J. Agr. Sci. Tech. (2023) Vol. 25(3): 623-634

Production and Stabilization of Functional Butter with *Artemisia absinthium* L. Essential Oil Incorporation

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ABSTRACT

This study aimed to evaluate the effect of *Artemisia absinthium* L. Essential Oil (EO) incorporation at various concentrations on the properties and oxidative stability of butter during 2 months of refrigerated storage. The obtained results on peroxide value, acidity, fatty acids, antiradical scavenging activity, and physical and microbiological properties confirmed the effectiveness of *Artemisia* EO incorporation in fat rich dairy foods. New produced butters can be classified as functional products due to their strong antioxidant activity, better oxidative stability, and richness in essential unsaturated fatty acids when compared to the control. All quality parameters were improved with *Artemisia* EO enrichment, however, descriptive sensorial analysis showed that the lowest concentration of about 45 ppm of the product was the most preferred in terms of color, odor, taste, firmness and overall acceptability. Due to its positive effects on all butter properties, *Artemisia absinthium* EO can be used as natural antioxidant and antimicrobial agent in food industries.

Keywords: Butter quality, Functional product, Oxidative stability.

INTRODUCTION

Butter is the most popular semi-solid fat-rich dairy product (Ceylan and Ozcan, 2020; Staniewski *et al*., 2021). Butter contains more than 80% milk fat, the most complex edible fats in terms of its fatty acid composition, influencing its characteristics. In fact, mainly 16 fatty acids are responsible for the main physical properties of butter, such as melting and solidification temperature and butter hardness. It represents an emulsion of the water-in-oil type with complex rheological properties (Staniewski *et al*., 2021). Butter has an important shelf life for producers and consumers (Ceylan and Ozcan, 2020) but, during storage, odor and taste of butter can be affected due to lipolysis and oxidation, causing the formation of peroxides and leading to rancidity (Abid *et al*., 2017). For manufacturers, it is important to delay the butter oxidation process as much as possible. Moreover, consumers' demand is increasing in terms of milk fat-based products with high nutritional value (Ceylan and Ozcan, 2020), which incite to develop new products using natural antioxidants improving oxidative stability of butter (Abid *et al*., 2017). Medicinal plants and Essential Oils (EOs) are gaining increasing interest for their important role in food preservation, not only as flavorings but also as natural antioxidants, because of their relatively safe status, their wide acceptance by consumers as well as their biological activities (Aati *et al*., 2020).

In the Mediterranean region, the genus Artemisia belonging to the family Asteraceae is represented by several species. Among the different species of Artemisia, *Artemisia absinthium* L. (wormwood) is a perennial medicinal plant of the Tunisian shrub vegetation. Its aromatic leaves are familiar and are traditionally used for flavoring and as antidiabetic, analgesic and antiulcer agents (Aati *et al*., 2020). Furthermore, the obtained essential oil from

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[DOI: 10.22034/jast.25.3.623]

the leaves of *Artemisia absinthium* L. is known for its interesting biological properties like antimicrobial, insecticidal, antiseptic, antioxidant and neuroprotective activities (Riahi *et al*., 2015).

Enrichment of food products with *Artemisia absinthium* EO is still limited and, to the best of our knowledge, there is no study about its incorporation in butter. In this context, this study aimed to assess the effect of *Artemisia* EO incorporation during butter manufacturing on oxidative stability, physico-chemical, textural, microbiological and sensorial properties of the final fat rich dairy product during two months of refrigerated storage.

MATERIALS AND METHODS

Plant Material and Essential Oil Extraction Description

The leaves of *Artemisia absinthium* L. (wormwood) growing wild were collected in January from the region of Bizerte located in the north-east of Tunisia and identified by Prof. Abdelhamid Khaldi, a specialist in botany, and certified specimens (VS1-QS2020/01) were deposited at the Herbarium run by INRGREF.

Wormwood leaves were air-dried at room temperature, then, hydrodistilled for 3 hours at a temperature of 50°C using a Clevenger-type apparatus (Nahita blue model 655, France). Obtained oil was dried using anhydrous sodium sulfate to obtain deep blue EO in yield of 0.55% (v/w), which was stored in sterile amber glass at 4°C (Aati *et al*., 2020). The main compounds of the obtained *Artemisia* EO were α-Thujone (51.17%), Camphor (28.82%), Chamazulene (6.81%), and Terpinen-4-ol (5.72%).

Butter Manufacturing

Butter manufacturing process was carried out in the Society of Milk and Dairy Products (SLD in Tunisia according to the industrial plan. Briefly, raw milk was preheated at 30°C and then centrifuged. Obtained cream containing 35% fat was pasteurized at 92°C for 20 seconds and then cooled down to 18°C. Cream was inoculated with commercial freeze-dried mesophilic butter culture (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, L*actococcus lactis* subsp. *lactis biovar*. *diacetylactis* and *Leuconostoc mesenteroides* subsp. *cremoris*) provided by Chr. Hansen (Hoersholm, Denmark). After ripening, the cream was subject to churning and washing with tap water. The butter was then mixed and divided into four batches. One batch served as control (CB). *Artemisia absinthium* EO was added to the remaining batches, at raison of 45 ppm (BA_1), 90 ppm (BA_2) and 135 ppm (BA_3) before a second mixing of the final products. Each 250 g of the produced butters were wrapped into industrial aluminum foil and stored at 6°C for two months. Sampling was performed at 0, 20, 40 and 60 days for three replicates with three repetitions for each analysis.

