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

Cristina Caleja [a,b,c], Lillian Barros [a,b], Amilcar L. Antonio [a], M. Beatriz P.P. Oliveira [c], Isabel C.F.R. Ferreira [a,*]

[a] Mountain Research Centre (CIMO), ESA, Polytechnic Institute of Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal
[b] Laboratory of Separation and Reaction Engineering (LSRE), Associate Laboratory LSRE/LCM, IPB, Campus de Santa Apolónia, 1134, 5301-857 Bragança, Portugal
[c] REQUIMTE/LAQV, Faculty of Pharmacy, University of Porto, Rua Jorge Viterbo Ferreira, No. 228, 4050-313 Porto, Portugal

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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,

* Corresponding author.
E-mail address: iferreira@ipb.pt (I.C.F.R. Ferreira).

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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).