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ORIGINAL PAPER

Development and characterization of cracker fillings with natural antioxidants

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Abstract

The addition of herbs to cracker fillings was evaluated. Oregano and basil showed a high content of phenolic compounds and a high antioxidant capacity, so these herbs were used in the fillings. All the products developed presented a desirable low water activity (< 0.75), but the addition of oregano or basil significantly decreased the luminosity of the products and increased their adhesiveness. Nevertheless, the addition of herbs positively increased the proportion of bioaccessible antioxidant compounds in the filling matrix. The highest bioaccessible antioxidant content was found in oregano fillings (10.80 ± 0.2; 15.80 ± 0.5 and 14.00 ± 0.10 µmol Trolox/g by DPPH, ABTS and FRAP assays respectively). Besides, sensory analysis showed that the oregano fillings developed in the present work presented excellent sensory characteristics and a high purchase intention. The results revealed that a well-accepted healthy filling with a high antioxidant capacity can be developed by the addition of herbs.

Keywords Antioxidants · Cracker fillings · In vitro digestion · Herbs

Introduction

Antioxidants have become one of the most important topics in food science. These bioactive compounds can control food oxidation during storage time and limit the development of rancid flavours that make foodstuffs unacceptable [1]. Moreover, antioxidants can protect biological tissues from oxidation, which has been associated with mutagenesis, carcinogenesis and degenerative diseases [2].

Vegetables, fruits and cereal products are some of the most important sources of natural antioxidants in diets [3]. Herbs (dry plant materials normally added to improve food flavour) are considered to be excellent sources of bioactive compounds and, because of their long history of safe usage, they are generally recognized as safe (GRAS) [4]. Furthermore, these products are well known for their medicinal properties such as antimutagenic, anticarcinogenic, and cardioprotective activities. These effects are attributed to the phenolic composition of herbs [5]. Thus, the addition of these dry plant materials to different foodstuffs is a worthwhile alternative to increase the amount of bioactive compounds in diets.

Nonetheless, as many food products are thermally treated, labile antioxidants can be destroyed [6, 7]. This is especially important in cereal-based foods, such as bread, biscuits and crackers, which are subjected a strong heating procedure that can destroy the polyphenolic compounds [3]. Results obtained by Patrignani et al. [2] suggested that at the beginning of the biscuit cooking procedure, an important proportion of phenolic compounds with antioxidant capacity are lost.

A possible solution to overcome this problem is to incorporate antioxidants in non-thermally treated food matrixes. The filling addition onto a cracker (or shell) to make a sandwich cracker is a secondary process that does not require the use of high temperatures and offers a great variety of products [8, 9]. Sandwich biscuits are the fastest growing section of biscuits in the United States but they are associated

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with an unhealthy diet [10]. Therefore, some studies have focused on the improvement of their formulation in order to have healthier products [10–12]. Nevertheless, the addition of natural antioxidants to cracker fillings has not been studied yet.

Although most sandwich biscuits are associated with sweet flavours, there are some salty sandwich crackers available in the market that have excellent acceptability (such as cheese sandwich biscuits) [8]. However, no information is available on the physical characteristics of these products or on the benefits that the addition of antioxidants might have on their formulation.

The objective of this study was not only to evaluate the effect of natural antioxidants on a salty cracker filling formulation, but also to develop and characterize a healthy product with good acceptability. In order to do this, the present work was divided into three sections: first, different herbs and species were selected and their antioxidant capacity was analysed. Then, the addition of herbs was evaluated in the cracker fillings, and their physical characteristics as well as the bioavailability of antioxidants in the food matrix were determined. Finally, the sensory acceptability of the cracker fillings developed was evaluated.

Materials and methods

Materials

Whey protein concentrate (80% protein; 7% carbohydrates; 4% lipids; 1% ash and 8% moisture, purchased from Arla Foods, Martinez, Buenos Aires); corn starch (91% carbohydrates; 0.26% protein; 0.05% lipids; 0.37% ash and 8.32% moisture, supplied by Unilever de Argentina S.A., Buenos Aires, Argentina) and a salt solution (NaCl 0.6 g/mL) were used for the cracker filling formulation. The shortening generally used in filling formulations (rich in saturated and trans-fatty acids) [10] was replaced with high oleic sunflower oil (Molino Cañuelas, Cañuelas, Buenos Aires, Argentina).