Physicochemical Analyses

The obtained butters were analyzed for acidity, pH and peroxide value. Titratable acidity of the control and treated butters was performed by preparing a solution containing 10 mL of melted butter, 10 mL of distilled water and phenolphthalein. The solution was titrated with 0.1N NaOH until appearance of a stable purple color. Acidity was expressed in terms of % lactic acid (Park *et al*., 2014). The pH values were performed by directly probing the butter with a glass electrode (Ceylan and Ozcan, 2020). The Peroxide Values (PV) expressed as meq O_2 kg^{-1} fat were determined according to the method of Asdagh and Pirsa (2020). Briefly, 2 g of the butter were mixed with a solution of acetic acid and chloroform. Then, potassium iodide saturation solution was added to the mixture that was kept in dark for 5 minutes before

[DOI: 10.22034/jast.25.3.623]

being titrated with sodium thiosulphate (0.01N) in presence of starch.

Radical Scavenging Activity of Butter

The antioxidant activity of *Artemisia* EO in butter was performed using DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by Asha *et al*. (2015). A total of 0.2 mL of butter was added to 3.8 mL of ethyl acetate and 1 mL of DPPH solution in ethyl acetate. A reference sample was also prepared. The absorbance was measured at 520 nm wavelength. Radical scavenging activity was expressed as percentage inhibition and was calculated using the following formula:

% Radical scavenging activity = $[(A_{Control} - A_{Sample})/(A_{Control})]\times 100$

Where, $A_{Control}$: Absorbance of the Control sample, A_{Sample}: Absorbance of the analyzed Sample.

Fat Content and Fatty Acids Profiles

The fat content was determined according to the reference procedure (IDF 80-3/ISO 3727-3, 2003). Fatty Acids (FAs) composition of the control and enriched butter samples was determined by a Gas Chromatography after conversion of fatty acids to methyl esters. Gas chromatograph (Agilent 6890N Series, Hewlett-Packard Co., Avondale, PA, USA) equipped with a hydrogen Flame Ionization Detector (FID) and a capillary column (Omega wax TM 320 (30 m×0.32 mm×0.25 µm). The column temperature was programmed from 180 to 240°C at 5 °C/min and the injector and detector temperatures were set at 250°C. Identification and quantification of FAs was performed by comparing the retention times of peaks with those of standards analyzed under the same conditions. Peak areas of triplicate injections were measured with an HP computing integrator. The results are expressed as w/w (%) total fatty acids in the lipid fraction (Abid *et al*., 2017).

Textural Measurement

Texture profile assessment was carried out using a Texture Analyzer TA-HDi (Stable Micro Systems, UK). Samples of rectangular 5×3 cm block of butter transferred from a refrigerator (6°C) was determined at room temperature (20±2°C) using a cylindrical probe (diameter 1 cm^2) with compression speed of 40 mm min^{-1} and a compression rate of 2 cm (Samet-Bali *et al*., 2009). For each replicate, the hardness of three samples of each butter was performed.

Color Measurement

For color parameters determination, a colorimeter (Minolta Chroma Meter CR-300, Minolta, Osaka, Japan) was used after calibration with a white tile. Based on the CIE Lab color space, the results were expressed as L*, a* and b*. L* varies from 0% (dark) to 100% (light); a* ranges from −100% (green) to 100% (red); and b* changes from −100% (blue) to 100% (yellow) as described by Abid *et al*. (2017).

Microbiological Analyses

The enumeration of coliforms was performed on Violet Red Bile Lactose **(**VRBL) (Biokar Diagnostics Beauvais, France) agar according to ISO 4832 (ISO, 2006), while the enumeration of yeasts and molds was carried out on Sabouraud with chloramphenicol (Biokar Diagnostics Beauvais, France) after incubation at 30°C for 3 days (Mehdizadeh *et al*., 2019).

Sensory Evaluation

At the beginning of storage, the sensory properties of the produced butters were performed by panelists consisting of 15 trained individuals. The studied butters were coded and served to the panel in a randomized order inside a uniformly

[DOI: 10.22034/jast.25.3.623]

illuminated room, at approximately 14°C. In this study, to determine sensorial quality of each butter sample, the studied descriptors were color intensity, odor intensity, acidic taste, bitterness, spreadability and overall acceptability (Ceylan and Ozcan, 2020) based on a scale from 0 (low intensity) to 9 (high intensity) (Mallia *et al*., 2008).

Statistical Analysis

Results of descriptive analyses were presented as mean and standard deviation (SD). A one-way Analysis Of Variance (ANOVA) in SPSS software version 26.0 (SPSS IBM 2020) was used on the obtained results performed in three replicates. Duncan's test was performed at a significance level of 5% to highlight significant differences among the samples and during storage time.

