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Research Article
Micro Sunflower Oil-Water-Emulsion as Fat Replacer in Biscuits

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Abstract
The emulsification process of sunflower oil in water using high speed homogenizing was studied in presence of 30% oil, 3% emulsifier (Tween 40) supplemented with 0.4-0.8% vitamin E (α-tocopherole). The obtained emulsion was stable according its physico-chemical properties, particle size, zeta potential, Poly-Disperse Index (PDI), surface tension and interfacial tension. Biscuit samples were also prepared with and without α-tochopherole. The DPPH scavenging activity was measured during storage 9 month and compared to BHT. Increasing concentrate of α-tochopherol 0.8% has an excellent effect of on DPPH scavenging activity where as it changes from 91.87-74.22% before and after the storage months period. The physico-chemical properties of oil (PV, IV, FFA, CD and CT) extracts from biscuits with or without vit. E were evaluated. The results showed that presence of 0.8% vit. E gives the lower changes in PV (0.13) compared to 0.46 of control oil and hence improve stability. In comparison to shortening dough, the microemulsion prepared biscuits has more volume and height.

Key words: Sunflower oil, tween 40, microemulsion, storage stability, antioxidant activity, organoleptic characteristics, rancidity analysis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

High fat consumption is associated with the development of various metabolic disorders such as obesity and diabetes, which, in turn, increase the risk of cardiovascular diseases and some types of cancer (Micha and Mozaffarian, 2010). Sunflower oil, in particular, besides their technological aspects, have interesting nutritional benefits, anti-oxidant and anti-cholesterolemic activities due to the gelator features (Bot *et al*., 2009) and a polyunsaturated fatty acid rich profile, brought by the sunflower oil. However, different approaches can be taken to minimize their dissolution rate by lowering the size of sunflower oil particles (micro particles). Texture, flavor and appearance are the main quality attributes of cookies. Fat is very important ingredient of cookies because it contributes texture and pleasing mouthfeel and positively impacts flavor intensity and perception. The addition of shortening is done principally to stabilize air cells that are generated by mixing (Given, 1994). Presence of fat contributes to the reduction of elastic nature of dough and shrinking of the dough during moulding (Maache-Rezzong *et al*., 1998). The type and amount of fat added to the dough has strong effect on viscoelastic properties (Baltsavias *et al*., 1997). Baltsavias *et al*. (1997) also reported that reducing the fat content as substituting liquid oil for solid caused a marked decrease in the stiffness of the dough which implies that fat is crucial structure component. More recently, Goldstein and Seetharaman (2011) studied the effect of a novel monoglyceride stabilized oil on cookie properties. They produced an emulsified shortening and found interesting benefits on cookie performance, especially on texture, compared to traditional fats (Tarancon *et al*., 2013). Refined sunflower oil was selected because of its high nutritional value. Sunflower oil seeds are rich source of linoleic acid, which is one of the nutritionally essential fatty acids (Jacob and Leelavathi, 2007). Homogenization pressure and recirculation will affect mean particle size and large diameter droplet. Particle size will decrease with increasing homogenization pressure from 400-1300 bar when homogenization recirculation is fixed when the homogenization pressure is fixed at 1000 bar (Peng *et al*., 2015). In view of the aforementioned information the possibility of making a good quality biscuit by using sunflower-oil-water emulsion and studying their effects on antioxidant activity (DPPH), physical properties and on final characteristics.

MATERIALS AND METHODS

Materials: Wheat flour (72% extraction) was purchased from local market. Commercially available sugar powder, milk solution, food grade sodium choride, sodium bicarbonate and sunflower oil. Sunflower oil as the disperse phase in these experiments was obtained from ARMA Oils Company, Egypt. Sunflower oil is light in taste and appearance and has high vitamin E content. Nonionic surfactants tween 40 (polyoxyethylene (20) sorbitan monopalmitate, T_{40}) with technical grade was purchased from Merck. Hydrophile-lipophile balance (HLB) of tween 40 is 15.4. This high HLB number indicates that the surfactant will travel into the water and oil phase. Distilled water was used as the continuous phase. Properties of the emulsifier are listed in Table 1. All chemicals were used without further purification before preparation of O/W emulsions. Tween 40, polyoxyethylene sorbitan monopalmitate has the following chemical structure, Fig. 1.

