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Effect of Technological Treatments and Polysaccharide Ingredients on Oxidative Stability of Innovative Freeze-Dried Walnut Products

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Many confectionery companies employ semi-finished lipid products, such as nut pastes, as an ingredient for the preparation of several foods. Nevertheless, during the storage, nut pastes have some drawbacks due both to the onset of rancidity, both to the separation between oil and semi-solid phase. The development of stable semi-finished walnut products in form of powder would allow to extend the walnut use in various preparations by increasing their convenience.
As is well known, walnuts are highly nutritious fruits with well evidenced health benefits. In spite of natural antioxidant content walnut kernels are rich in polyunsaturated fatty acids and vulnerable to oxidation. In order to preserve thermo sensitive substances, a freeze-drying (lyophilizing) technique was developed by addition of polysaccharide matrices (including dietary fiber) in a walnut paste obtained by roasted kernels. This study aims to provide information on the impact of technological treatments and ingredient combinations on oxidative stability of freeze-dried walnut products.
In preliminary tests, kernels were roasted at 110 °C for 5 min, 10 min, and 15 min. Residual moisture, pH and total acidity, peroxide value, and polyphenol contents were measured, as quality evaluation, in order to select the optimum operating conditions for the paste preparation. Optimal paste was obtained by milling 10 min roasted kernels, as it ensured a low residual humidity without affecting peroxides and polyphenols. Water and different combinations of maltodextrins DE12, tragacanth gum, and barley betaglucan were added to optimal walnut paste by creating emulsions which were then lyophilized.
Quality evaluation was carried out on samples before and after freeze-drying treatment, and on freeze-dried products after 7 day, 60 °C storage. The formulation, containing maltodextrin and tragacanth gum, supplemented with betaglucans (at a ratio of 1:100 with the optimal walnut paste), showed no differences in the values of pH, acidity and polyphenols measured throughout treatment. All of the samples kept peroxide values < 10 meqO_2/Kg expressed on dry matter. In particular, the addition of betaglucans gave the lyophilized samples a firm texture, without altering the oxidative chemical-physical parameters.

1. Introduction

Despite the important functional and sensory profile, the use of walnuts on industrial scale is limited by their high susceptibility to oxidation which leads to losses in terms of both nutritional value, and palatability.
The high content of polyunsaturated fatty acids (PUFA) is beneficial to the consumer health but it favors the rancidity and the development of undesirable volatile compounds and off-flavors (Calvo et al., 2011).
As known, walnuts are an important source of fat: the main components of the oil fraction are monounsaturated fatty acids (oleic acid), and omega-3 and omega-6 polyunsaturated fatty acids (linoleic acid and α-linolenic acid). Omega-3 and omega-6 account for about 70-80 % of total fatty acids and they make the walnut oil one of the vegetable oils richest in PUFA (Vaidya and Eun, 2013).

