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A comparative study between natural and synthetic antioxidants: Evaluation of their performance after incorporation into biscuits

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ABSTRACT

Currently, the food industry is focused in replacing the use of synthetic by natural antioxidants. The present study focused on the use of fennel and chamomile extracts, rich in phenolic compounds, as natural antioxidants in biscuits and compared their performance with a synthetic antioxidant widely used, the butylated hydroxyl anisole (BHA). The complete nutritional profile, free sugars, fatty acids and antioxidant activity were determined immediately after baking and also after 15, 30, 45 and 60 days of storage. The results showed that the incorporation of natural and synthetic additives did not cause significant changes in colour or in nutritional value of biscuits when compared with control samples. Both natural and synthetic additives conferred similar antioxidant activity to the biscuits. Therefore, natural additives are a more convenient solution for consumers who prefer foods "free" from synthetic additives. Additionally, natural additives were obtained by aqueous extraction, an environment friendly and safe process.

1. Introduction

The affordable cost together with the good nutritional quality, availability in different tastes and long shelf life are some of the reasons which turn the biscuits into the most popularly consumed bakery items all around the world (Gandhi et al., 2001). To maintain its high consumption, the biscuit texture, colour, and sensory parameters should be in line with consumer's expectations (Bajaj, Urooj, & Prabhasankar, 2006), which increasingly demand minimally processed foods and avoid the presence of synthetic additives (Carocho, Morales, & Ferreira, 2015).

Crackers, cookies and biscuits are widely consumed and stored for extended periods of time before consumption, thus, keeping quality of these baked foods is of great economic importance (Reddy, Urooj, & Kumar, 2005). Antimicrobials, antioxidants and antibrowning agents are among the additives mostly used by the food industry to preserve products for longer periods (Carocho, Barreiro, Morales, & Ferreira, 2014). In the last century, butylated hydroxyl anisole (BHA) has been used as antioxidant in foods (EFSA, 2011; Freitas & Fatibello-Filho, 2010). However, the use of this synthetic molecule has been associated with a possible toxicity, and it has been reported that it has some side effects such as carcinogenesis, which has led to some restraint in its use (Branen, 1975; Ito, Fukushima, Hassegawa, Shibata, & Ogiso, 1983; Reddy et al., 2005).

Some authors have developed studies in biscuits where they intended to compare the use of natural antioxidants from plant or fruit extracts with synthetic BHA. For example, the incorporation of fresh mango peel extracts in biscuits improved their antioxidant properties, in comparison with BHA (Ajila, Leelavathi, & Prasada Rao, 2008; Ajila, Naidu, Bhat, & Prasada Rao, 2007). The same tendency was demonstrated by Reddy et al. (2005) who used ethanolic extracts from three plant foods as sources of natural antioxidants: amla (*Emblica officianalis* Gaertn), drumstick leaves (*Moringa oleifera* Lam.) and raisins (*Vitis vinifera* L.) for application in biscuits. The addition of these extracts gave an excellent antioxidant effect to the biscuits compared with the effect of BHA. Bajaj et al. (2006) have also studied the effects of different forms of mint (*Mentha spicata* L.), namely powder and ethanolic extracts, in biscuits.

Natural extracts from plant origin could provide alternatives to synthetic preservers, namely antioxidants, also providing bioactive properties and bringing additional value to the final products (Pasqualone et al., 2015; Rasooli, 2007; Ye et al., 2013). Some bakery, dairy and meat products have already been developed incorporating natural extracts from aromatic plants, spices and fruit powder, for antioxidant purposes (Bajaj et al., 2006; Caleja,

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Barros, Antonio, Ciric, Barreira, et al., 2015; Caleja, Barros, Antonio, Ciric, Soković, et al., 2015; Reddy et al., 2005; Shah, Don Bosco, & Mir, 2014).