Five dehydrated culinary products were purchased in a local supermarket: basil (*Ocimum basilicum),* onion (*Allium cepa*), green onion (*Allium fistulosum*), oregano (*Origanum vulgare*) and Provencal mix. The latter is a mixture of garlic (*Allium sativum*) and parsley (*Petroselinum crispum*). All ingredients were of food grade. The moisture content of all the dehydrated culinary products was < 8%.

Chemicals and reagents

Sodium carbonate, sodium acetate and acetic acid were purchased from Biopack. TPTZ reagent (2,4,6-tris(2-pyridyl)-s-triazine) was supplied by Fluka Chemicals. DPPH reagent (1,1-diphenyl-2-picrylhydrazyl), gallic acid, Folin-Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), ABTS reagent (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and potassium persulphate were purchased from Sigma-Aldrich. All these chemicals were of analytical grade.

Extraction of bioactive components

Herbs were ground in an electric coffee grinder (TopHouse, PCML-2013ST, China) and sieved (500 µm). Then, 1 g of ground herbs was placed in a baker and extracted with 10 mL of warm deionized water (45 °C). After 5 min of stirring at ambient conditions, the samples were centrifuged (10 min at 10,000×*g*) and filtered (0.45 µm pore size). Aqueous herbal extracts were stored at − 20 °C until analysis [2]. In the present work, water was used as the extraction solvent as recommended by Wong et al. [13]. According to these authors, plant extracts made with water are more nutritionally relevant than extracts prepared with organic solvents and have no ecological limitations.

Antioxidant capacity determination and total phenolic content

The ferric reducing capacity of each sample was determined according to the procedure described by Benzie and Strain [14]. Briefly, 1.8 mL of freshly prepared FRAP reagent was mixed with 200 µL of properly diluted aqueous samples. After 30 min, the sample absorbance was measured at 593 nm. The DPPH assay as described by Brand-Williams et al. [15] was used to evaluate the free radical scavenging of herbs. A fresh DPPH solution (30 mg/L in ethanol) was added to 25 µL of diluted aqueous samples. After 60 min of reaction time, the absorbance was determined at 515 nm. Finally, the radical scavenging activity of herbs was determined as recommended by Re et al. [16] (ABTS assay). The reaction was started by the addition of 10 µL of the aqueous herbal extract to 1 mL of the fresh ABTS solution (initial absorbance of 0.70 ± 0.02 at 734 nm). After 6 min of reaction time, the absorbance was measured at 734 nm. All the determinations were performed at least in duplicate, and the results were expressed as µmol Trolox equivalents per g of dry herb.

The total phenolic content (TPC) of samples was determined in duplicate according to Patrignani et al. [17]. Briefly, 25 µL of the extract and 50 µL of Folin-Ciocalteu reagent were added to 2325 µL of distilled water. Then, 100 µL of a Na_2CO_3 solution (20% prepared in NaOH 0.1 M) was added. After 90 min of reaction at room temperature (25 °C), the absorbance was measured at 750 nm, and results were expressed as mg of gallic acid per g of dry herb [18].

Preparation of fillings

Three formulations were prepared using high oleic sunflower oil (12 mL), 9 mL of NaCl solution, 11.3 g of whey protein concentrate, 3 g of corn starch and 0.5 g of the selected herb (oregano or basil). The final composition per 100 g of filling was 33.5 g of oil, 25.1 g of NaCl solution, 31 g of whey protein concentrate, 8.4 g of corn starch and 1.4 g of the selected herb. A control filling (without herb addition) was also made as an equivalent to the salty cracker fillings commercially available.

Liquid ingredients (oil and the salt solution) were mixed together for 1 min and then added to the dry solid ingredients. They were then mixed for 2 min to obtain a homogeneous cream [9]. The mixture was placed in a polyethylene bag and sheeted with a rolling pin until a thickness of 0.3 cm. Finally, the fillings were cut with a 3 cm diameter round pastry cutter.

Water activity of the fillings

Water activity of the cracker fillings was measured in quadruplicate with a water activity meter (AquaLab Series 3, Decagon Devices, Pullman, Washington, USA) at 25 °C. The water activity of a salty cracker made as described by Bilbao et al. [19] was also determined.