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Walnuts are rich in other bioactive compounds besides the fats. In particular, walnuts kelners have one of the highest phenolic content (mainly polyphenols) among nuts. They have been reported to display strong antioxidant and free radical-scavenging activity, able to maintain the oil stability (Martinez et al., 2011).
Phenolic compounds are located in high concentration especially in the pellicle of the kernel. Genetic, environmental, and storage factors can affect the content of non-flavonoid polyphenols (notably ellagic tannins) (Vaidya and Eun, 2013).
Technological processes can be detrimental to the walnut particles containing the fat, increasing the risk of oxidative alterations. In these conditions the lipids, no longer protected by the membranes, may come in contact with oxygen, light, lipolytic enzymes (endogenous and exogenous), and metals naturally present in plant tissues, which act as catalysts, and undergo the oxidation process. Roasting of kelners can improve the oxidative stability of oils, increasing the extraction yield, enhancing characteristic flavor, and inactivating enzymes (Vaidya and Eun, 2013). In order to avoid or limit the oxidation process, several further techniques have been developed by protecting polyunsaturated fatty acids from environmental damage (Anwar and Kunz, 2011). Encapsulation can be designed as a valid methodology to address this issue. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating wall or embedded in a homogeneous or heterogeneous matrix, to give small capsules (Calvo et al., 2011). The efficiency of the process depends mainly on the composition and structure of the coating material (including proteins, hydrocolloids, and hydrolyzed starches) that must provide a barrier also preventing contact with other ingredients present in the matrix. Therefore, the use of these materials, combined with an appropriate dehydration technique, is an effective tool to extend the shelf life of walnut. Freeze drying is one of the most useful processes for drying thermosensitive substances because it minimizes the product damages due to decomposition, and the changes in structure, texture, appearance and flavor (Ratti, 2001).
In the present study walnut paste, deriving from the selected roasting process, was added with water and different combinations of polysaccharide matrices (maltodextrins, gum, and betaglucans) by creating emulsions to be lyophilized. In order to enhance the properties of the whole fruit (not just of the oil phase), products were developed from the whole walnut kernels, keeping the pellicle (high in phenolics) (Martinez et al., 2010). Betaglucans were supplemented with the dual aim of replacing the common coating materials, and of improving the nutritional value of the product. This technique is intended to develop and to achieve a stable and sensorially pleasant powdered walnut product, with minimal change in the natural polyphenol content. On this basis, the impact of ingredients and technological treatments on oxidative stability was evaluated.

2. Materials and methods

2.1 Materials

Shelled walnuts (*Juglans regia* L.) were purchased from the local market (I frutti del Convento, Alfano F.lli, Italy). Maltodextrin DE 12 (Glucidex® 12) was supplied from Roquette Italia (Italy), Tragacanth gum powder (CEROTRAG 888) was procured from Roeper (Germany), and betaglucans (Glucagel™) were provided from DKSH Italia (Italy). All chemicals used in the analytical determinations were high-purity commercially available reagents.

2.2 Analytical determinations

Residual moisture, pH, total acidity, peroxide value, and polyphenol content were set as quality evaluation analysis. Moisture content was determined according to the method AOAC 931.04 (AOAC, 2005), pH and total acidity were performed following the methods recommended by the Office International du Cacao, du Chocolat et de la Confiserie (OICCC, 1972). Peroxide value was determined as described by the regulation (Commission Regulation 2568/91) on the oil fraction extracted according to Calvo et al. (2011). Polyphenols content was measured on defatted sample powder as reported by Belscak et al., 2009. Official methods of analysis (AOAC, 2005) were used to determine fat (method AOAC 948.22), proteins (method AOAC 950.48), crude fibre (method AOAC 953.53), and ash (method AOAC 22.010). For the analysis of reducing and non-reducing sugars (glucose, fructose, maltose, lactose and sucrose) the volumetric method of Luff- Shoorl was applied (Egan et al., 1981).

2.3 Preliminary tests

Walnut kelners were roasted in a forced convection oven at 110 °C for 5, 10, and 15 min. Each roasted and unroasted walnut samples were ground in a planetary micro mill (Pulverisette, Fritsch, Germany) at 800 rpm for 3 min. Quality evaluation analysis were carried out in order to select the operating conditions for the optimal paste preparation. Optimal paste was stored at -18 °C in dark vessel saturated with nitrogen until the next phase.

2.4. Microencapsulation process

In order to obtain a fine and stable emulsion, optimal walnut paste was added with water and homogenized for 5 min at 25 °C. Combination of matrices and their compositions are summarized in Table 1.