In particular, aqueous extracts prepared from *Foeniculum vulgare* Mill. (fennel) and *Matricaria recutita* L. (chamomile) were successively incorporated as natural antioxidants and antimicrobials for cottage-cheese (Caleja, Barros, Antonio, Ciric, Barreira, et al., 2015; Caleja, Barros, Antonio, Ciric, Soković, et al., 2015; Caleja, Ribeiro, et al., 2016) and yogurts (Caleja, Barros, et al., 2016), being those properties attributed to phenolic compounds namely di-caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid and luteolin-*O*-glucuronide in fennel (Caleja, Barros, Antonio, Ciric, Soković, et al., 2015) and quercetin-3-*O*-glucoside and 5-*O*-caffeolylquinic acid in chamomile (Caleja, Barros, Antonio, Ciric, Barreira, et al., 2015). Furthermore, the infusions of the abovementioned plants have been traditionally used for the treatment of hypertension, neurological diseases, and allergies (Matić et al., 2013; Ranpariya, Parmar, Sheth, & Chandrashekhar, 2011; Rather, Dar, Sofi, Bhat, & Qurishi, 2012).

In order to generalize the use of fennel and chamomile aqueous extracts (prepared by decoction) as natural antioxidants, the present work evaluated their performance, in two different doses, in a novel food matrix (biscuits), and compared the results with a widely used synthetic antioxidant (BHA), in relation to the storage time.

2. Materials and methods

2.1. Preparation of the natural antioxidants from plant origin and synthetic antioxidant

Commercial samples of *Matricaria recutita* L. (chamomile flowers) and *Foeniculum vulgare* Mill. (fennel aerial parts) were provided by Américo Duarte Paixão Lda. (Vale da Trave, Santarém, Portugal). The dried samples were powdered (~20 mesh; Ultra Centrifugal Mill ZM 200, Porto, Portugal) and decoctions were prepared by adding 5 g of plant material to 200 mL of distilled water (Milli-Q water purification system, TGI Pure Water Systems, Greenville, SC, USA), heated (heating plate, VELP Scientific, Usmate, Italy) and boiled for 5 min. The mixture was left to stand for 5 min, filtered, and then frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA). The prepared ingredients were previously characterized in terms of antioxidant compounds; the fennel extract was rich in quercetin-3-*O*-glucuronide, 5-*O*-caffeolylquinic acid and 1,5-di-*O*-caffeolylquinic acid (Caleja, Barros, Antonio, Ciric, Soković, et al., 2015), while the chamomile extract presented di-caffeoyl-2,7-anhydro-3-deoxy-2-octulopyranosonic acid, 5-*O*-caffeolylquinic acid, luteolin-*O*-glucuronide and myricetin-3-*O*-glucoside as major phenolic compounds (Caleja, Barros, Antonio, Ciric, Barreira, et al., 2015).

Butylated hydroxyl anisole (E320-BHA) was used as synthetic additive being supplied by Merck-Schuchardt (Darmstadt, Germany).

2.2. Preparation of the biscuits by incorporation of natural and synthetic antioxidants

To prepare the biscuits, a traditional recipe was followed: one egg was thoroughly mixed with 125 g of sugar. The antioxidants were dissolved in 60 mL of water and added to the mixture. Then, 300 g of wheat flour were sequentially added to the mixture while mixing vigorously with a hand mixer at 450 W during 8 min (Bosch, Munich, Germany). After 15 min of rest the dough with the intended consistency was reduced to 5 mm thickness and cut by a round biscuit cutter with 50 mm internal diameter. Six lots of biscuits (30 per lot, 6 biscuits for each storage time) were prepared: i) control biscuits – without any antioxidant, designated by C; ii) two lots of biscuits with fennel extract (80 mg and 800 mg, designated by Fen and Fen10, respectively); iii) two lots of biscuits with chamomile extract (80 mg and 800 mg, designated by Cham and Cham10, respectively); iv) biscuits with synthetic additive, BHA (80 mg). The biscuits were baked in an electric oven for 10 min at 180 °C. All samples were lyophilized, finely crushed and analyzed, in triplicate, immediately after preparation and after fifteen, thirty, forty-five and sixty days of storage (at room temperature and packed in a sealed plastic bag covered with aluminum paper).

