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Encapsulated Walnut Paste with Grape Skin Extract Addition: Oxidative Stability and Use in Biscuits

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Thanks to their nutritional, healthy and sensory attributes, walnuts are raw materials of great interest for the agri-food sector. Nevertheless, a prolonged shelf-life is a fundamental requirement for promoting their industrial use. Rapid onset of rancidity can be avoided by adding preservative compounds and/or by applying stabilization processes able to make the fat component less prone to oxidation. The aim of this study was to evaluate the effect exerted by the addition of natural antioxidants (from grape skin) and/or by the application of an encapsulation technique on walnut paste. With the purpose of using them in bakery products, the developed formulations were subjected to thermal stress and used as ingredients in biscuits preparation: the impact of each treatment on fat fraction stability and antioxidant capacity was therefore assessed. In detail, walnut paste was obtained by roasting and grinding of kernels. An aliquot was enriched with 5000 ppm (w/w) grape skin extract in powder form. Both formulations were then encapsulated through a freeze-drying method, using maltodextrin DE12 and tragacanth gum as well materials. The chemical and oxidative analyses were performed on samples just after preparation, on the ones exposed to thermal stress (for 15 min at 180 °C) and on biscuits (baked at 180 °C for 15 min) that included just prepared samples. The results showed that the just prepared walnut paste supplemented with grape skin extract exhibited a greater antioxidant capacity, that was, however, reduced by heat treatment. Encapsulation procedure decreased the antioxidant capacity with a less evident effect on compounds revealed by FRAP and ORAC tests. The nutritional profile of just baked biscuits (intended as antioxidant capacity, phenolic profile, and tocopherol content) was not statistically influenced by the different formulations. In terms of induction period, encapsulated samples (and related biscuits) displayed higher values: the encapsulation process seemed to exert a barrier effect against oxidative agents and to protect the antioxidant properties of the grape skin extract, increasing the stability of both pasta and biscuits. In conclusion, recipe inclusion of encapsulated samples in just baked biscuits did not exert any benefits, neither in terms of oxidative stability, nor in terms of antioxidant properties. However, maltodextrins and gums, used as wall materials in the encapsulated samples, could help preserve the whole matrix prolonging the shelf-life of baking products containing them.

1. Introduction

Walnuts arise great interest all around the world: they became very popular for their nutritional qualities and for the positive effect that they have on human health. This fruit represents a natural functional food, it is consumed in different way and it might be a perfect ingredient for the confectionary industry also for its nutraceutical properties (Ling et al., 2014). Walnuts are rich in fibers, proteins and minerals and they are also characterized by a high antioxidant capacity due to the phenolic compound presents in their seed coat and tocopherols in the lipid fraction (Salcedo et al., 2010). For their composition, walnuts can be easily subjected to rancidity (hydrolytic, oxidative or enzymatic), that can also occur because of inadequate processing and/or storage conditions (Martínez et al., 2011). Oxidation takes place on lipid fraction and it can lead to organoleptic decay, worsening

of their nutritional quality, increase risk for consumers' health and a shorter product's shelf-life (Laguerre et al., 2007). Considering the characteristics of this nut fruit and its susceptibility to oxidation, this study aims to extend the shelf-life of a walnut paste by applying two different techniques: the addition of an antioxidant extract obtained from grape skin and the encapsulation of the emulsion though the use of coating materials. Previous studies have shown how the addition of antioxidants to fat matrices helps to prevent lipid oxidation (Martínez et al., 2012). The choice of using a grape skin extract would allow the use of processing residues that would otherwise be disposed of and it could also extend the shelf life of the product, especially if combined with an encapsulation technique (Spigno et al., 2013). Encapsulation is used to protect sensitive compounds from their degradation; in this case, a freeze-drying was applied starting from an emulsion of walnut paste added with coating materials to create a protective lattice around the lipid droplets (Calvo et al., 2011). For this purpose, tragacanth gum and maltodextrins were used: their combination favors the encapsulation efficiency and improves the rheological properties of the product.
With the aim of using them in bakery products, the developed formulations were subjected to thermal stress and used as ingredients in biscuits preparation: the impact of each treatment on fat fraction stability and antioxidant capacity was therefore assessed.