RESULTS AND DISCUSSION

Effect of *Artemisia* EO Incorporation on Physicochemical Properties of Butter

Titratable acidity, pH and PV values of the control and butters enriched with *Artemisia absinthium* EO was determined during 2 months of refrigerated storage and are given in Table 1.

It was found that the highest initial acidity

Table 1. Physicochemical properties, oxidative stability and antioxidant activity of the control and butters enriched with *Artemisia absinthium* EO during 2 months of storage at 6°C.

Analyses	Storage period (Days)	Butter samples*			
		CB	BA_1	BA_2	BA_3
pH (at 20 °C)	0	$4.96 \pm 0.01^{a\,A}$	$5.18 \pm 0.01^{b\,A}$	5.28 ± 0.00^{c} A	$5.32 \pm 0.03^{c\,A}$
	20	$4.87 \pm 0.01^{a\,B}$	$5.09 \pm 0.01^{b\,B}$	5.10 ± 0.01^{c} B	$5.23 \pm 0.01^{d\,B}$
	40	$4.86 \pm 0.01^{a\,B}$	4.93 ± 0.00^{b} C	4.98 ± 0.01^{c} C	$5.20 \pm 0.01^{b\,B}$
	60	$4.80 \pm 0.01^{a\,C}$	$4.79 \pm 0.03^{a\,D}$	4.96 ± 0.03^{b} C	$5.06 \pm 0.06^{b\,C}$
Acidity (% Lactic acid)	0	$0.10 \pm 0.01^{b\,A}$	$0.09 \pm 0.01^{a.b\,A}$	$0.08 \pm 0.01^{a.b\,A}$	$0.07 \pm 0.00^{a\,A}$
	20	$0.14 \pm 0.02^{b\,AB}$	$0.1 \pm 0.00^{a\,AB}$	$0.09 \pm 0.01^{a\,A\,B}$	$0.08 \pm 0.01^{a\,AB}$
	40	$0.15 \pm 0.03^{b\,BC}$	$0.11 \pm 0.01^{a.b\,BC}$	$0.09 \pm 0.01^{a\,BC}$	$0.08 \pm 0.01^{a\,B}$
	60	$0.16 \pm 0.01^{b\,C}$	$0.14 \pm 0.01^{a.b\,C}$	$0.12 \pm 0.04^{a.b\,C}$	$0.09 \pm 0.01^{a\,C}$
Peroxide value (meq O2 kg^{-1} fat)	0	$0.29 \pm 0.00^{c\,A}$	$0.19 \pm 0.01^{b\,A}$	$0.15 \pm 0.00^{a\,A}$	$0.14 \pm 0.01^{a\,A}$
	20	$0.55 \pm 0.01^{d\,B}$	$0.25 \pm 0.00^{c\,B}$	$0.19 \pm 0.00^{b\,B}$	$0.15 \pm 0.01^{a\,A}$
	40	$0.69 \pm 0.01^{c\,C}$	$0.28 \pm 0.00^{b\,B}$	$0.26 \pm 0.01^{b\,C}$	$0.21 \pm 0.00^{a\,B}$
	60	$0.78 \pm 0.03^{c\,D}$	$0.4 \pm 0.04^{b\,C}$	$0.37 \pm 0.01^{b\,D}$	$0.29 \pm 0.01^{b\,C}$
Fat (% w/w)	0	$83.65 \pm 0.21^{a\,A}$	$84 \pm 0.02^{b\,A}$	$85 \pm 0.04^{c\,A}$	$85 \pm 0.00^{c\,A}$
	20	$84 \pm 0.00^{a\,B}$	$84 \pm 0.00^{a\,B}$	$85.75 \pm 0.15^{b\,B}$	$86 \pm 0.07^{b\,B}$
	40	$85.5 \pm 0.07^{a\,C}$	$85.5 \pm 0.04^{a\,C}$	$86 \pm 0.03^{a\,C}$	$86.5 \pm 0.07^{a\,C}$
	60	$86 \pm 0.02^{a\,D}$	$86.5 \pm 0.20^{a\,D}$	$86.5 \pm 0.25^{a\,D}$	$87 \pm 0.02^{a\,D}$
Antioxidant activity (%)	0	10.79 ± 3.21^{aA}	52.97 ± 1.9^{bB}	57.46 ± 1.07^{bB}	60.98 ± 8.9^{bB}
	20	10.1 ± 2.8^{aA}	31.89 ± 0.54^{bB}	42.38 ± 2.7^{bB}	50.6 ± 7.23^{bAB}
	40	8.76 ± 1.61^{aA}	25.38 ± 7.49^{bAB}	34.48 ± 0.28^{bcAB}	40.16 ± 7.23^{cA}
	60	4.68 ± 0.14^{aA}	12.2 ± 0.95^{bA}	21.745 ± 2.23^{cA}	32.65 ± 2.02^{dA}

$^{(A-D)}$ and $^{(a-d)}$: Values are means of three replicates and ±standard deviation of n= 3. Means with different superscripts are significantly different (P< 0.05). Lowercase letters (a, b, c) represent the statistical difference between samples; Uppercase letters (A, B, C) represent the statistical difference between the same sample during storage period.
* Symbols are defined in the text and under Figure 1.

