oat flour with 3% chamomile and 3% marjoram increased the arrival time as compared to the arrival time of the control (1.5 min). On the other hand, adding oat flour with essential oil (25% chamomile or 0.1% marjoram) decreased the arrival time comparing to the control.

Data obtained by Peymanpour et al. (2012) proved that as the oat level in the flour increased, the time needed for the preparation of good dough was also increased, due to a weaker formation of gluten matrix. Since pentosans and *ß*glucans benefit form high water binding capacities, their presence in the oat flour caused slightly higher water absorption capacities, for dough made of oat as part of the formula, in comparison with control. Adding of oat, oat with herbs and oat with herbs EOs increased both dough development time and dough stability as compared to the control. The highest dough development and stability values were for the addition of oat with marjoram oil which recorded 10, 15 *min,* respectively. Salehifar and Shahedi (2007) reported that dough development time increased whereas dough stability decreased as the oat flour level increased. The longer development time of the oat-wheat dough might be due to the higher water absorption and larger particle size of oat flours. Dough resistance and dough extensibility decreased as a consequence of oat flour addition, probably due to the effects of gluten dilution, water retention and higher levels of fat. Finally, obtained results show that the addition of oat flour and oat flour with 0.1% marjoram oil decreased the mixing tolerance index, while the addition of oat with 0.25 chamomile oil had no effect on the same parameter as compared to the control which recorded 60 *Bu*.

Data represented show also that the dough weakening of the control (100% wheat) was 140 *Bu*, while the addition of 20% oat flour and oat flour with all herbs essential oils decreased the dough weaking, in the same time the addition of oat flour with all herbs powder increased the dough weaking as compared to the control. According to extensibility data obtained in this study, it is important to mention that the decrease in the gluten content of the dough and the increase of bran proportion (ending up with weaker formation of gluten matrix) could not maintain the dough extensibility. According to Salehifar and Shahedi (2007) oat flour had pronounced effects on dough properties yielding a higher water absorption and dough development time and lower dough stability and extensibility compared with the wheat flour. Also D'Appolonia and Kunerth (1990) mentioned that Farinograph characteristics and absorption increased with increasing the oat flour proportion in the formula.

Table 8: Farinograph parameters of wheat flour dough

Samples	Water absorption (*%*)	Arrival Time (*min*)	Dough development time(*min*)	Dough stability (*min*)	Mixing tolerance index (*BU*)	Dough Weakening(*BU*)
Control WF dough	65	1.5	2.0	3.0	60	140
OF dough	64.5	1.5	2.5	9.5	50	120
ChPO dough	70	2.0	6.0	6.5	140	170
MPO dough	72	4.5	7.0	5.0	130	200
ChOO dough	68	1.0	4.0	12.0	60	100
MOO dough	72	1.0	10	15	50	80

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat, and MOO: Marjoram Oil Oat.

Effect of six months storage period at room temperature (25°C) and fridge temperature (3°C) on crispiness of herbal oat biscuits:

Crispiness is an important sensory attribute in many types of food especially biscuit as it consider a factor of quality which could be affected by moisture and fat of biscuit. The mean scores of crispiness in prepared biscuit samples at zero time and during storage at room temperature (25°C) and fridge temperature (3ºC) for 6 months were recorded in Table 9.

Table 9: Crispiness of biscuits during six months storage period at room temperature (25°C) and fridge temperature (3°C).