2. Materials and methods

2.1. Materials

Walnuts in shell (*Juglans regia* L., var. Howard, California, USA) were purchased from a local market in Piacenza (Italy). The extract of natural antioxidants, added to the pastes, was obtained from grape pomace of the Barbera variety according to the procedure reported in Dordoni et al. (2019c).

2.2. Experimental plan

After manual shell removal, the kernels were roasted and lipoxygenases activity (LOX) was measured. Toasted walnut kernels were ground and refined until paste. An aliquot of paste was enriched with grape skin extract in powder form (Dordoni et al., 2019a); both formulations were encapsulated. The obtained samples were, therefore: walnut paste (P), walnut paste added with grape skin extract (PE), freeze-dried walnut paste (L) and freeze-dried walnut paste added with grape skin extract (LE). The samples were subjected to accelerated oxidative stability tests by using an Oxidation Test Reactor (Oxitest) device. They were, also, analyzed for color, acidity, and pH. Oil fraction and defatted powder were separated by a cold extraction procedure: peroxides, conjugated dienes and trienes, and tocopherols were measured on the lipid fraction, whereas total phenolics and antioxidant capacity (through ABTS, ORAC, and FRAP assays) were evaluated on defatted matter. All the samples were evaluated after their preparation, after the application of a thermal stress and in the biscuits obtained by including the different samples.

2.3. Walnut paste preparation

Kernels were roasted in a forced convection oven at 160 °C for 15 min, ground by an electric domestic grinder (La Moulinette, Moulinex) and refined to paste using a planetary micro mill at 700 rpm for 2 min. An aliquot of walnut paste was handily enriched with 5000 ppm (w/w) grape skin extract. Walnut paste (P) and the walnut paste added with the extract (PE) were collected in plastic and opaque containers, saturating head space with nitrogen to limit the exposure to light and to air; the pastes were finally stored at -18 °C until use.

2.4. Encapsulation procedure

Aliquot of the above-mentioned walnut paste were encapsulated according to the process reported by Dordoni et al. (2019b); briefly, pastes were added with maltodextrin DE 12 (Glucidex® 12, Roquette) and tragacanth gum powder (CEROTRAG 888, C.E. Roeper GmbH), mixed for 1 min, and emulsified with water for 30 s though an immersion blender. The obtained compounds (L and LE) were poured into special silicone molds and stored at -18 °C for 24 h. Finally, the samples were lyophilized (ALPHA 1-2 LD, Christ) for 72 h (Dordoni et al., 2017).

2.5. Thermal stress

In order to simulate baking conditions, 100 g of each sample (P, PE, L, LE) were weighed in open glass containers and kept in forced conventional oven at 180 °C for 15 min. The treated samples (P T, PE T, L T, LE T) were then left at room temperature before analysis (see paragraph 2.2).

2.6. Biscuit preparation

Biscuits were prepared by mixing 55 g of eggs with 30 g of white sugar; therefore, 35 g of sample (P, PE, L, or LE) and 110 g of soft wheat flour were added. The obtained dough was left at 4°C for 10 min, then it was rolled

out maintaining a thickness of 5 mm. Once 5 cm diameter biscuits were shaped, they were baked in forced convention oven at 180 °C for 15 min. After grinding, the biscuits (B P, B PE, B L, B LE) were evaluated for the parameters detailed in paragraph 2.2.

2.7. Oxidative quality index

Lipoxygenases activity (LOX) was measured on un-roasted and toasted walnuts following the method indicated by Fortunato et al. (2006). The Oxidation Test Reactor (Oxitest) was used, at 90 °C and 6 bar oxygen pressure, to evaluate the accelerated oxidative stability of the samples. Color was monitored through a portable colorimeter (CR-310 Konica Minolta). Total acidity and pH were evaluated following the methods reported by the Office International du Cacao, du Chocolat et de la Confiserie (OICCC, 1972). The conjugated dienes and trienes and the peroxide value (PV) were determined according to the European Union Commission Regulation (1991). The quantification of tocopherols was carried out following the method reported by Calvo et al. (2011). Total phenolic content was determined on defatted samples (Bela• ak et al., 2009) through the Folin-Ciocalteau method (Ribéreau-Gayon et al., 2000). The ABTS assay was carried out according to Re et al. (1999). The ORAC was analyzed through the method reported by Huang et al. (2002), while the FRAP assay was measured as indicated by Pulido et al. (2000). Results are reported as mean values of three replicates with their corresponding standard deviations. Data were then statistically elaborated through SPSS® software (SPSS Inc., version 23.0, Chicago, IL, USA).