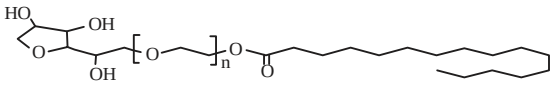

Fig. 1: Chemical structure of tween 40

Table 1: Properties of tween 40 emulsifier

Molecular formula	$C_{62}H_{123}O_{26}$
Synonym	Polyoxyethylene-sorbitan monopalmitate polysorbate 40
Content	~90% palmitic acid
Form	Viscous liquid
Specific gravity	~1.08 g mL^{-1}
Viscosity @ 25°C	600 cps
Acid No	0-2.0
Saponification No.	43-49
Hydroxyl No.	89-105
HLB	15.6
Critical Micelle Concen. (CMC)	0.027 mM
Molecular weight	1277
Flash point	>200°C

Methods

Emulsion preparation

High speed homogenizer: Raw emulsion was prepared by adding 30 wt% sunflower oil slightly to a solution of the nonionic surfactant tween 40 with concentration 3 wt% in distilled water under continuous stirring using high speed homogenizer of 19000 rpm at room temperature for 5 min. stirring. The overall mass of the emulsion was 500 g.

Physical characterization techniques of microemulsion

Particle size measurement and particle size distribution: Microemulsion particle sizes were measured using dynamic light scattering (Nano ZS, Malvern and Worcestershire, United Kingdom), at a scattering angle of 173°C using a 633 nm laser

with each measurement was being the average of 16 runs, each of 10 sec duration. All samples were measured in distilled deionized water. Microemulsions were diluted to give a scattering intensity of less than 500 cps (~0.0075 wt%) to avoid the effects of multiple scattering. Samples were measured after 5 min equilibration at 25°C and results are reported as the average of 3 measurements.

Zeta potential: Zeta potential is a scientific term for electrokinetic potential in colloidal systems (Mills *et al*., 1993). In colloidal chemistry literature, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A value of 30 mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from highly charged surfaces (Preetz *et al*., 2010). Zeta potential value can be related to the stability of colloidal dispersions, indicating the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist to aggregation. Briefly, zeta potentials from 0 to ±30 mV indicate instability, while higher values more than ±30 mV indicates more stability (ASTM., 1985).

Polydispersity Index (PDI): The PDI can be assessed using a particle size analyzer (Nano ZS90, Malvern Instrument and Worcestershire, UK). It is a measure of the broadness of the size distribution derived from the cumulative analysis of dynamic light scattering. The polydispersity index indicates the quality or homogeneity of the dispersion (Li *et al*., 2011). Also it is an indicator of aggregation in the particles and it has values between 0-1. Lower values, closer to zero, denotes the monodisperse system. Higher values show a polydisperse system which have greater tendency to aggregation than monodisperse system.

Surface tension and interfacial tension analysis: Surface tension and interfacial tension were measured using the pendant drop method with the Data Physics OCA20 contact angle system (Data Physics Instruments, Germany). A drop of the emulsifier solution (1% by mass) to be analyzed was formed at the bottom of a capillary column using the manual dosing system. The surrounding medium was the atmosphere for surface tension determination or MCT oil for interfacial tension determination. Charged Coupled Device (CCD) camera photographed the contour of the drop, from which the tension values were calculated using the SCA22 software automatically.

Preparation and evaluation of biscuits: Three blends samples including control which was made with 100% shortening for comparison (Table 2). Biscuit samples were processed and backed quality (diameter, height, spread ratio and volume) according to the procedure described in AACC (2010) and organoleptic characteristics of biscuits were evaluated. The colors were measured with hunter lab colorimeter model D25.