Samples	Room temperature (25°C).			
	At zero time	After two months	After four months	After six months
WF biscuit (Control)	0.240^{e}±0.03	0.265^{e}±0.09	0.280^{e}±0.03	0.297^{e}±0.05
OF biscuit	0.232^{f}±0.01	0.248^{f}±0.01	0.263^{f}±0.01	0.280^{f}±0.01
ChPO biscuit	0.273^{a}±0.02	0.291^{a}±0.05	0.30^{a}±0.06	0.318^{a}±0.012
MPO biscuit	0.252^{b}±0.01	0.283^{c}±0.09	0.291^{c}±0.02	0.309^{c}±0.08
ChOO biscuit	0.250^{c}±0.04	0.284^{b}±0.02	0.293^{b}±0.04	0.310^{b}±0.04
MOO biscuit	0.247^{d}±0.02	0.272^{d}±0.01	0.281^{d}±0.01	0.306^{d}±0.02
LSD at 0.05	0.0023	0.0019	0.0019	0.0016
	Fridge temperature (3°C).			
Samples	At zero time	After two months	After four months	After six months
WF biscuit (Control)	0.240^{e}±0.03	0.240^{e}±0.04	0.252^{e}±0.06	0.248^{e}±0.01
OF biscuit	0.232^{f}±0.01	0.239^{f}±0.01	0.250^{f}±0.01	0.227f±0.02
ChPO biscuit	0.273^{a}±0.02	0.281^{a}±0.02	0.291^{b}±0.03	0.294^{a}±0.01
MPO biscuit	0.252^{b}±0.01	0.258^{b}±0.06	0.272^{c}±0.01	0.275^{b}±0.03
ChOO biscuit	0.250^{c}±0.04	0.252^{d}±0.03	0.329^{a}±0.07	0.265^{d}±0.01
MOO biscuit	0.247^{d}±0.02	0.253^{c}±0.01	0.264^{d}±0.02	0.267^{c}±0.04
LSD at 0.05	0.0023	0.002	0.0018	0.0016

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at $P < 0.05$.

Crispiness increased by increasing the storage period during storage at room temperature (25°C), this result may be due to the slightly increase in moisture content of biscuits during storage period at room temperature and the slight decrease of biscuits fat contents also as the crispiness represented a relationship between the moisture and fat contents of biscuit. Meanwhile, the addition of oat, herbs or their essential oils increased the crispiness during fridge storage comparing to the wheat biscuit control. Also, it could be observed that crispiness scores were more stable during fridge storage period as it increased slightly as compared to the storage at room temperature and these results may be due to the fridge temperature which decreased the changes in both moisture and fat contents as compared to storing at room temperature which increased changes in both.

Overall, 20% oat flour supplemented biscuit had softer texture and the addition of herbs or their essential oils to the oat biscuit increased the crispiness value during the storage period when compared to controls (without herbs or their EOs). According to results obtained by Salehifar and Shahedi (2007) the 40% oat flour supplemented bread had a significantly softer texture than all other breads. By increasing the oat flour level, the hardness of the breads decreased. This probably due to the higher levels of fat from oats compared with wheat.

Results are in accordance with Pamies *et al.* (2000) as they observed that an increase in water content altered the texture of starch based samples from crispy to crackly, whereas the texture of starch-sucrose samples remained unchanged in the same hydration range. As recorded by Chappalwar *et al.* (2013), the 40% oat and finger millet flour cookies were crispier as compared to 30% oat and finger millet flour cookies, so there may be a chance of breakage during transportation. From this observation it was concluded that 30% oat and finger millet flour cookies was good quality and easy to handle as compared to 40% oat and finger millet flour cookies.

Effect of six months storage period at room temperature (25°C) on acid value (AV), peroxide value (PV) and thiobarbituric acid (TBA) of lipid extracted from of herbal oat biscuits:

The changed occurred in the chemical quality attributes, acid value (AV), peroxide value (PV) and thiobarbituric acid (TBA) of lipid extracted from different type of biscuit samples during storage for 6 months at room temperature (25°C) and fridge temperature (3°C) were represented in Table 10.

Table 10: Acid value (AV), peroxide value (PV) and thiobarbituric acid concentration (TBA) of biscuits lipid extracts during six months storage period at room temperature (25 °C) and fridge temperature (3 °C) .