[DOI: 10.22034/jast.25.3.623]

was obtained in the control. Also, acidity values decreased with the increase of *Artemisia* EO concentration. During storage, acidity increased significantly ($P< 0.05$) to reach the highest value (0.16±0.01%) in the control. These changes were mainly attributed to the release to fatty acids as a result of lipolysis as described by Ceylan and Ozcan (2020).

When pH values of the studied butters were examined, observations on acidity evolution were confirmed with a decrease in pH values during storage. The lowest pH values were observed in the control compared to butters treated with different concentrations of *Artemisia* EO. These results were in agreement with those of Ozkan *et al*. (2007) reporting a decrease in pH and an increase in acidity for traditional Tunisian butter and Turkish industrial butter stored at 4°C for, respectively, 6 weeks and 2 months. The highest EO concentration led to the highest ($P< 0.05$) pH values, which can positively affect the properties of butter while pH of the environment influences the characteristics of butter in the presence of lactic acid and carbonyl components according to Ceylan and Ozcan (2020).

The peroxide value is used as an oxidative index for early lipid oxidation when hydroperoxide is the primary formed product (Ozkan *et al*., 2007; Abid *et al*., 2017). Results showed that initial PV values were significantly ($P< 0.05$) different between the control and all tested butters incorporated with various *Artemisia* EO concentrations. However, the obtained initial PV values were lower than that (0.35 meq O_2 kg^{-1} fat) reported by Ozkan *et al*. (2007). During all refrigerated storage period, it should be noted that PV values increased significantly ($P< 0.05$) for all analyzed samples with the highest value noted in the control. Moreover, it can be seen that incorporation of *Artemisia* EO at various concentrations reduced significantly the oxidation rate of butter in terms of peroxides formation, confirming its antioxidant effect as described by Aati *et al*. (2020). Compared to the control (0.78±0.03 meq O_2 kg^{-1} fat), the highest concentration of *Artemisia* EO was the most efficient in retarding the formation of oxidation products with PV value not exceeding 0.29±0.01 meq O_2 kg^{-1} fat at the end of storage. These findings were in line with those of Ozkan *et al*. (2007) and Abid *et al*. (2017) reporting the intense antioxidant effect of *Satureja cilicica* EO and tomato extract in butter, respectively.

Effect of *Artemisia* EO Incorporation on Radical Scavenging Activity of Butter

The antioxidant activity of *Artemisia* EO in butter during refrigerated storage was evaluated by DPPH assay and results are shown in Table 1.

The initial antiradical activity of control butter (10.79±3.21%) was significantly lower when compared to those of enriched butters. During refrigerated storage, radical scavenging activity decreased significantly ($P< 0.05$) in the control and enriched butters as reported before by Asha *et al*. (2015) on ghee samples. As expected, throughout storage, no significant differences ($P> 0.05$) were observed between enriched butters (BA_3, BA_2 and BA_1) showing intense antioxidant activities due to their richness in natural antioxidant compounds. However, butter incorporated with the highest EO concentration exhibited stronger ($P< 0.05$) radical scavenging activity (32.56±2.02%) than other analyzed butter samples after two months of refrigerated storage. Thus, *Artemisia* EO could successfully be used as natural antioxidant in foods to provide protection against oxidative diseases as suggested before by Riahi *et al*. (2015).

Effect of *Artemisia* EO Incorporation on Fat Content and Fatty Acids Profile of Butter

Fat content (%) of the control and enriched butters were determined during 60 days of storage (Table 1). The initial fat content of control butter (83.65±0.21%) was lower ($P<$

[DOI: 10.22034/jast.25.3.623]

0.05) than those noted for enriched butters and reaching the same content of about 85±0.00% for BA_2 and BA_3 butters incorporated with the two highest *Artemisia* EO concentrations. This result was in perfect accordance with previous studies reporting that butter contains more than 80% milk fat (Staniewski *et al*., 2021). At the end of storage, no significant differences (P> 0.05) were observed between all analyzed butter samples in terms of fat content.

Table 2 shows the Fatty Acids (FAs) composition of the control and enriched butters at initial and final days of storage at 6°C. From each butter sample, 14 fatty acids were quantified with the major detected FAs were palmitic acid followed by oleic acid, myristic acid and stearic acid. These findings were in line with those of Staniewski *et al*. (2021) showing a similar FAs composition in butter samples. Moreover, Ceylan and Ozcan (2020) reported the importance of butter consumption for human energy metabolism due to its rich biochemical composition. It was reported that the high contents of some Saturated Fatty Acids (SFA) (palmitic and stearic acids) and particularly Unsaturated Fatty Acids (UFA) were essential in human nutrition due to their functional properties and positive effects on health. In this study, compared to the control, the enriched butters had higher contents of monounsaturated and polyunsaturated fatty acids. It was observed that incorporating *Artemisia* EO led to higher contents in stearic (C18: 0) and oleic (C18: 1) acids, which will be beneficial for human health. Oleic acid was, in fact, used in body fat reserves as well as one of the components reducing the proportion of low-density lipoprotein (LDL) in the blood (Ceylan and

Table 2. Total fatty acids (g 100 g^{-1} milk fat & %) of the control and butters enriched with *Artemisia absinthium* EO during 2 months of storage at 6°C.