2.3. Evaluation of the colour parameters of the biscuit samples along storage time

The colour of the samples was measured in three different points on the top using a colorimeter (model CR-400, Konica Minolta Sensing Inc., Tokyo, Japan). The illuminate C was used and a diaphragm aperture of 8 mm and previously calibrated against a standard white tile. The CIE L^* (lightness), a^* (greenness/redness), b^* (blueness/yellowness) colour space values were registered using a data software "Spectra Magic Nx" (version CM-S100W 2.03.0006) (Fernandes et al., 2012).

2.4. Evaluation of the nutritional properties

Proximate composition with reference to the contents of protein (N × 5.70, AOAC 978.04), fat (AOAC 920.85) and ash (AOAC 923.03), was determined following AOAC methods (AOAC., 2005). Total energy was calculated following the equation: Energy (kcal) = 4 × (g proteins + g carbohydrates) + 9 × (g lipids). Fatty acids were determined, after Soxhlet extraction, by gas-chromatography coupled to flame ionization detector (GC-FID), identified by comparison with standards (standard 47885, Sigma-Aldrich, St. Louis, Missouri, USA) and expressed as relative percentages of each fatty acid (Barros et al., 2013). Free sugars were determined in defatted samples by HPLC coupled to a refraction index (RI) detector (Barros et al., 2013), identified by comparison with standards, and further quantified (g/100 g of biscuit) considering the internal standard (melezitose).

2.5. Evaluation of the antioxidant activity of the biscuit samples

All lyophilized samples (3 g) were extracted for 1 h, using a procedure previously described by Caleja, Barros, et al. (2016). After recovering, the extracts were dissolved in methanol in a final concentration of 200 mg/mL. The antioxidant activity evaluation was performed using two *in vitro* assays: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and reducing power (RP), following the experimental methodologies adopted by the authors (Caleja, Barros, et al., 2016; Caleja, Ribeiro, et al., 2016).

2.6. Statistical analysis

The experimental data was checked for normality (Shapiro-Wilk test) and homogeneity of variances (Levene's test) assumptions. When it was not possible to apply an analysis of variance (ANOVA) and for data that did not follow a normal distribution, non-parametric tests were performed to evaluate significant differences at a level of 5%, using the EXCEL software, Microsoft Office Professional Plus 2010, version 14.0.7159.5000, with the add-in Analysis ToolPak (Microsoft Corp., USA).

3. Results and discussion

The different types of biscuits (control, incorporated with BHA, fennel, fennel_10, chamomile, and chamomile_10) were analyzed in five storage times (0, 15, 30, 45 and 60 days) and the results are presented as the mean value of each storage time (ST) regardless of the incorporated additive, and also the mean of each additive (A) regardless of the storage time (Tables 1–4). In this way it is possible to determine the best additive regardless of cookie storage time and vice versa. So, the standard deviations presented in the tables should not be regarded as a measure of accuracy of the methodologies, since they include the results for one of the factors (ST or A).

Colour is an important quality attribute for consumer acceptance of bakery products. In bakery, colour can give indication when cooking is complete, besides that colour changes during storage provide information about its freshness (Mamat, Abu Hardan, & Hill, 2010). Table 1 presents the results for the different colour parameters of the biscuits (L^*, a^*, b^*). The total instrumental colour

Table 1
Colour parameters of the biscuits in relation to storage time (ST) and food additive (A) for top side.