Colour

The CIELab scale was used to determine the L*, a* and b* parameters of the cracker fillings. All determinations were performed in triplicate with a Minolta Chromameter CR 300 (Osaka, Japan), and the 2-degree standard observer angle was used [20, 21].

Filling texture

For the texture characterization of the fillings, a texture profile analysis (TPA) was performed [22]. The round fillings (0.3 cm high and 3.0 cm in diameter) were subjected to two consecutive cycles of compression until 40% of their height with a P/75 probe (75 mm diameter) in a TA.XT2i Texture Analyser (Stable Micro Systems, UK) at a constant temperature (25 °C) (see Supplementary material). The displacement speed during the analysis was 2 mm/s, and the time elapsed between two cycles was 5 s. The hardness, cohesiveness, adhesiveness and gumminess parameters were graphically obtained from the resulting texture profile analysis as described by Bourne [23]. For each filling formulation, the average of at least five determinations was calculated.

In vitro digestion of cracker fillings

In order to simulate the mastication process, an appropriate amount of lyophilized filling was minced using an electronic mincer (TopHouse, PCML-2013ST, China) as recommended by Patrignani et al. [2]. To simulate the physiological conditions of the digestion process, a standardized in vitro procedure was performed [24]. The soluble supernatants (bioaccessible fractions) were collected and filtered (0.45 μm pore size) [2]. The antioxidant capacity of the soluble fractions was determined by FRAP, DPPH and ABTS as described previously. All the determinations were performed at least in duplicate.

Sensory analysis

In order to simulate the usual consumption conditions, the fillings were provided in a sandwich cracker. Cracker fillings were prepared as aforementioned, while neutral flavour crackers ("the shells") were made as described by Bilbao et al. [19]. The biscuit sandwiching was performed manually by setting the filling onto the base followed by the addition of the top [8]. The panellists were advised to evaluate the fillings only.

Forty-six consumers (men and female) between 18 and 54 years of age were recruited among the students and personnel of the National University of La Plata. Samples were coded and supplied to the consumers who rated their "colour", "texture", "flavour" and "overall acceptance" on a nine-point hedonic scale (1 = dislikes much, 9 = likes much). The panellists also indicated the "purchase intention" towards the presented products [25].

Statistical analysis

Results were statistically evaluated by analysis of variance (ANOVA) at a 0.05 significance level. The least significant differences (LSD) were calculated by comparing the means at a 95% confidence level using the Fisher's test (InfoStat, 2012; Universidad Nacional de Córdoba, Argentina). In order to evaluate the strength of the relations between variables, Pearson's correlation coefficients were calculated. Pearson's coefficients can take on values in the interval [−1; 1]; positive regression coefficients indicate direct correlations, while large regression coefficients suggest strong associations [26].

Results and discussion

Antioxidant capacity of herbs

Significant differences were found in the antioxidant activity of the herbs analysed ($p \leq 0.05$) (Table 1). According to

FRAP and ABTS assays, the antioxidant capacity increased in the following order: onion < Provencal mix < green onion < basil < oregano. These results compare well with those of Hossain et al. [27] and Assefa et al. [28], who also indicated that the antioxidant capacity of oregano was higher than that of basil. Furthermore, the results of green onion antioxidant capacity by the ABTS assay (26 ± 5 µmol Trolox/g of dry herb) were in good agreement with those of Assefa et al. [29], who reported 33 µmol of Trolox equivalent per g of this dry species. However, these authors indicated that the antioxidant capacity of onion was higher than that of green onion by ABTS assay, which is slightly inconsistent with our results.

Onion did not present any detectable antioxidant activity by ABTS or FRAP assays (Table 1). This result was slightly unexpected, since onion is the main source of dietary quercetin, a well-known antioxidant with several health benefits such as prevention of cardiovascular and neurodegenerative diseases [30]. However, the antioxidant activity of onion could be determined by DPPH analysis. This supports the idea that, because of the complexity of oxidative reactions, a combination of tests must be used in order to obtain accurate results [31].