	Butter samples*							
	CB		BA_1		BA_2		BA_3	
Storage period (Days)	0	60	0	60	0	60	0	60
Fatty acids								
Butyric acid C4:0	2.30^{cA}	2.28^{aA}	2.38^{aA}	2.34^{aA}	2.47^{aA}	2.41^{aA}	2.63^{aB}	2.44^{aA}
Caproic acid C6:0	1.46^{aA}	2.18^{aA}	0.40^{aA}	1.65^{aB}	0.40^{aA}	1.81^{aB}	0.57^{aA}	1.16^{aA}
Caprylic acid C8:0	2.85^{bA}	2.84^{bA}	1.24^{aA}	1.28^{aA}	1.19^{aA}	1.48^{aA}	1.13^{aA}	0.98^{aA}
Capric acid C10:0	3.93^{aA}	3.92^{aA}	3.57^{aA}	3.25^{aA}	3.25^{aA}	3.45^{aA}	3.80^{aA}	3.75^{aA}
Lauric acid C12:0	4.96^{bA}	4.35^{bA}	3.61^{aA}	2.91^{aA}	3.56^{aA}	3.01^{aA}	3.50^{aA}	3.07^{aA}
Myristic acid C14:0	14.09^{bA}	13.47^{aA}	13.37^{aA}	12.87^{aA}	13.38^{aA}	13.01^{aA}	13.28^{aA}	13.50^{aA}
Palmitic acid C16:0	32.04^{aA}	33.03^{bB}	33.26^{bA}	33.72^{bA}	33.38^{bA}	33.52^{bA}	33.48^{bA}	34.11^{bA}
Palmitoleic acid C16:1	1.98^{aA}	2.21^{aA}	2.12^{aA}	2.47^{aA}	2.39^{aA}	2.65^{aA}	2.32^{aA}	2.53^{aA}
Stearic acid C18:0	10.34^{aA}	9.66^{aA}	12.47^{bA}	12.03^{bA}	12.49^{bA}	12.10^{bA}	12.83^{bA}	12.00^{bA}
Oleic acid C18:1	21.54^{aA}	23.32^{aB}	22.49^{aA}	25.65^{bB}	22.92^{aA}	25.55^{bB}	23.11^{aA}	26.29^{bB}
Linoleic acid C18:2	2.22^{aA}	2.32^{aA}	2.33^{aA}	2.36^{aA}	2.31^{aA}	2.50^{aA}	2.32^{aA}	2.49^{aA}
Linolenic acid C18:3	0.08^{aA}	0.68^{aA}	0.14^{aA}	0.65^{aA}	0.18^{aA}	0.69^{aA}	0.17^{aA}	0.72^{aA}
Arachidic acid C20:0	0.52^{aA}	0.52^{aA}	0.60^{aA}	0.21^{aA}	0.65^{aA}	0.20^{aA}	0.24^{aA}	0.24^{aA}
Eicosenoic acid C20:1	0.94^{aA}	0.92^{aA}	1.2^{aA}	1.17^{aA}	1.27^{aA}	1.17^{aA}	1.26^{aA}	1.25^{aA}
Saturated Fatty Acids (SFA)	72.49^{cA}	72.25^{cA}	70.9^{aA}	70.28^{aA}	70.77^{aA}	70.99^{abA}	71.46^{bA}	71.25^{bA}
Unsaturated fatty acids (UFA)	24.26^{aA}	29.45^{aB}	28.28^{bA}	32.3^{bB}	29.07^{cA}	32.56^{bB}	29.18^{cA}	33.28^{cB}
UFA/SFA	0.33^{aA}	0.41^{aB}	0.4^{bA}	0.46^{bA}	0.41^{bA}	0.46^{bA}	0.41^{bA}	0.47^{bA}

$^{(A-C)}$ and $^{(a-c)}$: Values are means of three replicates, standard deviation are in the range [±0.87 to ±1.97]. Means with different superscripts are significantly different (P< 0.05). Lowercase letters (a, b, c) represent the statistical difference between samples; uppercase letters (A, B, C) represent the statistical difference between the same sample during storage period.

* Symbols are defined in the text and under Figure 1.

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Ozcan, 2020). Moreover, the linoleic acid (C18: 2), an essential fatty acid known for its effect on body development (Mallia *et al*., 2008), was quantified in the analyzed butters at levels varying between 2.32% and 2.50%, at the end of storage. Thus, the incorporation of *Artemisia absinthium* EO was efficient for the reduction of food oxidation due to the strong antioxidant activity of its major and minor components (Riahi *et al*., 2015). It could be used as ingredient for the production of functional foods containing high content of fat to reduce lipid oxidation and improve their nutritional value.