3. Results and discussion

The walnut kernel roasting at 160 °C for 15 min allowed to reduce the LOX activity by -89 ± 6 %; similar results were mentioned in previous studies (Vaidya & Eun, 2013; Jung et al.,1997, and Lee et al.,2004). As for the accelerated oxidative stability test (Oxitest), the encapsulation process determined an increase of several hours in the induction times (in L, LE, L T, LE T, B L, B LE) (Figure 1); moreover, it seemed also to exert a protective effect on the enriched samples (LE and LE T). The applied thermal stress on just prepared samples (fluid or encapsulated) gave rise to a shorter induction time than the related biscuits, where the extract addition was less effective.

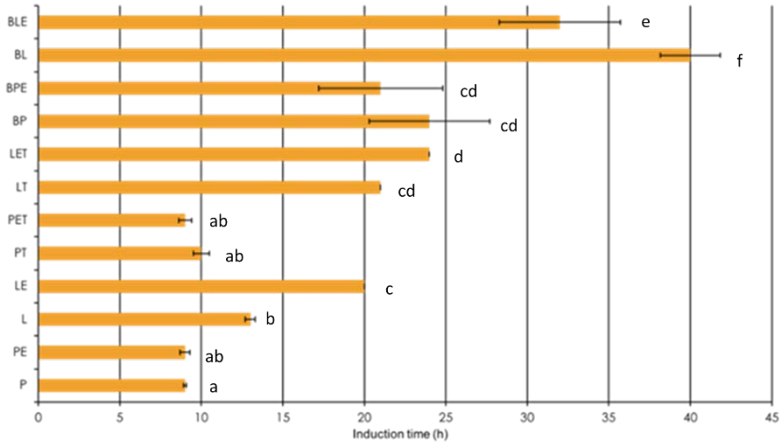

Figure 1: Induction time (h) for the different samples analyzed by Oxitest. At each bar top, different letters indicate statistically different values according to post-hoc comparison (Tukey's test) at p d 0.05. Values are expressed as mean ± sd (n = 6).

Peroxides showed no significant differences and always remained at low levels (< 5 meqO_2/kg_{oil}) (data not shown). However, conjugated dienes and trienes increased because of thermal stress and baking (Table 1). In the freeze-dried samples a slight increase of conjugated dienes was observed: the encapsulation procedure (with greater exposure to light and air) could be responsible of initial oxidative phenomena, or else, the lattice formation (given by maltodextrins and tragacanth gum) could slow down the release of the antioxidant compounds in the products during the short term.

Table 1: Evaluation of the oxidative state. Within each column, different superscript letters indicate statistically different values according to post-hoc comparison (Tukey's test) at p d 0.05. Values are expressed as mean ± sd (n = 6).

Sample	Dienes K_{232}	Trienes K_{270}	pH	Total acidity meq_{NaOH}
P	1.111±0.075^{ab}	0.175±0.005^{bc}	6.39±0.02^{c}	59.5±1.8^{cde}
PE	1.021±0.020^{a}	0.116±0.012^{ab}	6.29±0.03^{bc}	61.8±3.7^{cde}
L	1.218±0.055^{b}	0.078±0.008^{a}	6.30±0.15^{bc}	55.5±6.9^{c}
LE	1.397±0.035^{c}	0.107±0.008^{ab}	6.18±0.09^{ab}	59.8±7.5^{cde}
P T	1.503±0.085^{c}	0.219±0.010^{cd}	6.28±0.05^{bc}	52.3±2.5^{c}
PE T	1.505±0.055^{c}	0.220±0.080^{cd}	6.18±0.05^{ab}	71.2±2.7^{e}
L T	1.725±0.097^{d}	0.230±0.017^{cd}	6.21±0.01^{ab}	57.8±3.0^{cd}
LE T	2.071±0.076^{e}	0.288±0.014^{d}	6.04±0.04^{a}	70.0±4.1^{de}
B P	2.333±0.034^{f}	0.519±0.008^{e}	6.34±0.04^{bc}	23.0±2.2^{a}
B PE	2.480±0.041^{f}	0.616±0.006^{f}	6.35±0.02^{bc}	24.0±2.2^{a}
B L	2.775±0.028^{g}	0.881±0.022^{g}	6.26±0.05^{bc}	24.2±2.9^{a}
B LE	2.874±0.196^{g}	0.867±0.039^{g}	6.25±0.05^{bc}	37.7±5.3^{b}