According to results displayed in Table 1, the antioxidant activity determined by DPPH assay showed similar results to ABTS and FRAP assays, but basil presented a higher antioxidant activity than oregano ($p \leq 0.05$) (205 ± 5 µmol Trolox/g of dry herb and 178 ± 6 µmol Trolox/g of dry herb for basil and oregano, respectively, according to DPPH analysis). This result does not agree with that of Assefa et al. [28], who indicated that oregano presented a higher antioxidant activity than basil by the DPPH assay. According to these authors, the antioxidant activity was 95 µmol Trolox/g of dry herb and 215 µmol Trolox/g of dry herb for basil and oregano respectively. However, Assefa et al. [28] also indicated that the antioxidant capacity of basil was only 95.12 µmol Trolox/g of dry herb, significantly lower than the one found in the present work (205 ± 5 µmol Trolox/g of dry herb). Differences in the antioxidant content of aromatic herbs could be due to various reasons, including the extraction conditions, the sample pre-treatment and differences in the growth conditions of the plant material [32–34].

Phenolic compounds are the most well-known antioxidants in plant-based foods. The TPC of the different culinary spices analysed is listed in Table 1. The highest TPC was found in oregano (17.60 ± 0.50 mg of gallic acid/ g of dry herb), followed by basil (5.50 ± 0.50 mg of gallic acid/g of dry herb) and then green onion (2.55 ± 0.07 mg of gallic acid/ g of dry herb) ($p \leq 0.05$). On the other hand, the lowest TPC values were found in Provencal mix (1.52 ± 0.50 mg of gallic acid/ g of dry herb) and dry onion (0.91 ± 0.05 mg of gallic acid/ g of dry herb). By contrast, Shan et al. [35] reported higher TPC amounts in oregano and basil (36.4 mg of gallic acid/g of dry herb and 101.7 mg of gallic acid/g of dry herb respectively). However, Hossain et al. [27] determined that the TPC of basil was 30 mg of gallic acid/ g of dry herb, which is slightly lower than that of oregano (40 mg of gallic acid/g of dry herb). Differences in the phenolic content and antioxidant capacity can be partially attributed to the different extraction methods: Shan et al. [35] used methanol as extraction solvent and Hossain et al. [27] ethanol, while in the present work aqueous extracts were employed. According to Alothman et al. [36] the recovery of phenolic compounds is strongly dependent on the polarity of the solvent used. These authors reported higher phenolic and antioxidant extraction rates when ethanol or methanol was used instead of water. Nevertheless, an aqueous medium is more representative of physiological conditions, and several studies support the aqueous phenolic extraction [37]. In good line with our results, Cioroi and Dumitriu [38] extracted phenolic compounds of oregano and basil in water. These authors indicated that the TPC of basil was 5.16 mg of gallic acid/g of dry herb, which was similar to our results (5.50 ± 0.50 mg of gallic acid/g of dry herb). However, they found a slightly lower amount of phenolic compounds in oregano (according to these authors the TPC of oregano was only 8.59 mg of gallic acid/g of dry herb). As previously described, differences in the phenolic content and the antioxidant activity of herbs can be explained by a combination of factors, including the temperature, the

Table 1 Antioxidant capacity and TPC of different herbs expressed as g of dry herb

	ABTS (µmol Trolox/g)	DPPH (µmol Trolox/g)	FRAP (µmol Trolox/g)	TPC (mg gallic acid/g)
Onion	Nd	Nd	11.2 ± 0.3^{a}	0.91 ± 0.05^{a}
Provencal mix	5 ± 7^{a}	30 ± 6^{a}	24.2 ± 0.2^{b}	1.52 ± 0.03^{a}
Green onion	26 ± 5^{b}	134 ± 1^{b}	27.6 ± 0.1^{b}	2.55 ± 0.07^{b}
Basil	115 ± 7^{c}	205 ± 5^{d}	158.0 ± 5.0^{c}	5.50 ± 0.50^{c}
Oregano	234 ± 7^{d}	178 ± 6^{c}	213.0 ± 4.0^{d}	17.60 ± 0.50^{d}

Nd not detectable

*Results are expressed as mean ± standard deviation. Different letters within each column indicate significant differences among samples according to Fisher's test ($p \leq 0.05$)

Table 2 Pearson's correlation coefficients of antioxidant capacity (measured by DPPH, ABTS and FRAP assays) and TPC of different species

	TPC	DPPH	ABTS	FRAP
TPC	1			
DPPH	0.63*	1		
ABTS	0.97**	0.75*	1	
FRAP	0.90**	0.81**	0.97**	1

* Significant at $p \leq 0.05$

**Significant at $p \leq 0.01$

stirring conditions, e extraction procedure and unavoidable variations in the plant materials used.