Table 1: Formulation of the samples to be subjected to freeze-drying and shelf-life tests

Sample	Formulation
1	50 g walnut paste
2	50 g walnut paste+75 mL H_2O
3	50 g walnut paste+2.5 g maltodextrin DE12+0.25 g tragacanth gum+75 mL di H_2O
4	50 g walnut paste+2.5 g maltodextrin DE12+0.25 g tragacanth gum+0.5 g betaglucans+75 mL H_2O

In this research three biopolymers (maltodextrin, tragaganth gum and betaglucans) were employed in combination as coating material in walnut paste microencapsulation process. Maltodextrins are starch hydrolysis products generally used to improve drying during encapsulation: they are a filler matrix which is cheap, highly soluble in water and able to form stable emulsion (Anwar and Kunz, 2011). Tragacanth gum is a plant derived hydrocolloid exhibiting high resistance, stabilizing, emulsifying and gelling ability, even at low concentrations (0.2 - 1.3 %) (Farzi et al., 2013). Betaglucans are a dietary fibre with well evidenced health benefits. They are glucose polymers with branched structure and small size. These properties influence their solubility, enabling them to form viscous solutions (Mudgil and Barak, 2013).

2.5 Freeze-drying tests

The freeze drying method was initiated by a freezing process. Emulsions were placed into glass bottles and frozen at -18 °C for 24 h. A Christ Alpha 1-2 LD freeze dryer was used to lyophilized the emulsions. During the drying process, the ice condenser was set at lower than -50 °C, and the pressure was around 0.120 mbar. The frozen emulsion was dried for 72 h.

2.6 Shelf-life tests

Accelerated storage tests were carried out on walnut paste (as control) and dried samples. Aliquots (50 g) of each sample were placed in open glass vessels and stored at 60 °C for 7 days in the absence of light. Quality evaluation analysis were carried out on samples before and after freeze-drying treatment, and on freeze-dried products after storage.

2.7 Statistical analysis

Data represent mean values (n=6) ± SD. Optimal walnut paste characteristics and data from quality evaluation were analyzed by factorial ANOVA at $p \leq 0.05$. Significantly different samples were selected by post-hoc comparison with Tukey's test using SPSS software version 21 (IBM Corporation, New York, USA). Within the tables, different letters indicate statistically different values.

3. Results and discussion

3.1 Preliminary tests

The impact of the roasting process on the walnut kelners was assessed by measuring the chemical-physical parameters reported in Table 2 and in Figure 1. The thermal processes significantly reduced the residual moisture in the samples treated for 10 and 15 min, but it did not involve significant changes in pH and total acidity. Polyphenol content was not significantly reduced by heat treatment ≤10 min, while the peroxide values increased in the walnut sample that was exposed to 15 min roasting. Although the roasting treatment determined an increase in the peroxide number, it can be considered as a good method to increase the oxidative stability of the nuts during storage, as it demonstrated to slow down the oxidation process (Vaidya and Eun, 2013). Peroxide values <10 usually guarantee an excellent state of preservation in the oil (Commission Regulation 2568/91), therefore the effect of the applied roasting processes on the walnut paste was limited in all cases. The walnuts subjected to a 15 min treatment showed a too intense olfactory perception. The roasty aroma was already perceivable and pleasant in the samples roasted for 10 min, while it was totally missing in the samples treated for 5 min. Therefore the paste obtained by walnut kelners roasted at 110 °C for 10 min was selected as the main ingredient to prepare freeze-dried products, as it had low humidity, low oxidative damage, and agreeable flavor.

Table 2: Residual moisture, pH, and total acidity of walnut paste samples obtained by different roasting time. Within each column, different letters indicate statistically different values

Roasting parameters	Moisture (%)	pH	Total acidity (meq/100g d.m.)
110°C 5 min	3.20±0.18a	6.29±0.02a	86±6a
110°C 10 min	2.27±0.19b	6.30±0.01a	76±4a
110°C 15 min	2.12±0.16b	6.31±0.02a	79±4a