Effect of *Artemisia* EO Incorporation on Texture of Butter

Texture values of butter samples showing hardness are given in Table 3. During butter production, coalescence of fat globules is required to contribute to structure formation, firmness and sensory properties (Sert *et al*., 2020). Initial firmness values ranged from 1,810 g in the control to 4,461 g in BA3. These values were higher than those reported by Sert *et al*. (2020). It was observed that firmness of the analyzed butters increased with the increase of *Artemisia* EO concentration. During the two months of storage, the hardness of all analyzed butters decreased significantly to reach values varying from 1,350 g in the control to 2,725 g in BA3. These findings were probably attributed to the fat content and the composition in fatty acids. In fact, the main physical properties of butter, including melting and solidification temperature and, consequently, butter hardness are influenced by, respectively, fat content and fatty acids composition as suggested by Ceylan and Ozcan (2020) and Staniewski *et al*. (2021). In this study, *Artemisia* EO preserved good contents of FAs and improved the oxidative stability of enriched butters. As a result, the firmness of tested butter samples was positively affected by *Artemisia* EO incorporation as reported by Ceylan and Ozcan (2020), showing that

Table 3. Hardness and color parameters of the control and butters enriched with *Artemisia absinthium* EO during 2 months of storage at 6°C.

Analyses	Storage period (Days)	Butter samples*			
		CB	BA_1	BA_2	BA_3
Hardness (g)	0	1810 ± 127.28^{aB}	1830 ± 692.97^{aC}	4328 ± 0.00^{bC}	4661 ± 0.00^{bC}
	30	1700 ± 596.8^{aB}	1607 ± 238.3^{aB}	3100 ± 141.42^{bB}	3125 ± 35.36^{bB}
	60	1350 ± 212.13^{aA}	1550 ± 494.97^{abA}	2325 ± 176.78^{bcA}	2300 ± 0.00^{cA}
L*	0	62.49 ± 0.24^{bB}	61.57 ± 0.57^{aB}	60.36 ± 0.30^{aD}	58.93 ± 0.23^{aC}
	20	61.74 ± 0.43^{cB}	59.32 ± 1.36^{bB}	57.14 ± 0.58^{abC}	54.51 ± 3.20^{aB}
	40	60.28 ± 0.16^{cB}	58.67 ± 0.95^{cAB}	53.67 ± 1.68^{bB}	47.07 ± 1.14^{aA}
	60	58.44 ± 0.69^{bA}	56.52 ± 1.34^{bA}	47.35 ± 2.18^{aA}	45.6 ± 0.67^{aA}
b*	0	26.11 ± 0.79^{aA}	25.78 ± 0.43^{aB}	28.91 ± 0.89^{bC}	30.35 ± 0.74^{bB}
	20	26.44 ± 0.07^{cA}	23.92 ± 0.23^{bB}	24.72 ± 0.94^{bcAB}	20.37 ± 1.28^{aA}
	40	26.42 ± 0.49^{cA}	22.8 ± 0.97^{bAB}	23.88 ± 0.07^{bA}	19.44 ± 1.35^{aA}
	60	26.81 ± 0.19^{bA}	20.32 ± 0.48^{aA}	21.71 ± 0.91^{aA}	19.36 ± 0.46^{aA}
a*	0	-3.50 ± 0.39^{bA}	-3.67 ± 0.36^{bC}	-5.21 ± 0.58^{aB}	-5.53 ± 1.54^{aC}
	20	-2.80 ± 0.48^{cB}	-4.27 ± 0.28^{bB}	-5.80 ± 0.19^{aB}	-6.44 ± 0.12^{aB}
	40	0.82 ± 0.08^{cC}	-4.94 ± 0.02^{bB}	-6.87 ± 0.24^{aA}	-6.75 ± 0.09^{aB}
	60	1.17 ± 0.07^{cC}	-5.70 ± 0.20^{bA}	-7.04 ± 0.25^{aA}	-7.85 ± 0.04^{aA}

$^{(A-C)}$ and $^{(a-c)}$: Values are means of three replicates and ±standard deviation of n= 3. Means with different superscripts are significantly different (P< 0.05). Lowercase letters (a, b, c) represent the statistical difference between samples; uppercase letters (A, B, C) represent the statistical difference between the same sample during storage period.
* Symbols are defined in the text and under Figure 1.

[DOI: 10.22034/jast.25.3.623]

the increase of hardness was attributed to the increase of the total fat content. Moreover, this finding was probably assigned to the highest values of UFA/SFA ratios (0.46 to 0.47) noted in the enriched butters when compared to the control (0.41). In fact, according to Staniewski *et al*. (2021), the rheological properties of butter are significantly correlated not only with the content of individual FAs, but also with the ratios UFA/SFA, C14:0/C18:1 and C16:0/C18:1. These authors concluded that more desirable rheological properties of butter can be achieved when the ratio of UFA/SFA exceeds 0.54.

Effect of *Artemisia* EO Incorporation on Color Properties of Butter

Concerning CieLab coordinates (L*, a*, b*) of tested butters (Table 3), it can be seen that the difference between all color parameter values of different butter samples was statistically significant (P< 0.05) depending on the type of butter. The *L** value indicates the lightness or darkness of the color, *b** indicates the intensity of yellow and *a** indicates redness (Ceylan and Ozcan, 2020). The *b** parameter remained constant for the control butter during storage. However, the yellow color (b*) decreased slightly in all enriched butters during storage. This parameter decreased with the increase of *Artemisia* EO concentration, which was probably assigned to the initial color of used EO. Furthermore, during the two months of storage, Lightness (L*) values decreased in all the analyzed butters. These results were partially in accordance with those of Samet-Bali *et al*. (2009) reporting a decrease in yellowness and an increase in lightness of traditional butter oil at the first 5 days of storage followed by a stabilization during storage at 60°C. The incorporation of *Artemisia* EO was influential on color characteristics of the final product. This result was expected due to the richness of *Artemisia* EO with chamazulene molecule responsible for the typical greenish-blue color of the tested EO (Riahi *et al*., 2015).