Table 2 displays the correlation between the TPC and the antioxidant assays. Results indicate that the phenolic content of the species analysed (determined by Folin-Ciocalteu method) presented a strong and significant correlation with their antioxidant activity determined by DPPH, ABTS and DPPH ($p \leq 0.05$). This confirms that phenols are largely responsible for the antioxidant capacity of herbs [39]. Thus, it could be inferred that the TPC can be considered a good predictor of the antioxidant activities in basil, onion, green onion, oregano and Provencal mix. The strong correlation between the antioxidant contents determined by DPPH, ABTS and FRAP indicated that the compounds capable of reducing radicals were also able to reduce ferric ions [13]. This is consistent with Patrignani et al. [2], who indicated that antioxidants may exert their activity through different mechanisms.

On the whole, it could be concluded that among all the analysed culinary herbs, oregano and basil exerted the highest antioxidant activity. Both products are part of the *Lamiaceae* family; herbs of this family have already demonstrated an important antioxidant activity and are well recognized because of their health benefits such as antimutagenic activity [28]. Therefore, oregano and basil were considered the most suitable herbs to incorporate in the cracker fillings.

Cracker filling characterization

Water activity (a_w)

In order to prevent moisture migration from the fillings into the biscuit shells and avoid their softening, the water activity must be controlled [8]. Moreover, this parameter estimates the microbial growth and the susceptibility to non-enzymatic modifications such as lipid oxidation that could occur during the storage time of the fillings [40]. As reported by other authors, most food-spoiling bacterial growth ceases at an a_w below ≈ 0.90, and the yeast growth limit is 0.85 a_w, while mycotoxigenic moulds have a lower limit for growth at a water activity of ≈ 0.78 [41]. As detailed in Table 2, all the cracker fillings presented an a_w value below 0.75, therefore, it could be considered that microbial deterioration would be inhibited in the filling. Moreover, a low a_w value is desirable to control the moisture migration and avoid the adverse modifications of sandwich crackers during shelf life [9].

The a_w of the biscuits (or "shells") was 0.43 ± 0.03, significantly lower than that of the fillings. As described by Patrignani [42], during the storage time the sandwich cracker reaches the equilibrium at an intermediate value of a_w. In the present work, the final a_w of the sandwich crackers (shells and fillings) was 0.67 ± 0.1, and it could also be considered that microbial deterioration would be inhibited in the sandwich cracker.

Colour of cracker fillings

Colour is one of the most important attributes in food stuffs as it can influence the consumer acceptability and purchase intention [43]. Significant differences were found among the colour parameters L*, a* and b* of the cracker fillings ($p \leq 0.05$). According to Table 3, the luminosity of the fillings decreased when oregano or basil was added ($p \leq 0.05$). Similar results were observed by Chinprahast et al. [44] when oregano or basil was added to pasta. On the other hand, herbs also decreased the a* value, which is perceived as a greenish product. Control fillings presented the highest values of b* parameter, which indicates a more yellow colour (27.69 ± 2), followed by oregano fillings (20.02 ± 2), while in basil fillings the values of this parameter were the lowest (15.81 ± 2). This indicated that the addition of herbs, and especially basil, significantly decreased the yellow colour in the fillings.

Texture of biscuit fillings

Texture perception is one of the most important quality parameters of crackers [20]. Texture includes a variety of characteristics such as firmness, adhesiveness, cohesiveness and gumminess. In order to have a well-accepted sandwich cracker, these parameters must be delicately balanced. The filling should be adhesive to maintain shells together, but a

Table 3 Colour parameters (L, a*; b*) and water activity (a_w) of control, oregano and basil fillings

	a_w	L*	a*	b*
Control	0.736 ± 0.003^{a}	71 ± 1^{c}	-0.77 ± 0.3^{b}	27.69 ± 2^{a}
Oregano	0.739 ± 0.001^{ab}	53 ± 2^{b}	-1.58 ± 0.1^{a}	20.02 ± 2^{b}
Basil	0.742 ± 0.001^{b}	49 ± 1^{a}	-1.31 ± 0.2^{a}	15.8 ± 2^{b}

Results are expressed as mean ± standard deviation. Different letters within each row indicate significant differences among regions according to Fisher's test ($p \leq 0.05$)

too adhesive product could lead to an unacceptable sticky mouth feel [8]. Moreover, the filling should be firm and cohesive to maintain the biscuit shape, but it should break easily in the mouth [8, 9].