Chemical analysis

Preparation of lipid extract: The extraction of lipid from biscuits was carried out according to the method described by Baiano and Del Nobile (2005). Ten grams of biscuits were ground roughly and placed in a closed flask with 200 mL n-hexane. The flask was shaken for 1 h and then filtered through filter paper (Whatmann No. 1). The solvent was removed from the extracted lipids by rotary at 50°C. The obtained extracts were stored in amber colored airtight containers at -4°C, until further use.

Determination of antioxidant activity: Essential-linoleic acid emulsion system was used to determine the antioxidant activity of the extracts obtained from the three samples (Taga *et al*., 1984). Sunflower oil extracted (2 mg) was dissolved in chloroform (20 mL). To an aliquot (3 mL) of the solution was added linoleic acid (40 mg) and tween

Table 2: Biscuit baking formulation

Ingredients	+Vit. E 0.4%	+Vit. E 0.8%	Control biscuit with sunflower oil
Flour	100 g	100 g	100 g
Sugar	69.6 g	69.6 g	69.6 g
Sunflower oil	-	-	4%
Sunflower oil emulsion	4%	4%	-
Sodium bicarbonate	1.3	1.3	1.3
Salt	1.1	1.1	1.1
Milk solution (17.3 g skim milk powder 100 mL water)	12.4	21.4	21.4
Water	Variable	Variable	Variable
Emulsifier tween (40)	3%	3%	3%

40 (400 mg). Chloroform was removed using a rotary evaporator at 50°C, made up to 100 mL with oxygenated water mixed well.

Aliquots of the sunflower oil-linoleic acid emulsion were mixed with antioxidant extract (40 µL) and incubated at 50°C, for 60 min. The absorbance of the oxidized emulsion was measured in a spectrophotometer at 470 nm. The synthetic antioxidant of Butylated hydroxytoluene (BHT) was used as reference. Distilled water was used as control instead of antioxidant extract. The degradation rate of extracts was calculated as follows:

Antioxidant activity (AOA) was expressed using the following Eq. 1 and 2:

$$\text{Antioxidant activity} = \frac{\text{Degradation rate of control - Degradation rate of sample}}{\text{Degradation rate of control}} \times 100 \quad (1)$$

$$\text{Sample degradation rate} = \ln (a/b) \times 1/t \quad (2)$$

where, ln is the natural log, a is the initial absorbance (470 nm) at time zero, b is the absorbance at specific interval (60 min) and t is the time (min).

DPPH scavenging assay: The free radical scavenging activity of various extracts was performed according to the method of Tepe *et al*. (2005). One milliter of (0.1 mM) ethanolic solution of DPPH was added to 3 mL of different concentrations (10-250 µg mL^{-1}) for the ethanol and water extracts and (5-25 µL mL^{-1}) for the essential oil. The disappearance of DPPH reagent was read in spectrophotometer using (UV-visible spectrophotometer, UK) at 517 nm after 30 min of incubation at room temperature. Inhibition of free radical DPPH in percent (1%) was calculated using the following Eq. 3:

$$\text{Inhibition percentage} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \quad (3)$$

where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound. The IC_{50} defined as the concentration of extract providing 50% inhibition of DPPH calculated from the graph plotting inhibition percentage against extract concentration. Tests carried out in triplicate.

Analysis of rancidity parameters in sunflower oil extracted from bisuit: The lipids of ground biscuits were extracted using n-hexane. Stability of biscuit lipids were followed periodically at intervals of 1 week during storage for 6 weeks at ambient temperature, by determining antioxidant activity. Free Fatty Acid (FFA) values and Peroxide Values (PV) and Iodine Values (IV) were determined by following the recommended methods of AOCS (1989). Conjugated Dienes (CD) and Conjugated Trienes (CT) were determined according to recommended methods of IUPAC (Paquot and Hautfenne, 1987). For the determination of Conjugated Dienes (CD) and Conjugated Trienes (CT) sunflower oil samples were diluted with iso-octane to bring the absorbance with in the limits. The absorbance was measured at wavelength 232 and 268 nm for conjugated diene and triene values, respectively (Hitachi, U-2001, Model 7400 spectrometer, Tokyo, Japan, Paquot and Hautfenne, 1987). All these parameters were good indicators of lipid oxidation. Many scientists monitored the phenomenon of lipid oxidation to judge the extent of oxidation and antioxidant potential of plant extracts (Anwar *et al*., 2006, 2007; Frega *et al*., 1999; Ozkan *et al*., 2007). The above analyses were carried out in two replicates.