		L^*	a^*	b^*
Storage time (ST)	0 days	75.59 ± 2.59 a	5.24 ± 2.37 a	29.73 ± 3.09 a
	15 days	74.91 ± 2.44 a,b	5.08 ± 1.50 a	28.32 ± 2.10 b
	30 days	76.15 ± 1.58 a	4.69 ± 1.44 a	29.13 ± 2.45 a
	45 days	76.60 ± 2.24 a	4.50 ± 1.81 a	28.16 ± 2.25 b
	60 days	76.01 ± 2.16 a	4.45 ± 1.75 a	28.29 ± 1.74 b
Additive (A)	C	75.35 ± 2.15 a,b	5.81 ± 2.54 a	30.61 ± 3.10 a
	BHA	76.48 ± 2.11 a	3.80 ± 1.14 b	28.21 ± 1.69 b
	Fen	76.53 ± 2.04 a	5.07 ± 1.31 a	29.54 ± 2.07 a
	Fen10	74.71 ± 2.64 b	5.61 ± 1.39 a	27.42 ± 1.92 b
	Cham	76.78 ± 2.01 a	4.22 ± 1.71 b	27.13 ± 1.95 b
	Cham10	75.26 ± 1.98 b	4.24 ± 1.57 b	29.46 ± 1.52 a

The results are presented as mean ± SD. L^*: Lightness, a^*: red-green, b^*: yellow-blue. Control (C); Butylated hydroxyl anisole (BHA); Fennel in the same amount of BHA (Fen) or Fennel ten times more (Fen10); Chamomile in the same amount of BHA (Cham) or Chamomile ten times more (Cham10).
In each column, within each storage period and within each additive, different letters mean statistical significant differences ($p < 0.05$).

Table 4
Antioxidant activity (EC_{50} values expressed in mg/mL) in relation to storage time (ST) and food additive (A).

		DPPH scavenging activity	Reducing power (RP)
Storage time (ST)	0 days	128.55 ± 62.28 b	83.41 ± 32.15 a
	30 days	149.24 ± 47.91 b	88.38 ± 35.56 a
	60 days	171.40 ± 31.68 a	99.04 ± 36.66 a
Additive (A)	C	>200 a	135.13 ± 3.69 a
	BHA	88.32 ± 29.38 e	53.54 ± 9.91 d
	Fen	191.73 ± 9.68 b	121.43 ± 16.64 b
	Fen10	120.73 ± 35.00 c,d	62.60 ± 3.52 c
	Cham	185.15 ± 8.78 b	113.39 ± 11.07 b
	Cham10	104.77 ± 31.41 d	55.57 ± 2.42d

The results are presented as mean ± SD. Control (C); Butylated hydroxyl anisole (BHA); Fennel in the same amount of BHA (Fen) or Fennel ten times more (Fen10); Chamomile in the same amount of BHA (Cham) or Chamomile ten times more (Cham10).
In each column, within each storage period and within each additive, different letters mean statistical significant differences (p < 0.05).

Table 2
Macronutrients, free sugars composition (g/100 g) and energy value (kcal/100 g) of the biscuits in relation to storage time (ST) and food additive (A).