Table 4 lists the texture parameters of the cracker fillings. No significant difference was found in the firmness, cohesiveness or gumminess of control and oregano fillings ($p > 0.05$). Nonetheless, basil fillings showed the highest values of hardness (37.3 ± 2.8 N), gumminess (16 ± 2 N) and the lowest values of cohesiveness (0.44 ± 0.02). Hardness is perceived as the strength required to compress the product between the teeth, gumminess is related to the energy required to disintegrate a product before swallowing it, while cohesiveness is strongly associated with the extent to which a material can be deformed and it is perceived as the degree to which a product can be compressed between the teeth before it breaks [45]. Therefore, compared to basil fillings, control and oregano fillings were softer, more cohesive, and less energy was required to disintegrate them.

On the other hand, as can be observed in Table 4, all the biscuit fillings were adhesive, which indicates that they could successfully maintain the shells together. Moreover, the incorporation of herbs to the cracker fillings significantly increased their adhesiveness ($p \leq 0.05$). Samples with herb addition presented similar adhesiveness to some commercial fillings, as reported by Battaiotto et al. [9]. It could be concluded that the addition of herbs to the cracker filling formulation increases their stickiness, making them more similar to commercial products.

The hardness of the cracker filling should be in line with the energy required to break the shells [8]. If the filling is too soft, it could squeeze out when bitten, but if it is too hard, then the consumer might reject it. Results obtained by Patrignani [42] indicated that the hardness of a well-accepted biscuit filling was 21.13 ± 4.31 N. This value is significantly lower than the hardness values found in basil filling (37.3 ± 2.8 N), but similar to the control and oregano filling firmness (16.7 ± 4.6 N and 20.7 ± 1.0 N respectively). Therefore, it could be concluded that basil fillings are not recommended for sandwich cracker production.

Antioxidant capacity of cracker fillings

The biological properties of antioxidants depend on their release from the food matrix during the digestion process. The first step for antioxidant bioavailability is the solubility within the intestinal tract (or bioaccessibility) for subsequent absorption [2]. Bioaccessible compounds can be released from the food matrix by the action of digestive enzymes and may be susceptible to absorption through the intestinal wall [46]. Therefore, the application of a gastrointestinal digestion process before the determination of antioxidant activity has been strongly recommended [2].

In the present work, the stability and release of bioaccessible antioxidant compounds were determined through the standardized static in vitro digestion method developed by Minekus [24] (Fig. 1). Results showed that the addition of herbs significantly increased the bioaccessible antioxidant fraction of cracker fillings ($p \leq 0.05$). The highest bioaccessible antioxidant amount was found in oregano fillings (10.80 ± 0.20 µmol Trolox/g; 15.80 ± 0.5 µmol Trolox/g and 14.00 ± 0.10 µmol Trolox/g by DPPH, ABTS and FRAP assays respectively). Pérez-Jiménez et al. [47] determined the antioxidant capacity of Spanish diet considering the

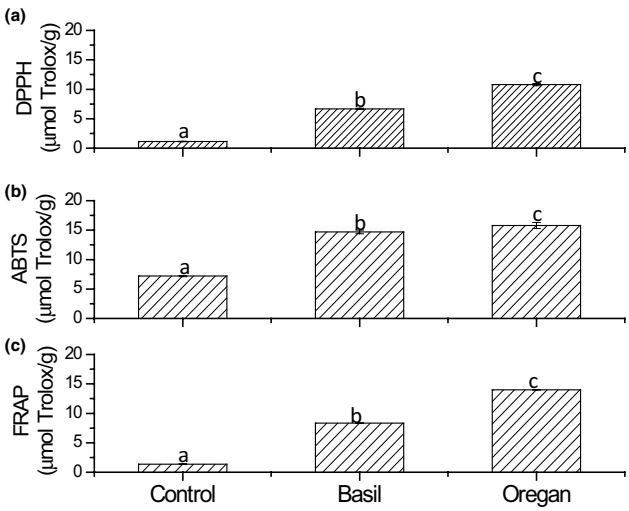

Fig. 1 Antioxidant activity of the bioaccessible fraction of control, basil and oregano cracker fillings. Determinations were performed with DPPH (**a**), ABTS (**b**) or FRAP (**c**) assays