Organoleptic characteristics: Organoleptic characteristics of biscuits were conducted to determine the acceptability of samples. Fifteen panelists were selected from among the postgraduate students in the Department of Food Science and Nutrition at National Research Centre. Sensory scores for different attributes like color, flavor, texture, taste and appearance were obtained (Smith, 1972).

Statistical analysis: The sensory data were subjected to ANOVA followed by Duncan's new multiple range test (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Physicochemical properties of sunflower oil: The physicochemical properties of sunflower oil are characterized and shown in Table 3. Sunflower oil is liquid at room temperature. The refined oil is clear and slightly amber-colored with a slightly fatty odor. The fatty acid composition of sunflower oil contains 5% palmitic acid, 6% stearic acid (saturated), 30% oleic acid (monounsaturated omega-9) and 59% linoleic acid (polyunsaturated omega-6). It is clear that

Table 3: Physico-chemical properties of sunflower oil

Density (25ºC)	0.98
Refractive index	1.464
Fatty acid (%)	0.2
Iodine value (g/100 g) fat	110
Saponification value (mg KOH/oil)	175
Viscosity, cp (25°C)	41.5

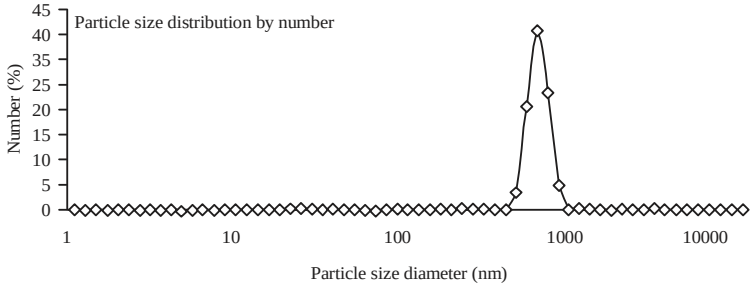

Fig. 2: Particle size distribution of micro-emulsion (30% oil, 3% tween 40, 19000 rpm), PDI = 0.517 and particle size = 626 nm

sunflower oil is a combination of monounsaturated and polyunsaturated fats with low saturated fat levels.

Physicochemical properties of sunflower oil in water emulsion: The properties of sunflower oil in water emulsion is characterized to its stability by measuring particle size, particle size distribution, Poly Disperse Index (PDI), zeta potential and interfacial tension.

Particle size and particle size distribution: The stabilization of emulsion is an important factor to provide a better product over conventional emulsions. The particle size distribution is shown in Fig. 2. It is clear that the particle size of the product is 626 nm, which in the range of micro size emulsion, so it is electrically stabilized and will resist to droplet aggregation.

Zeta potential and polydisperse Index (PDI): Zeta potential values of emulsions using 3% tween 40 emulsifier and 30% sunflower oil after 5 min emulsification time at 19000 rpm is about -33 mV. It means that using tween 40 in the emulsion process results in high zeta potential which related to the stability of colloidal dispersions, indicating the degree of repulsion between adjacent, similarly charged particles in dispersion. For small enough molecules or particles, a high zeta potential will confer stability, i.e., the solution or dispersion will resist to aggregation. Briefly, zeta potentials from 0 to ±30 mV indicate instability, while higher values more than ±30 mV indicates more stability (ASTM., 1985).

The polydisperse index (PDI = 0.517) is in the suitable range which indicates the high quality and homogeneity of the dispersion.