Effect of *Artemisia* EO Incorporation on Microbiological Quality of Butter

The microbiological characteristics of the analyzed butters are shown in Table 4. Coliform count is one of the most important contamination indicators. The highest initial coliform and yeast and molds counts were found in the control with respective values of about 1.70±0.05 and 1.50±0.01 log CFU/g. Initial microbial counts of all tested butters were very close (P< 0.05) and lower than those found in previous research (Samet-Bali *et al*., 2009; Sert *et al*., 2020). During 60 days of refrigerated storage, coliforms and fungal counts increased slightly (P> 0.05) in the control. However, *Artemisia* EO incorporation decreased the counts of these undesirable microorganisms and even inhibited their proliferation during storage, confirming the intense antibacterial and antifungal effects of the compounds present in *Artemisia* EO (Riahi *et al*., 2015). These findings showed that enrichment of butter with *Artemisia* EO can contribute to the improvement of its shelf life.

Effect of *Artemisia* EO Incorporation on Sensorial Properties of Butter

The results of descriptive sensorial analysis are depicted in Figure 1. Many authors showed that the color, firmness, and spreadability of butter are the most important sensory parameters that affect consumers' preference (Ceylan and Ozcan, 2020; Sert *et al*., 2020).

No significant differences were found regarding the color of the control and the butter incorporated with the lowest EO concentration with assigned note of about 4.58. However, the two other enriched butters were distinguished by a darker color tending towards green. This finding was attributed to the typical greenish-blue color of chamazulene molecule present in *Artemisia* EO (Riahi *et al*., 2015). The panelists attributed high intensities of bitterness of about 5 and 6 for butters BA_2 and BA_3, respectively, incorporated with the

[DOI: 10.22034/jast.25.3.623]

Table 4. Microbiological quality (log CFU/g) of the control and butters enriched with *Artemisia absinthium* EO during 2 months of storage at 6°C.

Analyses	Storage period (Days)	Butter samples*			
		CB	BA_1	BA_2	BA_3
Total coliforms	0	1.70 ± 0.05^{aA}	1.71 ± 0.01^{aB}	1.70 ± 0.01^{aC}	1.69 ± 0.01^{aB}
	20	1.78 ± 0.01^{cA}	1.4 ± 0.01^{bA}	1.11 ± 0.01^{bB}	$<1^{aA}$
	40	1.85 ± 0.01^{cA}	1.34 ± 0.01^{bA}	1.06 ± 0.01^{bAB}	$<1^{aA}$
	60	1.88 ± 0.01^{cA}	1.3 ± 0.01^{bA}	0.85 ± 0.01^{bA}	$<1^{aA}$
Yeasts and molds	0	1.50 ± 0.01^{aA}	1.50 ± 0.03^{aC}	1.50 ± 0.05^{aB}	1.47 ± 0.02^{aB}
	20	1.54 ± 0.03^{bA}	1.12 ± 0.01^{bB}	$<1^{aA}$	$<1^{aA}$
	40	1.58 ± 0.01^{bA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$
	60	1.65 ± 0.02^{bA}	$<1^{aA}$	$<1^{aA}$	$<1^{aA}$

$^{(A-C)}$ and $^{(a-c)}$**:** Values are means of three replicates and ±standard deviation of n= 3. Means with different superscripts are significantly different (P< 0.05). Lowercase letters (a, b, c) represent the statistical difference between samples; uppercase letters (A, B, C) represent the statistical difference between the same sample during storage period.

* Symbols are defined in the text and under Figure 1.