		Moisture	Fat	Protein	Ash	Carbohydrates	Energy	Sucrose
Storage time (ST)	0 days	4.08 ± 0.55 a	1.45 ± 0.07 b	8.16 ± 0.53 a	1.03 ± 0.11 b	87.58 ± 2.22 a	386.81 ± 2.22 a	30.85 ± 0.62 b
	15 days	3.99 ± 0.46 a	1.51 ± 0.05 b	8.46 ± 0.44 a	1.04 ± 0.14 b	85.00 ± 0.50 b	387.43 ± 1.76 a	32.96 ± 1.07 a
	30 days	4.33 ± 0.50 a	1.31 ± 0.10 c	8.35 ± 0.57 a	1.37 ± 0.15 a	84.64 ± 0.50 b,c	383.74 ± 2.46 b	31.68 ± 1.76 b
	45 days	4.49 ± 0.98 a	1.45 ± 0.27 b,c	8.50 ± 0.49 a	1.40 ± 0.12 a	84.17 ± 0.90 c	383.68 ± 4.00 b	30.93 ± 0.86 b
	60 days	4.21 ± 1.63 a	1.74 ± 0.17 a	8.49 ± 0.56 a	1.06 ± 0.12 b	84.50 ± 1.11 b,c	387.64 ± 6.83 a,b	31.02 ± 0.63 b
Additive(A)	C	3.91 ± 0.54 a	1.63 ± 0.20 a	8.28 ± 0.29 b	1.15 ± 0.21 a	85.02 ± 0.65 a	387.91 ± 2.58 a	31.92 ± 0.93 a
	BHA	4.86 ± 1.28 a	1.55 ± 0.16 a	8.10 ± 0.35 b	1.29 ± 0.26 a	84.68 ± 2.07 a	383.15 ± 4.68 b	30.30 ± 1.08 b
	Fen	3.64 ± 0.63 a	1.54 ± 0.16 a	8.99 ± 0.60 a	1.12 ± 0.22 a	85.53 ± 1.95 a	388.67 ± 2.83 a	31.78 ± 1.22 a
	Fen10	3.51 ± 0.70 a	1.48 ± 0.22 a,b	8.78 ± 0.31 a	1.14 ± 0.15 a	85.56 ± 1.41 a	388.77 ± 3.91 a	31.45 ± 0.38 a
	Cham	4.56 ± 0.37 a	1.41 ± 0.24 a,b	8.15 ± 0.36 b	1.19 ± 0.20 a	85.31 ± 1.86 a	384.05 ± 2.91 b	31.41 ± 2.00 a
	Cham10	4.83 ± 0.69 a	1.35 ± 0.14 a	8.04 ± 0.36 b	1.20 ± 0.19 a	85.16 ± 2.09 a	382.62 ± 2.91 b	32.07 ± 1.08 a,b

In each column, within each storage period and within each additive, different letters mean statistical significant differences ($p < 0.05$). Control (C); Butylated hydroxyl anisole (BHA); Fennel in the same amount of BHA (Fen) or Fennel ten times more (Fen10); Chamomile in the same amount of BHA (Cham) or Chamomile ten times more (Cham10).

Table 3
Fatty acids composition of the biscuits (expressed as relative percentage of each fatty acid) in relation to storage time (ST) and food additive (A).

		C16:0	C16:1	C18:0	C18:1n9	C18 :2n6	C18 :3n3	SFA	MUFA	PUFA
Storage time (ST)	0 days	20.51 ± 0.93 a	1.91 ± 0.19 a	3.89 ± 0.42 a	28.63 ± 1.42 a	39.72 ± 2.27 a	2.57 ± 0.37 a	25.33 ± 1.25 a	31.15 ± 1.54 a	43.52 ± 2.52 a
	15 days	20.57 ± 0.82 a	1.97 ± 0.18 a	3.82 ± 0.40 a	28.76 ± 1.63 a	39.65 ± 2.43 a	2.54 ± 0.37 a	25.32 ± 1.16 a	31.32 ± 1.72 a	43.35 ± 2.68 a
	30 days	20.63 ± 0.98 a	1.99 ± 0.20 a	3.77 ± 0.40 a	28.61 ± 1.56 a	39.73 ± 2.42 a	2.56 ± 0.36 a	25.27 ± 1.27 a	31.20 ± 1.64 a	43.54 ± 2.67 a
	45 days	20.94 ± 0.94 a	1.93 ± 0.16 a	3.92 ± 0.45 a	28.51 ± 1.62 a	39.46 ± 2.47 a	2.44 ± 0.36 a	25.27 ± 1.26 a	31.07 ± 1.73 a	43.17 ± 2.61 a
	60 days	20.85 ± 0.84a	1.86 ± 0.29 a	4.10 ± 0.35 a	27.51 ± 2.20 a	40.09 ± 2.41 a	2.52 ± 0.33 a	25.76 ± 1.36 a	29.97 ± 2.47 a	44.18 ± 2.99 a
Additive (A)	C	21.65 ± 0.18 b	2.01 ± 0.04 b	4.32 ± 0.10 a	30.46 ± 0.41 a	36.73 ± 0.19 e	2.07 ± 0.03 e	26.88 ± 0.15 a	33.07 ± 0.39 a	40.05 ± 0.31 a
	BHA	20.45 ± 0.63 d	2.10 ± 0.07 a	3.81 ± 0.29 c	29.36 ± 0.59 b	38.88 ± 1.03 c	2.63 ± 0.28 b	25.13 ± 0.90 d	32.10 ± 0.56 b	42.77 ± 1.21 c
	Fen	21.91 ± 0.23 a	2.08 ± 0.05 a	3.88 ± 0.11 b	28.01 ± 0.73 c	39.15 ± 0.42 c	2.15 ± 0.04 d	26.72 ± 0.32 b	30.67 ± 0.78 c	42.61 ± 0.57 c
	Fen10	19.81 ± 0.34 e	1.55 ± 0.08 d	3.76 ± 0.34 c	26.86 ± 1.20 d	42.51 ± 0.51 b	2.64 ± 0.03 c	24.51 ± 0.65 e	29.01 ± 1.31 d	46.48 ± 0.82 b
	Cham	19.73 ± 0.18 e	1.85 ± 0.14 c	3.37 ± 0.36 d	26.23 ± 0.58 e	42.97 ± 0.34 a	3.03 ± 0.08 a	24.00 ± 0.43 f	28.71 ± 0.73 e	47.29 ± 0.45 a
	Cham10	20.64 ± 0.18 c	2.00 ± 0.12 b	4.28 ± 0.22 a	29.49 ± 1.28 a,b	38.12 ± 0.68 d	2.65 ± 0.07 c	25.80 ± 0.44 c	32.09 ± 1.36 a,b,c	42.11 ± 1.02 c