Surface tension and interfacial tension: The measurements of surface tension and interfacial tension were made at room temperature. The interfacial tension was measured against MCT oil. Table 4 represents the surface tension of the emulsifier solution and interfacial tension of the oil and the emulsifier solution. As indicated, tween 40 dissolved in water has surface tension value of 38 which is much smaller than water and also showed low interfacial tension of 4.59 when mixed with oil, which was favorable for the formation of small droplets.

Table 4: Surface tension of emulsifier solution and interfacial tension of the emulsifier solution and oil

	Water	T 40
Surface tension/(mN/m)	72.20	38.00
Interfacial tension/(mN/m)	20.72	4.59

Baking quality of biscuits: One decisive aspect of final biscuit quality is the expansion of the dough during baking as this determines the size and texture of biscuit (Seker *et al*., 2010). The diameter, height, volume and spread ratio are shown in Table 5. Both the diameter and height were less than control. As a result, reducing the fat content of biscuit formulations generally leads to lower diameter and spread ratio, which has a negative effect on the final biscuit quality (Sudha *et al*., 2007). Abboud *et al*. (1985) had earlier reported that with the use of oil there was notice increase in the diameter of the cookies. Partial hydrogenation is applied to help produce vegetable bakery shortening having desirable plastic character (Given, 1994). In contrast, using nano sunflower oil emulsion in the biscuit formula samples having better volume and height than the control sample. For instance, biscuits in which up to 50% of the fat was replaced by emulsifiers did not expand properly after baking (Zoulias *et al*., 2002). This means that the presence of nano sunflower oil emulsion in the formula succeeded to raise dough volume and height.

Color of biscuits: Color is a major quality factor of the biscuits manufacturing industry. The L-value is a measure of the light-dark fraction of biscuit surface color. Control biscuits containing vegetable fat had the highest L-value (75.84). Data represented in Table 6 shows that, all cookies with micro sunflower oil emulsion had the lowest b and L values than

Table 5: Baking quality of biscuits

Samples	Diameter (cm)	Height (cm)	Spread ratio (cm)	Volume (cm^3)
Control	3.75	1.50	2.50	130
Biscuits with sunflower oil emulsion	3.20	2.50	1.30	73
With vit. E 0.4%	3.50	2.00	1.75	170
With vit. E 0.8%	3.20	1.50	2.13	150

Table 6: Color quality of biscuits

Samples	Lightness (L)	Redness (a)	Yellowness (b)
Control			
Crust	75.84	7.42	30.21
Crumb	60.38	11.58	34.78
Biscuits with sunflower emulsion			
With vit. E 0.4%			
Crust	67.51	10.14	30.33
Crumb	57.37	12.83	32.73
With vit. E 0.8%			
Crust	70.82	7.54	24.98
Crumb	60.70	11.77	33.66

Table 7: DPPH radical scavenging activity percentage of micro-emulsion sunflower before and after processing compared to BHT

	DPPH radical scavenging activity (%)				
Concentration (μg mL^{-1})	Micro-emulsion sunflower oil with vit. E 0.4%	Micro-emulsion sunflower oil with vit. E 0.8%	Sunflower oil emulsified extracted from biscuit with vit. E 0.4%	Sunflower oil emulsified extracted from biscuit with vit. E 0.8%	BHT
10	54.37±0.18	68.58±0.24	51.62±0.18	65.11±0.24	63.21±0.22
20	59.71±0.20	70.62±0.20	54.13±0.32	68.52±0.16	66.18±0.18
50	63.48±0.31	76.18±0.19	59.52±0.25	72.91±0.25	69.62±0.22
100	70.54±0.28	81.51±0.24	64.68±0.21	78.63±0.20	72.41±0.32
150	75.09±0.25	87.24±0.32	70.06±0.33	84.86±0.26	76.84±0.31
200	79.43±0.21	91.88±0.22	75.41±0.26	88.07±0.17	80.29±0.29
250	85.46±0.18	94.63±0.27	82.17±0.29	91.06±0.18	84.07±0.19

Data expressed as mean of 3 replicates±standard deviation, mean in the same column showed the same small letters are not significantly different ($p \leq 0.05$)

control sample indicating low degrees of yellowness and had more (a) values resulting more red than those control biscuits (O'Brien *et al*., 2003).