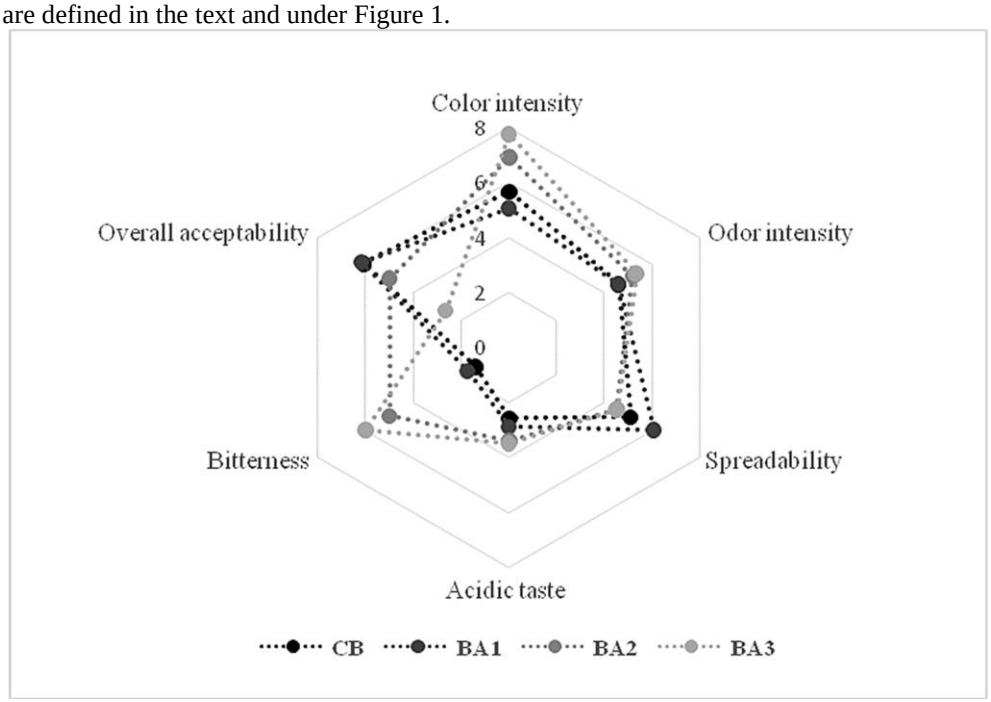

Figure 1. Sensorial properties of control and enriched butters with *Artemisia absinthium* EO during 2 months of storage at 6 °C. **CB**: Control Butter; **BA_1**: Butter treated with 45 ppm of *Artemisia absinthium* EO; **BA_2**: Butter treated with 90 ppm of *Artemisia absinthium* EO; **BA_3**: Butter treated with 135 ppm of *Artemisia absinthium* EO.

two highest EO concentrations, while the butter BA_1 enriched with the lowest EO concentration and the control were devoid of bitterness. This result can be assigned to the presence of bitter molecules in wormwood such as thujone, sesquiterpene lactones including absinthin and its isomeric form as reported by Bach *et al*. (2016).

Concerning the texture, the butter incorporated with the lowest EO concentration seemed to be the most preferred in terms of spreadability with a score of about 6.05 followed by the control and the other enriched butters. Thus, the butter BA_1 incorporated with 45 ppm EO concentration was the most appreciated by consumers.

[DOI: 10.22034/jast.25.3.623]

CONCLUSIONS

In this study, incorporation of *Artemisia absinthium* essential oil in butter was shown to be an efficient treatment to improve quality of fat rich dairy products in terms of physicochemical, textural, microbiological and sensorial properties. During refrigerated storage, a strong antioxidant effect of *Artemisia* EO was observed in butter by preventing the decrease of lipid content and retarding the formation of peroxides and the oxidative deterioration. Changes in fatty acids showed that the new functional butter enriched with *Artemisia* EO had interesting nutritional value when compared to the control by increasing the content of unsaturated fatty acids. As confirmed by sensorial analysis, the findings revealed that low concentration of *Artemisia absinthium* EO could be used in butter as natural antioxidant and antimicrobial agent, with no changes in color, odor, taste, and overall acceptability.

ACKNOWLEDGEMENTS

This study was sponsored by the «Tunisian Ministry of Higher Education and Scientific Research» from University of Carthage. This work was performed in the Research Laboratory: Technological Innovation and Food Safety (LR22- AGR01) «Higher Institute of Food Industries of Tunisia- ESIAT».

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تولید و پایدار سازی کره عملکردی در ترکیب با اسانس درمنه آبسینتیوم *Artemisia absinthium* L.

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چکیده

هدف این پژوهش بررسی اثر ترکیب اسانس درمنه آبسینتیوم (EO) در غلظت‌های مختلف بر ویژگی ها و پایداری اکسیداتیو کره طی ۲ ماه نگهداری در یخچال بود. نتایج به‌دست‌آمده در مورد مقدار پراکسید، اسیدیته، اسیدهای چرب، فعالیت ضد رادیکال و خواص فیزیکی و میکروبیولوژیکی، موثر بودن ترکیب اسانس درمنه EO را در غذاهای لبنی غنی از چربی تایید کرد. کره های تولید شده جدید را می توان به دلیل فعالیت آنتی اکسیدانی قوی، پایداری اکسیداتیو بهتر و غنی بودن از اسیدهای چرب غیراشباع ضروری در مقایسه با شاهد به عنوان محصولات عملکردی (functional)گروه بندی کرد. همه پارامترهای کیفی با غنی‌سازی با اسانس Artemisiaبهبود یافتند، با این حال، تجزیه و تحلیل حسی توصیفی (descriptive sensorial analysis) نشان داد که کمترین غلظت محصول (حدود ۴۵ قسمت در ملیون) از نظر رنگ، بو، طعم، سفتی و مقبولیت کلی، بیشترین ارجحیت را داشت. با توجه به اثرات مثبت اسانس درمنه آبسینتیوم (EO) بر تمامی

[DOI: 10.22034/jast.25.3.623]

ویژگی های کره، این اسانس می تواند به عنوان آنتی اکسیدان طبیعی و عامل ضد میکروبی در صنایع غذایی مورد استفاده قرار گیرد.

[DOI: 10.22034/jast.25.3.623]