The results are presented as mean ± SD. Palmitic acid (C16:0); Palmitoleic acid (C16:1); Stearic acid (C18:0); Oleic acid (C18:1n9); Linoleic acid (C18:2n6); α-linolenic acid (C18:3n3); SFA-Saturated fatty acids; MUFA-Monounsaturated fatty acids; PUFA-Polyunsaturated fatty acids. Control (C); Butylated hydroxyl anisole (BHA); Fennel in the same amount of BHA (Fen) or Fennel ten times more (Fen10); Chamomile in the same amount of BHA (Cham) or Chamomile ten times more (Cham10).
In each column, within each storage period and within each additive, different letters mean significant statistical differences ($p < 0.05$).

is the contribute of L^*, a^* and b^* values (Fernandes et al., 2012), where for this particular case the contribute of a^* value is less relevant. With storage time, it was observed a slight diminish of yellow colour (b^*-value) and no statistical significant changes of lightness (L^*-value). For different additives, the lower values of L^* were presented by the biscuits with fennel (Fen10) and chamomile (Cham10); and the higher values for b^* parameter were presented by the control (C), fennel (Fen) and chamomile (Cham10) samples. Some authors affirm that the colour of biscuits may result from Maillard reactions between reducing sugars and amino acids (Köksel & Gökmen, 2008; Mundt & Wedzicha, 2007; Pasqualone et al., 2014). The colour of the additive may influence the biscuits colour. For example, incorporation of bee pollen caused a darkening in the surface of the biscuit (Krystyjan, Gumul, Ziobro, & Korus, 2015), but in this study it was not possible to observe any difference between samples (with or without additives), what could be considered a positive aspect, if it is intended to maintain the traditional aspect of the biscuits.Table 2 shows the content of macronutrients, sucrose and energy of the biscuits during storage and with different additives. It is possible to verify that the carbohydrates are the most abundant macronutrients in biscuits. The results are presented as the mean value of each storage time (ST) regardless of the additive incorporated, and also the mean of each additive (A) regardless of the storage time. With this type of statistical analysis and representation, the best additive (control, BHA, fennel decoction and chamomile decoction) could be determined independently of the storage time, but also the influence of the storage time irrespectively of the additive type. When it was observed a variation in both factors the samples were not classified. Regarding carbohydrates, samples with additives, synthetic or natural, were similar along storage time, except immediately after cooking. For proteins, the samples with chamomile additive were similar to control samples, but the higher protein content observed in samples with fennel additive highlights its higher protective role regarding this parameter. Carbohydrates did not vary with additive type. Only some differences were observed with storage time, with lower values registered after 15 days of storage. One free sugar was detected in the studied samples: sucrose, which was also one of the ingredients used in biscuits preparation.Table 3 shows the six most abundant fatty acids detected in biscuits samples. Although twenty-three fatty acids were identified in the studied samples, seventeen were detected only in trace amounts (data not shown). The most abundant fatty acids were linoleic acid (C18:2n6) and oleic acid (C18:1n9). The prevalence of these fatty acids would be expected since the literature confirm the predominance of oleic and linoleic acids in the composition of hen eggs (Belitz, Grosh, & Schieberle, 2009) and flour (Nikolić, Sakač, & Mastilović, 2011). Unsaturated fatty acids (polyunsaturated followed by monounsaturated) were found in higher amounts than saturated fatty acids.