DPPH radical scavenging: In the DPPH radical scavenging method, relatively stable radical DPPH has been widely used in the determination of the antioxidant activity of single compounds as well as the different sunflower oil extracts through the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate the antioxidant activity. Data presented in Table 7 showed the percentage scavenging activities of extracted sunflower essential oil and after micro-emulsion from biscuits samples compared to BHT.

The results showed that the highest percentage of DPPH radical scavenging activity was 94.63% for sunflower oil emulsification with vit. E 0.8% extracted in 250 μg mL^{-1} concentration. Within the test range of concentrations (10-250 μg mL^{-1}), all extracts exhibited a dose-dependent and scavenging activity of various degrees. Ethanol extracts of sunflower oil showed a strong correlation between their antioxidant activities and concentrations (68.58 and 94.63 μg mL^{-1}, respectively, $p \leq 0.05$), while the corresponding for the synthetic antioxidant (BHT) was 84.07. Sunflower oil exhibited highest antioxidant activity compared to other extracts and BHT, (Table 5) and the results indicated that the DPPH scavenging activities (%) were increased significantly with increasing the concentration from 10-250 μL mL^{-1} essential oil ($p \leq 0.5$). At a concentration of 200 μg mL^{-1}, the extracted showed the highest (91.88±0.22%) radical scavenging activity compared to other extracts and BHA. However, the synthetic antioxidant BHT can not be used beyond a concentration of 200 ppm, there is no such limit in using the antioxidants from natural sources (Alvarez-Figueroa and Blanco-Mendez, 2001).

DPPH scavenging activity: The Addition natural substances of α-tochopherol to sunflower oil was increases the antioxidant properties influenced the capacity of DPPH scavenging. The DPPH scavenging activity of sunflower oils

Table 8: DPPH scavenging activity (%) of micro-emulsion sunflower before and after processing

Sunflower oil extracts	DPPH scavenging activity (%)
Micro-emulsion sunflower oil with vit. E 0.4%	78.43±0.274[a]
Micro-emulsion sunflower oil with vit. E 0.8%	93.61±0.253[a]
Micro-emulsion sunflower oil extracted from biscuit treated with vit. E 0.4%	76.82±0.312[a]
Micro-emulsion sunflower oil extracted from biscuit treated with vit. E 0.8%	91.87±0.270[a]
Sunflower oil extracted from biscuit without treatment (control)	34.22±0.193[b]

Data expressed as mean of 3 replicates±standard deviation, mean in the same column showed the same small letters are not significantly different ($p \leq 0.05$). According to our results, sunflower oil emulsion with vit. E had a significantly higher value than sunflower oil (control) ($p<0.05$)

Table 9: Antioxidant activity of micro-emulsion sunflower oil extracted from biscuit samples during storage periods

	Antioxidant activity of micro-emulsion sunflower oil (%) during storage				
Biscuit samples	0 day	60 days	120 days	180 days	270 days
Sunflower oil micro-emulsion extracted from biscuit with vit. E 0.4%	76.82±0.31	74.21±0.24	68.94±0.25	61.75±0.10	54.51±0.2
Sunflower oil micro-emulsion extracted from biscuit with vit. E 0.8%	91.87±0.27	91.16±0.19	90.07±0.17	89.13±0.13	74.28±0.3
Sunflower oil extracted from biscuit control	34.22±0.19	31.55±0.21	25.18±0.22	19.24±0.24	12.36±0.2

Antioxidant activity by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method

after and before emulsion is shown in Table 8. All sunflower oils extracted from biscuit had a very strong DPPH scavenging activity and increases with increasing the concentration of α-tocopherol from 0.4-0.8% except those without α-tocopherol (control) was 34.22±0.193% of DPPH scavenging activity at the concentration of 250 mg mL^{-1}.