Table 4 Texture parameters (firmness, cohesiveness, gumminess, adhesiveness of control, oregano and basil filling)

	Firmness (N)	Cohesiveness	Gumminess (N)	Adhesiveness (N cm)
Control	16.7 ± 4.6^{a}	0.53 ± 0.03^{b}	9 ± 2^{a}	-0.05 ± 0.02^{b}
Oregano	20.7 ± 1.0^{a}	0.53 ± 0.03^{b}	11 ± 1^{a}	-0.24 ± 0.08^{a}
Basil	37.3 ± 2.8^{b}	0.44 ± 0.02^{a}	16 ± 2^{b}	-0.16 ± 0.06^{a}

Results are expressed as mean ± standard deviation. Different letters within each row indicate significant differences among regions according to Fisher's test ($p \leq 0.05$)

bioaccessible fractions. According to these authors, the Trolox equivalent antioxidant capacity of well-known antioxidant food groups such as vegetables (18 µmol Trolox/g of dry matter), nuts (18 µmol Trolox/g), fruits (17 µmol Trolox/g) or legumes (9 µmol Trolox/g) could compare well with the antioxidant capacity found in the bioaccessible fraction of cracker fillings in the present work. Moreover, the antioxidant capacity of the cracker fillings with herbs was higher than the antioxidant capacity of cereal products such as commercial biscuits, wheat bread and wheat bran bread found in previous studies [48, 49]. Nonetheless, it should be considered that sandwich crackers have a long shelf life and during storage time phenolic compounds could undergo oxidation [50]. Thus, the antioxidant capacity of the food product might change. Future work should consider and evaluate the stability of the antioxidants in the fillings during storage time in appropriate conditions.

The present results mainly show that the incorporation of herbs, especially oregano, in a food matrix such as a cracker filling would be a good alternative to increase the amount of bioaccessible antioxidant compounds in diets. Moreover, these compounds presented a high amount of polyphenols, which has been associated with beneficial health effects such as the prevention of degenerative diseases, including cancer and cardiovascular affections [51].

Sensory analysis

The acceptability of the oregano filling developed was evaluated through a sensory analysis with untrained panellists and compared to the control filling (similar to commercial products). Scores assigned showed that the salty fillings presented good global acceptability (the mean scores were 6.82 ± 2.1 and 7.32 ± 1.57 for control and oregano fillings respectively). No significant differences were found in the general acceptability or texture of the cracker fillings analysed ($p > 0.05$).

The colour of control fillings was ranked better than the colour of oregano fillings ($p \leq 0.05$). This could be accounted for by the greenish colour of the oregano fillings compared to the yellow colour of control products similar to the ones commercially available nowadays. On the other hand, the flavour acceptability of oregano fillings was significantly higher than that of the control fillings ($p \leq 0.05$). Therefore, it could be concluded that the flavour of the products was improved by the addition of oregano. This is in good agreement with Chinprahast et al. [44], who indicated that although the addition of herbs into food products may adversely affect the colour, their taste and odour improved. The histograms with the panellists' score responses to texture, taste, colour and overall acceptability attributes of the samples are shown in Fig. 2.

Finally, significant differences were found in the purchase intention of the products (Fig. 3). According to the results, the buying preference of oregano sandwich crackers was significantly higher than that of products with the control fillings (69% of the panellists indicated that they would buy the oregano filling, 26% said that they would buy control products, while 5% answered that they did not have any preference for any particular product).