In the present study, the samples with natural additives revealed higher values of PUFA, and lower values of SFA (probably related with a higher inhibition of lipid peroxidation). As can be observed in Table 3 PUFA with storage time and biscuits additive, its variation is dominated by the sample type or additive, and not by the storage time.

The results of reducing power (RP) and DPPH radical scavenging activity of the biscuits samples during storage are presented in Table 4. As expected, the control sample showed the lower reducing power capacity and the EC_{50} value of samples fortified with plant extracts decreased with the increase of the quantity of the extract. Natural additives with higher concentration revealed a strong RP (i.e., lower EC_{50} values). It was also possible to verify that storage time had no influence in RP and just had influence after 60 days in DPPH scavenging activity. Along storage time, after 60 days, there was a slight tendency for antioxidant activity reduction (higher EC_{50} values). However, relevant variations could be observed within a longer period of time. The antioxidant activity was higher (lower EC_{50} value) when the concentration of natural additive was higher (Fen10 and Cham10) for both *in vitro* assays, DPPH and Reducing Power, but in the same order of magnitude as the synthetic additive BHA. However, the presence of the natural extracts can bring some additional benefits (Caleja, Barros, Antonio, Ciric, Barreira, et al., 2015; Caleja, Barros, Antonio, Ciric, Soković, et al., 2015) and without deleterious effects. A similar study incorporating fresh peel extracts of two different varieties of mango in biscuits demonstrated higher antioxidant properties, when compared to BHA (Ajila et al., 2007, 2008). Alternative and promising natural antioxidants have been used by other authors, but obtained by methanolic extraction (Bajaj et al., 2006; Reddy et al., 2005). These natural additives were obtained by aqueous extraction, a more environment friendly and safer process to obtain food additives (Reddy et al., 2005). A recent study described the high antioxidant activity of functional biscuits prepared with wheat purple, due to its high levels of bioactive compounds (Pasqualone et al., 2015). Other previous studies have proven the capacity of fennel and chamomile extracts to bring antioxidant activity to cottage cheese and yogurt (Caleja, Barros, Antonio, Ciric, Barreira, et al., 2015; Caleja, Barros, Antonio, Ciric, Soković, et al., 2015; Caleja, Barros, et al., 2016).

4. Conclusions

Bakery industry is in constant innovation and biscuits are products worldwide appreciated and consumed by different categories of consumers. Therefore, the production of this type of product enriched with natural sources of antioxidants may be attractive for consumers who are increasingly concerned about the choice of healthy foods. This study revealed that the addition of fennel and chamomile decoctions improves the antioxidant activity of biscuits, bringing beneficial advantages without adverse effects, contrary to what has been reported for the synthetic additive BHA. It should be noted that the incorporation of these aqueous extracts caused no significant changes in appearance or nutritional profile of biscuits thus proving that the use of such extracts can be important for the release of new healthier pastry products on the market.

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