Room temperature (25°C).												
Samples	At zero time			After two months			After four months			After six months		
	AV	PV	TBA	AV	PV	TBA	AV	PV	TBA	AV	PV	TBA
Control WF biscuit	0.56±0.01	2.9±0.09	0.013±0.001	$0.71^{a}\pm0.01$	$4.2^{a}\pm0.09$	$0.030^{a}\pm0.001$	$0.80^{a}\pm0.03$	$4.8^{a}\pm0.15$	$0.050^{a}\pm0.00$	0.90±0.05	$5.3^{a}\pm0.22$	$0.075^{a}\pm0.003$
OF biscuit	0.56±0.05	2.9±0.07	0.013±0.0	$0.68^{b}\pm0.05$	$4.0^{b}\pm0.07$	$0.028^{b}\pm0.0$	$0.75^{b}\pm0.01$	$4.5^{b}\pm0.12$	$0.045^{b}\pm0.03$	0.83±0.07	$5.0^{b}\pm0.25$	$0.060^{b}\pm0.001$
ChPO biscuit	0.56±0.03	2.9±0.19	0.013±0.002	$0.64^{d}\pm0.03$	$3.5^{d}\pm0.19$	$0.024^{d}\pm0.002$	$0.71^{d}\pm0.01$	$4.2^{c}\pm0.25$	$0.040^{c}\pm0.003$	0.77±0.02	$4.6^{cd}\pm0.19$	$0.051^{c}\pm0.005$
MPO biscuit	0.56±0.07	2.9±0.15	0.013±0.001	$0.65^{c}\pm0.07$	$3.6^{c}\pm0.15$	$0.025^{c}\pm0.001$	$0.72^{c}\pm0.04$	$4.3^{c}\pm0.17$	$0.039^{d}\pm0.001$	0.78±0.01	$4.7^{c}\pm0.16$	$0.048^{d}\pm0.003$
ChOO biscuit	0.56±0.03	2.9±0.11	0.013±0.001	$0.62^{e}\pm0.03$	$3.3^{f}\pm0.11$	$0.022^{e}\pm0.001$	$0.68^{e}\pm0.09$	$4.2^{c}\pm0.13$	$0.039^{d}\pm0.003$	0.73±0.07	$4.6^{cd}\pm0.15$	$0.049^{cd}\pm0.004$
MOO biscuit	0.56±0.07	2.9±0.13	0.013±0.003	$0.61^{f}\pm0.07$	$3.4^{e}\pm0.13$	$0.021^{f}\pm0.003$	$0.67^{f}\pm0.05$	$4.1^{c}\pm0.10$	$0.040^{c}\pm0.002$	0.72±0.03	$4.5^{e}\pm0.11$	$0.050^{c}\pm0.005$
LSD at 0.05	NS	NS	NS	0.017	0.170	0.0026	0.021	0.169	0.002	NS	0.170	0.002

Fridge temperature (3°C).												
Samples	At zero time			After two months			After four months			After six months		
	AV	PV	TBA	AV	PV	TBA	AV	PV	TBA	AV	PV	TBA
Control WF biscuit	0.56±0.01	2.9±0.09	0.013±0.001	$0.65^{a}\pm0.01$	$3.5^{a}\pm0.09$	$0.020^{a}\pm0.001$	$0.68^{a}\pm0.02$	$3.7^{a}\pm0.05$	$0.025^{a}\pm0.003$	$0.80^{a}\pm0.05$	$4.2^{a}\pm0.02$	$0.030^{a}\pm0.005$
OF biscuit	0.56±0.05	2.9±0.07	0.013±0.0	$0.63^{b}\pm0.05$	$3.4^{b}\pm0.07$	$0.018^{b}\pm0.0$	$0.66^{b}\pm0.03$	$3.5^{b}\pm0.03$	$0.022^{b}\pm0.001$	$0.72^{b}\pm0.09$	$4.1^{a}\pm0.06$	$0.028^{b}\pm0.001$
ChPO biscuit	0.56±0.03	2.9±0.19	0.013±0.002	$0.62^{b}\pm0.03$	$3.2^{c}\pm0.19$	$0.016^{c}\pm0.002$	$0.65^{bc}\pm0.02$	$3.3^{c}\pm0.01$	$0.019^{bc}\pm0.005$	$0.70^{c}\pm0.05$	$3.7^{c}\pm0.03$	$0.023^{c}\pm0.001$
MPO biscuit	0.56±0.07	2.9±0.15	0.013±0.001	$0.62^{b}\pm0.07$	$3.4^{b}\pm0.15$	$0.017^{bc}\pm0.001$	$0.65^{bc}\pm0.03$	$3.5^{b}\pm0.11$	$0.018^{c}\pm0.001$	$0.69^{c}\pm0.01$	$3.9^{b}\pm0.04$	$0.022^{c}\pm0.009$
ChOO biscuit	0.56±0.03	2.9±0.11	0.013±0.001	$0.58^{c}\pm0.03$	$3.0^{d}\pm0.11$	$0.016^{c}\pm0.001$	$0.61^{c}\pm0.02$	$3.0^{d}\pm0.01$	$0.019^{bc}\pm0.009$	$0.66^{cd}\pm0.04$	$3.5^{d}\pm0.02$	$0.022^{c}\pm0.003$
MOO biscuit	0.56±0.07	2.9±0.13	0.013±0.003	$0.57^{d}\pm0.07$	$3.0^{d}\pm0.13$	$0.014^{d}\pm0.003$	$0.60^{d}\pm0.05$	$3.1^{d}\pm0.003$	$0.015^{d}\pm0.006$	$0.65^{d}\pm0.02$	$3.6^{cd}\pm0.001$	$0.018^{d}\pm0.001$
LSD at 0.05	NS	NS	NS	0.0165	0.170	0.002	0.165	0.170	0.002	0.170	0.170	0.006