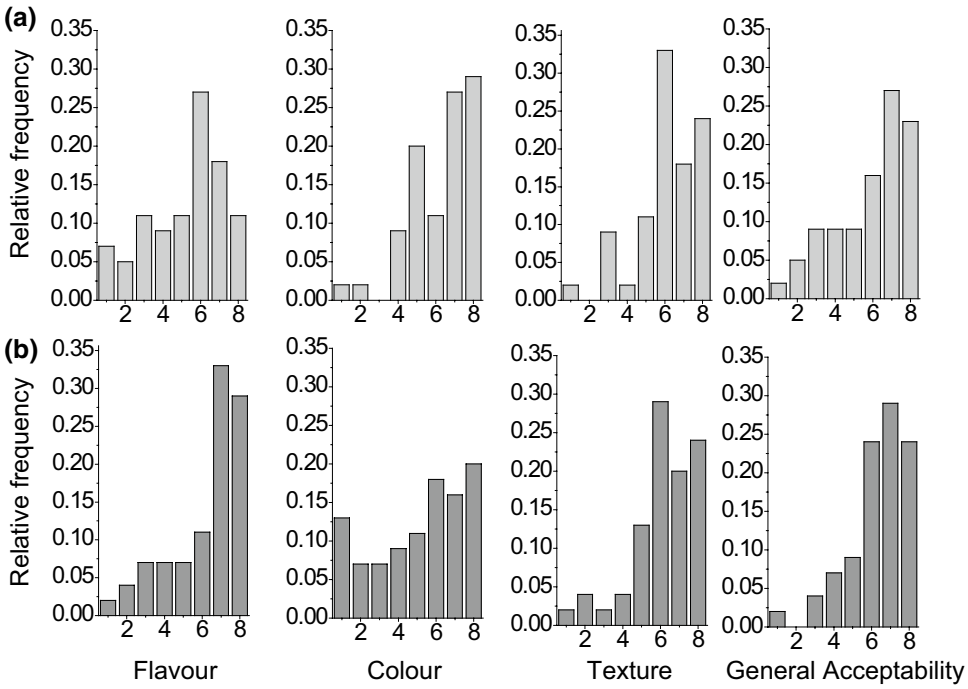

Fig. 2 Sensory evaluation histograms of flavour, colour, texture and general acceptability of control fillings (**a**) and oregano fillings (**b**)

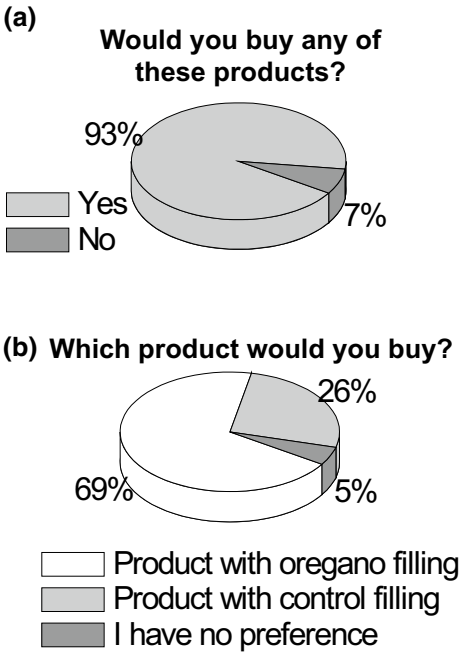

Fig. 3 Pie charts of purchase intention (**a**) and preference (**b**) towards the control fillings or oregano fillings

Conclusions

Among the culinary herbs analysed, oregano and basil presented the highest content of phenolic compounds and antioxidant capacity. Therefore, they were considered the most suitable herbs to be incorporated in the cracker fillings.

The addition of herbs decreased the luminosity of the fillings and significantly increased their adhesiveness. Besides, compared to basil fillings, control and oregano fillings were softer, more cohesive, and less energy was required to disintegrate them.

The in vitro digestion process showed that the addition of herbs, especially oregano, to a food matrix such as a cracker filling could increase the amount of bioaccessible antioxidant compounds. Nonetheless, future work should evaluate the stability of these bioaccessible antioxidants on the fillings during storage time in appropriate conditions.

Sensory analysis indicated that the cracker fillings developed in the present work presented good sensory characteristics and a high purchase intention. Therefore, the present results provide relevant evidence that oregano can successfully be added to salty cracker fillings in order to obtain a well-accepted product.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

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