Storage stability of antioxidant activity of sunflower oil extracted from biscuit samples: The storage stability of antioxidant activity of sunflower oil extracted from samples biscuit control and others samples contained vit. E were evaluated in Table 9. It is clear that higher antioxidant activity is recorded for sunflower oil extracted from biscuit samples contained vit. E than that of sunflower oil extracted from biscuit control.

Rancidity analysis of sunflower oil extracts from biscuit samples: Formation of Free Fatty Acids (FFA) might be an important measure of rancidity of foods. The FFAs are formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture (Frega *et al*., 1999). Table 10 shows the changes in free fatty acid values. Free Fatty Acid (FFA) value of sunflower oil for blank and after emulsification with vit. E 0.8% was found to be 0.060±0.01 and 0.061±0.01, respectively at zero time storage. After one month, the FFA values were promoted to 0.067±0.01 and 0.062±0.02, respectively. While the FFA value for the samples containing vit E after two month of storage protocol was found to be 0.064±0.02. This value was same as essential oil containing sample. After the completion of six-month storage protocol FFA value for blank solution was increased to 0.071±0.01. This change in FFA contents was significant according to statistical analysis.

Peroxide Value (PV) is a widely used measure of the primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation (Ozkan *et al*., 2007). Changes in peroxide values are showed in Table 10. Peroxide value of blank and sunflower oil extracted after emulsification from biscuit with vit. E 0.08% was 1.00±0.01 and 0.90±0.02, respectively. It was increased to 1.33±0.03 and 0.99±0.03, respectively, at the end of six month trial. These changes were significant indicating the noticeable phenomenon of lipid oxidation. The results were less in the increase for samples compared control.

Iodine Value (IV) for blank and sunflower oil sample was calculated as 131.7±0.2 and 131.0±0.3 it was subjected to 114.4±0.3 and 130.7±0.2, respectively, after six month storage. These variations were statistically significant in blank. While, variation in sunflower oil samples extracted from biscuit containing vit. E 0.8% was not huge indicating the presence of antioxidants in the form of essential oil (sunflower oil samples having 200 μg mL^{-1}).

Conjugated Diene (CD) and Conjugated Triene (CT) is a good measure of oxidative state of oils (McGinely, 1991). Conjugated Dienes (CD) and Conjugated Trienes (CT) were determined by measuring the specific extinction co-efficient at 232 and 268 nm, respectively. Samples were diluted with iso-octane to bring the absorbance within the limits from 0.22±0.01 to 0.40±0.03 after six months, while the CD was more increase in the blank from 0.23±0.01 to 0.47±0.02 at the same time storage. Variation trends in CD and CT are represented in Table 10 indicated that the significant increase was observed in CD and CT for controlled. The vit. E in micro-emulsion sunflower oil extracted from biscuit sample exhibited prominent antioxidant worth.

Table 10: Analysis of rancidity parameters in micro-emulsion sunflower oil extracted from biscuit samples during storage periods

Time storage	FFA		PV		CD		CT		IV	
(Days)	A	B	A	B	A	B	A	B	A	B
0	0.060±0.01	0.061±0.01	1.00±0.01	0.90±0.02	0.23±0.01	0.22±0.01	0.19±0.01	0.18±0.01	131.7±0.2	131.0±0.3
30	0.067±0.01	0.062±0.02	1.14±0.01	0.91±0.01	0.29±0.02	0.24±0.01	0.26±0.02	0.19±0.02	129.0±0.3	131.0±0.4
60	0.072±0.02	0.064±0.02	1.19±0.01	0.92±0.02	0.36±0.02	0.26±0.01	0.34±0.01	0.21±0.01	124.6±0.2	130.9±0.3
90	0.078±0.01	0.068±0.01	1.23±0.02	0.94±0.03	0.39±0.01	0.29±0.01	0.39±0.01	0.22±0.02	121.3±0.3	130.9±0.2
120	0.083±0.01	0.069±0.01	1.27±0.03	0.96±0.02	0.47±0.02	0.31±0.02	0.44±0.01	0.24±0.01	119.2±0.2	130.8±0.2
150	0.089±0.02	0.070±0.02	1.30±0.02	0.97±0.02	0.52±0.02	0.34±0.01	0.48±0.02	0.27±0.02	117.1±0.2	130.8±0.2
180	0.094±0.02	0.071±0.01	1.33±0.03	0.99±0.03	0.57±0.02	0.36±0.02	0.53±0.01	0.29±0.02	114.4±0.3	130.7±0.2
270	0.162±0.01	0.075±0.02	1.46±0.02	1.03±0.01	0.78±0.02	0.40±0.03	0.79±0.02	0.31±0.02	102.8±0.2	125.2±0.1
II	0.102	0.014	0.46	0.13	0.55	0.18	0.60	0.13	28.9	5.8