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05.

Result show that AV, PV and TBA recorded the same scores (0.56 ± 0.01, 2.9 ± 0.09 and 0.013 ± 0.001, respectively) in all samples at zero time. Each of AV, PV and TBA values increased gradually during the storage period in all biscuit samples. It could be noticed that scores of all chemical quality attributes of oat biscuit containing herbs EOs was lower than scores of oat biscuits containing examined herbs powder. Meanwhile, both of oat biscuit containing herbs powder or its EOs have lower AV, PV, and TBA increment comparing to WF and OF biscuits, also the changes in AV, PV and TBA value was lower in OF biscuit than WF biscuit control. This result may be due to the antioxidant affection of oat, used herbs and its essential oils.

Results indicated that the increments in AV, PV and TBA value for samples stored at low temperature (3ºC) were less than that occurred for the samples stored at room temperature(25ºC). This means, the temperature plays an important role in decrease the changing in chemical quality attributes of biscuit lipids during storage. Also, it could be noticed that the addition of oat with examined herbs powder or their essential oils decreased the changes occurred in AV, PV and TBA, especially the oat biscuit containing herbs EOs which had a strong antioxidant activity than herbs power.

Processed foods containing fats and oils, oxidize slowly during storage, various oxidation products cause rancidity and deterioration of the sensory properties of the food products. Auto oxidation of fats and oils in processed foods maybe prevented by the use of oxidation inhibitors or antioxidants (Adegoke *et al*., 1998). Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens (Dillard & German, 2000). Results are in agreement with those obtained by Salehifar and Shahedi (2007) as they mentioned that lipase, the major enzyme in oats, causes the rapid release of free fatty acids in damaged or milled oats which can result in off flavours. In industry the action of lipase is controlled by heating the oat groats. Also Wagner et al. (2007) and Yilmaz & Toledo (2005) reported that the observed increase in antioxidant activity (in both chemical and physiological extracts) that occurs during baking might be explained by the increase of extractable (or bioavailable) phenolic content due to the influence of high temperature.

Additionally, literature data indicates that increased antioxidant potential might be attributed to the production of certain Millard reaction products that results from a condensation reaction between amino acids (or proteins) and reducing sugars or lipid oxidation products under the influence of high temperature and might occur during baking.

Total microbial count (cfu/ml) of herbal oat biscuits:

Different studies have demonstrated the effectiveness of EOs and their active compounds to control or inhibit the growth of pathogenic and spoilage microorganisms and reported its dependence on pH, chemical structure and concentration of EOs or active compound, besides the number and type of microorganisms. As shown in the Table 11, no total bacterial count were detected in all biscuit samples during storage at room temperature and low temperature at zero time and until the fourth month of storage.