Total acidity and pH values are related to above mentioned parameters and generally depend on the sample formulations. From the trends (Figure 1 and Table 1), it is possible to note the protective action of the extract on products' oxidative stability, while encapsulation causes a protective effect even under a thermal stress.
The antioxidant properties of walnuts are mainly related to tocopherols, in the lipid fraction, and phenolic compounds, in the kernel pellicle (Table 2 and Table 3). The biscuit samples showed the lowest antioxidant capacity, due to the addition of further ingredients for the dough formation. The grape skin extract addition caused an increase of the antioxidant values in the un-treated samples (non-freeze-dried and non-heated). Encapsulation procedure decreased the antioxidant capacity with a less evident effect on compounds revealed by FRAP and ORAC tests. In general, different behaviors were recorded depending on the various assays.

Table 2: Evaluation of the antioxidant capacity. Within each column, different superscript letters indicate statistically different values according to post-hoc comparison (Tukey's test) at p d 0.05. Values are expressed as mean ± sd (n = 6).

Sample	Phenolic content $mg_{GAE}/100g$	ABTS $mg_{Trolox}/100g$	FRAP $mmol_{Fe(II)}/100g$	ORAC $\mu mol_{Troloxeq}/100g$	TEAC mol_{Trolox}/mol_{GAE}
P	204.61±26.28^{bc}	302.52±24.17^{b}	4.67±0.52^{b}	1668.71±209.83^{bc}	1.39±0.23^{ab}
PE	390.46±39.28^{e}	589.39±92.93^{d}	9.09±0.93^{d}	2964.78±1893.56^{d}	1.22±0.65^{a}
L	197.83±45.46^{bc}	299.73±47.21^{b}	10.13±0.98^{de}	1382.51±432.11^{b}	1.35±0.18^{ab}
LE	143.07±45.87^{b}	246.11±36.22^{b}	11.68±2.23^{e}	3685.66±1177.89^{e}	5.22±1.75^{c}
P T	326.38±75.44^{d}	670.09±208.64^{d}	10.05±2.39^{de}	2226.54±1400.86^{c}	1.41±0.19^{ab}
PE T	259.49±32.34^{c}	452.25±39.03^{c}	8.47±0.95^{cd}	1859.00±365.65^{bc}	1.22±0.27^{a}
L T	144.74±18.73^{b}	283.29±18.69^{b}	6.68±0.79^{c}	1531.17±199.76^{b}	1.84±0.22^{b}
LE T	153.87±17.59^{b}	305.70±11.18^{b}	7.01±0.75^{c}	1648.63±207.92^{bc}	1.78±0.23^{ab}
B P	55.50±5.51^{a}	62.52±9.71^{a}	1.01±0.13^{a}	573.62±269.32^{a}	1.90±0.63^{b}
B PE	51.87±9.27^{a}	60.12±8.94^{a}	0.96±0.16^{a}	486.42±193.50^{a}	1.54±0.59^{ab}
B L	59.05±7.24^{a}	81.65±14.73^{a}	0.87±0.08^{a}	363.82±196.42^{a}	1.44±1.14^{ab}
B LE	63.87±7.19^{a}	106.79±5.88^{a}	1.32±0.25^{a}	540.41±121.55^{a}	1.38±0.32^{ab}

Tocopherol content values recorded for the δ, γ and α vitamers were shown in Table 3, while β-tocopherol always remained below the detection limit. As is known, ^{3}-tocopherol constitute the major homologous. The addition of grape skin extract did not significantly affect the tocopherol content, as well as the thermal stress did not negatively impact. Despite the dilution effect, biscuit samples revealed a comparable ± vitamer content mainly due to the ingredients added for the dough (i.e. eggs).

Table 3: Evaluation of the tocopherol content. Within each column, different superscript letters indicate statistically different values according to post-hoc comparison (Tukey's test) at p d 0.05. Values are expressed as mean ± sd (n = 6).