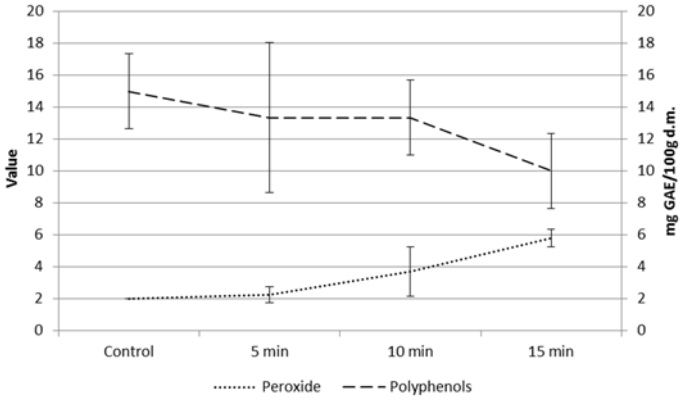

Figure 1: Peroxide values and polyphenol contents of walnut paste samples obtained by different roasting time at 110°C

3.2 Walnut paste characteristics

Optimal walnut paste characteristics are given in Table 3. Walnut is known as a source of high lipid content, so the fat was the major component in the paste. Proteins were present in high amounts. The examined walnut paste showed an important content of crude fibre, comparing to walnut proximate composition previously reported in literature (Ozcan, 2009). It should be noted that, unlike traditional processes, in the optimal walnut paste production the kelner pellicle was preserved after roasting. The mineral content matched the literature data, as well as the residual moisture reached values similar to those of hazelnut paste (D'Addio et al., 2013).

Table 3: Physical and chemical properties of the walnut paste obtained by roasting kelners for 10 min at 110 °C

Properties	Values (% w/w)
Moisture	2.27±0.19
Fat	68.78±1.37
Proteins	16.14±0.80
Crude fibre	9.12±0.12
Ash	1.73±0.03
Sugars	0.11±0.01

3.3 Freeze-drying and shelf life tests

The impact of different treatments and formulations on sample specifications are illustrated in Table 4. A walnut paste aliquot (sample 1) was stored as control in order to evaluate the oxidation process in the absence of additional ingredients and/or treatments: results showed an increase in pH and a significant reduction in acidity and polyphenol content after storage.

Table 4: Analysis on samples before and after freeze-drying treatment, and on freeze-dried products after 7 day 60 °C storage. For each parameter different superscript letters indicate statistically different values within each column, and different subscript letters indicate statistically different values within each row

Phases	Parameters (m.u.)	Samples 1	2	3	4
Before freeze-drying	pH	$6.23\pm0.01^{b}_{c}$	$6.71\pm0.03^{a}_{a}$	$6.65\pm0.02^{a}_{a}$	$6.46\pm0.00^{a}_{b}$
	Total acidity (meq/100g d.m.)	$69.04\pm0.00^{a}_{a}$	$71.07\pm14.36^{a}_{a}$	$81.22\pm7.18^{a}_{a}$	$77.06\pm5.74^{a}_{a}$
	Polyphenols (mg GAE/ 100g d.m.)	$27.26\pm0.12^{a}_{a}$	$16.22\pm0.95^{a}_{b}$	$25.07\pm0.95^{a}_{a}$	$16.81\pm0.95^{a}_{b}$
	Peroxide value	<5	<5	<5	<5
After freeze-drying	pH		$6.60\pm0.02^{ab}_{a}$	$6.59\pm0.02^{ab}_{a}$	$6.42\pm0.01^{a}_{b}$
	Total acidity (meq/100g d.m.)		$51.29\pm5.80^{a}_{b}$	$57.97\pm7.19^{a}_{b}$	$76.32\pm0.42^{a}_{a}$
	Polyphenols (mg GAE/ 100g d.m.)		$13.45\pm2.71^{a}_{a}$	$16.80\pm1.13^{b}_{a}$	$21.21\pm0.24^{a}_{a}$
	Peroxide value		<5	<5	<5
After 7 day 60°C storage	pH	$6.62\pm0.00^{a}_{a}$	$6.48\pm0.02^{b}_{b}$	$6.52\pm0.00^{b}_{ab}$	$6.44\pm0.05^{a}_{b}$
	Total acidity (meq/100g d.m.)	$53.02\pm4.24^{b}_{a}$	$64.00\pm5.66^{a}_{a}$	$64.07\pm0.00^{a}_{a}$	$72.10\pm2.83^{a}_{a}$
	Polyphenols (mg GAE/ 100g d.m.)	$17.72\pm1.41^{b}_{a}$	$21.41\pm3.70^{a}_{a}$	$24.61\pm0.23^{a}_{a}$	$17.55\pm2.23^{a}_{a}$
	Peroxide value	$14.69\pm2.41_{a}$	$8.89\pm0.71_{b}$	$9.75\pm0.36_{b}$	$9.48\pm1.50_{b}$