It could be noticed that adding examined herbs essential oils to the oat biscuits prevent any bacterial count from detecting among all storage period for six months of both room temperature and fridge temperature. Meanwhile, the addition of herbs powder controlled the bacterial growth in prepared biscuits among storage period at both room temperature and fridge temperature. These results are in agreement with Roby *et al.* (2012) who stated that the chamomile essential oil exhibit different degrees of antimicrobial activities depending on the doses applied.

Also Sellamia *et al*. (2009) reported that the essential oil of *O. majorana* is known for its strong antimicrobial activity, so it could be used by food industries as natural preservatives. It is also evident that all essential oil antimicrobials do not necessarily act in the same manner. With any one agent, changes in its concentration may change the nature of the inhibitory action. These effects may be related to the influence of concentration upon solubility in the membrane (Holley and Patel, 2005). Storage temperature also influences the antimicrobial effectiveness of EOs, as the bactericidal activity of different EOs or their active components against E. coli O157:H7 and Salmonella hadar in apple juice was higher at 37 °C than at 4 and 21°C (Friedman *et al*., 2004). Even though small amount of salt intake can lower the possibilities of heart-related diseases, certain additional supplements are required to retain the shelf-life of food products. Hence, there might be a high demand of alternative strategies to prolong the shelf-life of processed or cooked foods using PEOs (Burt, 2004). The active PEO volatiles during the preservation of food stuffs work consistently to make the foods free from microbial load and reduce their population by contact methodology (Gutierrez *et al*., 2009). Besides, incorporation of PEOs or their volatiles with polymer molecules can be a good strategy in food preservation technology to limit the propagation of foodborne pathogens (Appendini & Hochkiss, 2002).

Table 11: Total microbial count (cfu/ml) for herbal oat biscuits.

Samples	Storage time (months) at room temperature (25 ◦ c)			
	0	2	4	6
Control WF biscuit	0 ± 0.0	0^{a}± 0.0	0^{b} ± 0.0	0.66^{bc}± 1.15
OF biscuit	0 ± 0.0	0^{a}± 0.0	1.0^{ab} ± 1.0	1.33^{bc}± 1.52
ChPO biscuit	0 ± 0.0	0^{a}± 0.0	0^{b} ± 0.0	3.0^{b}± 1.0
MPO biscuit	0 ± 0.0	0.33^{a} ± 0.57	1.66^{a} ± 0.57	11.0^{a}± 3.60
ChOO biscuit	0 ± 0.0	0^{a}± 0.0	0^{b} ± 0.0	0^{c} ± 0.0
MOO biscuit	0 ± 0.0	0^{a}± 0.0	0^{b} ± 0.0	0^{c} ± 0.0
Samples	Storage time (months) at fridge temperature (3 ◦ c)			
	0	2	4	6
Control WF biscuit	0 ± 0.0	0 ± 0.0	0.33^{ab} ± 0.57	0.33^{b} ± 0.57
OF biscuit	0 ± 0.0	0 ± 0.0	0^{b}± 0.0	0^{b} ± 0.0
ChPO biscuit	0 ± 0.0	0 ± 0.0	0.33^{ab} ± 0.57	1.0^{a} ± 1.0
MPO biscuit	0 ± 0.0	0 ± 0.0	0.66^{a} ± 0.57	1.3^{a} ± 0.57
ChOO biscuit	0 ± 0.0	0 ± 0.0	0^{b} ± 0.0	0^{b} ± 0.0
MOO biscuit	0 ± 0.0	0 ± 0.0	0^{b} ± 0.0	0^{b} ± 0.0

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05.

Yeasts and molds count (cfu/ml) of herbal oat biscuits:

Yeasts and Molds colony formed unit per gram of biscuit samples were carried out before the storage and after storage for 2, 4 and 6 Months. The results were recorded in Table 12. No yeast or molds count were detecting after backing in all biscuit samples and also during storage in the room temperature and fridge temperature (3°C) until four months. The addition of 0.25% ChO and 0.1% MO to the oat biscuit was a strong addition which prevented detecting any yeasts or molds count among the storage period (six months) at room temperature and under cooling. In the same time the addition of tests herbs controlled the growth of yeasts and molds in the prepared biscuits among the storage period in the both storage conditions.