Sample	α-tocopherol mg/100g	γ-tocopherol mg/100g	δ-tocopherol mg/100g
P	1.15±0.67^{a}	47.68±27.72^{abcd}	0.29±0.40^{ab}
PE	0.76±0.00^{a}	33.24±2.58^{abcd}	0.01±0.00^{a}
L	1.84±0.21^{a}	66.49±6.72^{d}	0.01±0.14^{a}
LE	1.33±0.18^{a}	46.32±4.72^{abcd}	0.02±0.11^{a}
P T	1.51±0.76^{a}	48.50±18.12^{bcd}	1.19±0.08^{b}
PE T	0.99±0.04^{a}	38.12±7.39^{abcd}	0.48±0.67^{ab}
L T	1.50±0.16^{a}	68.01±4.00^{d}	7.46±0.36^{d}
LE T	1.40±0.13^{a}	49.12±5.18^{cd}	5.42±0.39^{c}
B P	1.73±0.31^{a}	8.56±1.30^{abc}	0.61±0.22^{ab}
B PE	1.29±0.24^{a}	7.62±1.48^{ab}	0.29±0.34^{ab}
B L	2.15±0.20^{a}	11.01±1.26^{abc}	0.90±0.01^{ab}
B LE	1.35±0.45^{a}	7.18±2.02^{a}	0.55±0.19^{ab}

As for the color, samples were mainly influenced by the different formulations. In particular, the encapsulated pastes showed a greater brightness (L) mainly given by the inclusion of maltodextrins; L parameter slightly reduced in the thermal stressed and in the antioxidant enriched samples. As a matter of fact, values were greatly influenced by the presence of the grape extract, causing a reduction of b and an increase of a parameter. The same behavior was observed in the thermal stressed samples: nevertheless, encapsulated pastes (L and LE) showed less susceptibility to browning.

Table 4: Evaluation of the color. Within each column, different superscript letters indicate statistically different values according to post-hoc comparison (Tukey's test) at p d 0.05. Values are expressed as mean ± sd (n = 6).

Sample	L	b	a
P	45.3±0.4^{b}	17.0±0.4^{c}	5.4±0.1^{bc}
PE	38.3±0.2^{a}	8.4±0.1^{a}	10.7±0.1^{e}
L	62.5±0.0^{fg}	22.3±0.0^{d}	3.6±0.0^{ab}
LE	53.1±0.0^{d}	10.8±0.0^{ab}	4.6±0.0^{abc}
P T	47.3±0.0^{bc}	22.1±0.2^{d}	2.2±0.1^{a}
PE T	37.1±0.0^{a}	15.6±0.0^{c}	7.3±0.0^{cd}
L T	60.1±0.00^{ef}	22.5±0.0^{d}	3.7±0.0^{ab}
LE T	49.6±0.0^{c}	11.8±0.0^{b}	4.3±0.0^{ab}
B P	67.5±2.2^{h}	29.2±1.8^{ef}	5.6±2.3^{bcd}
B PE	63.9±1.5^{g}	26.9±2.2^{e}	5.5±1.7^{bc}
B L	62.1±0.6^{fg}	31.5±0.9^{f}	8.3±0.7^{de}
B LE	58.4±0.2^{e}	26.9±2.2^{e}	7.1±1.4^{cd}

4. Conclusions

The present study aimed to evaluate the effects exerted by the addition of natural antioxidants from grape skin and by the application of an encapsulation procedure on walnut paste to be used in bakery products. The experimental results showed that the addition of grape skin extract contributed to enhance the antioxidant capacity of just prepared fluid paste but did not bring significant benefits under thermal stress. The encapsulation technique exerted a protective effect against oxidation by preserving antioxidant properties and increasing the stability of both pasta and biscuits. Recipe inclusion of encapsulated samples in just baked biscuits did not determined any advantages, neither in terms of oxidative stability, nor in terms of antioxidant properties. However, it produced a strong increase of the biscuit induction times. In the long terms, maltodextrins and gums used as coating agents in the encapsulated sample, could contribute to preserve the whole matrix prolonging the shelf-life of baking products containing them. In the future the effect on nutritional and sensory properties could be investigated.

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