A: Sunflower oil (control), B: Sunflower oil extracted after emulsification from biscuit with vit. E 0.8%, II: Increase from initial, FFA: Free fatty acid, PV: Peroxide values, CD: Conjugated dienes, CT: Conjugated trienes, IV: Iodine values

Table 11: Organoleptic characteristics of biscuits

Samples	Appearance	Color	Texture	Flavor	Taste
Control	9.24±0.12^{a}	9.33±0.14^{a}	9.53±0.18^{a}	9.71±0.21^{a}	9.63±0.13^{a}
Biscuits with sunflower emulsion					
With vit. E 0.4%	6.53±0.31^{c}	9.12±0.14^{a}	5.31±0.34^{c}	7.70±0.25^{c}	8.10±0.18^{b}
With vit. E 0.8%	7.81±0.25^{b}	9.13±0.11^{a}	6.50±0.28^{b}	8.10±0.22^{b}	7.22±0.20^{c}
LSD*	0.85	ns**	1.25	0.96	0.80

*Least significant difference, **Not significant

Results in Table 10 show no differences moral and clear between samples biscuits stored either in samples or Control period of time 6 months, a duration of the period of validity of biscuits in the local markets, while it was noted that there were significant differences evident in the samples after 9 months storage.

The relative increases in the Peroxide Values (PV) of the micro-emulsified and control sunflower oil extracted, stored under accelerated conditions presented in Table 8. The micro-emulsified and control oils showed characteristic increases in PV, FFA, CD and CT was 0.13, 0.14, 0.18 and 0.3, respectively, increase small compared to initial oil, while less than in IV. At the end of the storage period of 270 days, the control had the highest level of PV (relative to the initial value), indicating a higher extent of primary oxidation. The samples of sunflower oil micro-emulsified extract showed the minimum increase in PV (0.13), while the control oil extracted of biscuit showed the maximum level (0.46).

Organoleptic characteristics of biscuits: The results for appearance, color, texture, flavor and taste of biscuits samples are shown in Table 11. The two samples were rated lower than the control sample. Significant differences at ($p<0.05$) were observed within two recipes and between the control sample. The surface color of baked product is, together with texture and taste, a very important element for the initial acceptability of biscuits by consumers. Significant difference was observed between control and two samples. Appearance and texture (Table 11) showed a progressive decreasae.

CONCLUSION

This study showed that efficiency of micro-emulsion sunflower oil with vit. E 0.8% improved storage of biscuits which extend the biscuits shelf life by inhibiting its oxidation. Improved dispersion of micro-emulsion of sunflower oil significantly reduced the micro-emulsion required for potential production of a natural preservative agent.

The results obtained by the use of the DPPH method showed that the micro-emulsion of sunflower oil contain vit. E 0.8% can be considered good sources of natural antioxidants. These properties are also very much needed by the food industry in order to find possible alternatives to synthetic antioxidant preservatives such as butylated hydroxytoluene (BHT) and phenolic compounds.

Further research is needed to better understand the stability and action mechanism of the micro-emulsions and to optimize formulation and dosing levels, from different points of view: costs, sensorial quality of the product and possibility of using the extract not only as preserving additive but also as an healthy-functional ingredient.

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