These results were in agreement with Rauha *et al.* (2000) who found that the growth of the yeast, *Candida albicans*, was affected by 500 mg of chamomile (*Matricaria recutita*) methanolic extract. Meanwhile, Ezzeddine *et al.* (2001) stated that among several essential oils marjoram (*O. majorana* L.) may be useful as antimicrobial agents, marjoram oil possesses antimicrobial properties against food borne bacteria and mycotoxigenic fungi and therefore it may have the greatest potential for use in industrial applications. Dietary herbs and spices have been traditionally used as food additives throughout the world not only to improve the sensory characteristics of foods but also to extend their shelf life by reducing or eliminating survival of pathogenic bacteria. Many herbs and spice extracts possess antimicrobial activity against a range of bacteria, yeast and molds (Tajkarimi *et al*., 2010). Fungi are the main agents of spoilage e of bakery products. Apart from visible growth, they also produce off-flavors and mycotoxin production which can be a concern. As with other foods, bakery products which contain natural preservatives are becoming more common. However, as with bacteria, fungi are more resistant to these natural antimicrobials when challenged in foods (Lopez-Malo *et al*., 2002).

Table 12: Yeasts and molds count (cfu/ml) for herbal oat biscuits.

Samples	Storage time (months) at room temperature (25 ◦ c)			
	0	2	4	6
Control WF biscuit	0 ± 0.0	0 ± 0.0	0^c ± 0.0	0^d ±0.0
OF biscuit	0 ± 0.0	0 ± 0.0	0^c ± 0.0	0.33^d ±0.57
ChPO biscuit	0 ± 0.0	0 ± 0.0	2.0^b ± 1.0	3.33^c±2.51
MPO biscuit	0 ± 0.0	0 ± 0.0	7.0^a ± 2.64	7.33^a±3.51
ChOO biscuit	0 ± 0.0	0 ± 0.0	0^c ± 0.0	5.0^b ±2.0
MOO biscuit	0 ± 0.0	0 ± 0.0	0^c ± 0.0	5.0^b±1.0
Samples	Storage time (months) at fridge temperature (3 ◦ c)			
	0	2	4	6
Control WF biscuit	0 ± 0.0	0 ± 0.0	0^b ± 0.0	0^c ± 0.0
OF biscuit	0 ± 0.0	0 ± 0.0	0^b ± 0.0	0^c ± 0.0
ChPO biscuit	0 ± 0.0	0 ± 0.0	1.0^b ± 1.0	2.0^b ± 1.73
MPO biscuit	0 ± 0.0	0 ± 0.0	2.3^a ± 0.57	4.66^a ± 1.52
ChOO biscuit	0 ± 0.0	0 ± 0.0	0^b ± 0.0	0^c ± 0.0
MOO biscuit	0 ± 0.0	0 ± 0.0	0^b ± 0.0	0^c ± 0.0

WF: Wheat Flour; OF: Oat Flour; ChPO: Chamomile Powder Oat; MPO: Marjoram powder oat; ChOO: Chamomile Oil Oat; MOO: Marjoram Oil Oat, and Mean ± SD values in each column with different superscript letters (a, b, c, d) are significant at P< 0.05

Conclusion

With the growing interest of consumers in health-related foods, the addition of oat *(Avena sativa L.)* to wheat flour affected the sensory characteristics and chemical composition in various ways. Biscuits containing oat flour (20%) was highly acceptable. The addition of herbs and essential oils of the two plant materials {chamomile *(Matricaria renmcutita* L.*)* and marjoram (*Origanum marjorana*)} gave an excellent flavor, baking quality, color characteristics and antioxidant effect on the oat biscuit. Natural antioxidants can play a very important role in shelf-life extending of food products containing fats and oils, such as biscuits. This study has shown the potential of developing new herbal oat biscuits with natural antioxidants which are safe and impart health benefits to the consumer.

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