The effects of freeze-drying process on the walnut paste were evaluated in the sample 2 (walnut paste+H_2O): unlike the sample 1, the pH decreased significantly after storage, while the acidity and polyphenol values were not significantly altered by technological treatments. Also in the sample 3 (walnut paste added with maltodextrins and tragacanth gum) the pH significantly decreased after storage. The polyphenol content was significantly reduced by freeze-drying process, but it increased after 7 days at 60 °C, reaching value similar to the initial one. In the formulation containing betaglucans (sample 4), no differences were evidenced in pH, acidity, and polyphenols values measured at various stages of treatment.

Comparing samples within each phase, further differences may be highlighted. Before freeze-drying pH showed a significant variability in the various formulations. This could be due to the properties of the components added to the matrix. In particular, the sample 1 (only walnut paste) had the lowest pH value. Data related to the acidity did not change with the formulations, while the walnut paste emulsion (sample 2) and the sample containing betaglucans (sample 4) had a polyphenols content significantly lower than the other samples. Immediately after freeze-drying the acidity variation did not have a significant relevance in samples 2 and 3, while it was significantly higher in the formulation with the betaglucans (sample 4). The same applied to the pH that was significantly lower in sample 4. Finally, the polyphenols content did not change in the different samples. After the lyophilisation, the residual moisture content was measured. The lowest moisture value (0.03±0.01 %) was found in the sample 4, containing betaglucans, that differed from the sample 2 (2.51±0.31 %) and 3 (1.68±0.47 %) values. This was not expected because previous studies (Skendia et al., 2010) showed that the water retention capacity in food increases with increasing amount of betaglucans added in the formulation. After 7 days at 60 °C, the residual moisture was not detectable in all the samples. After storage, walnut paste (sample 1) showed the highest pH among the samples; acidity and polyphenols content did not change in the different formulations. Peroxide numbers were very low (<5) in all the samples before and after lyophilisation. After storage, the peroxide value increased in the sample 1 (not freeze-dried walnut paste) compared to microencapsulated samples (3 and 4). A barrier effect exerted by polysaccharides against oxidation or as a result of sample dilution (by adding coating materials) were conceivable (Calvo et al., 2011). Nevertheless, a lower peroxides level was found in the sample 2 that was added with water only and lyophilized. The physical transformations determined by the freeze-drying process (i.e. pressure variations) can induce a positive impact on the antioxidant potential (resulting not only from polyphenols but also from tocopherols, etc.) (Vaidya and Eun, 2013) by increasing the oxidative stability. Hydrophobic interactions between polyphenols and polysaccharides in the samples are affected by the treatments. In samples 2 and 3, the polyphenols content after 60 °C storage was higher than the content found immediately after lyophilisation. This can be explained by assuming that the heat altered the polysaccharide network formed during freeze-

drying: in this way the hydrophobic interactions that limit the polyphenols extraction were reduced (Nazzaro et al., 2012).Compared to the analysis carried out immediately after freeze-drying, all the stored samples showed a reduction in pH and an increase in acidity. Predictably, during the 60°C storage phase the samples were subjected to an oxidative stress resulting in release of free fatty acids.

4. Conclusions

Only a small share of walnuts is usually allocated for industrial processing: besides the oil, there are relatively few walnut-based products which, in most of the cases, are flavored surrogates. Since walnuts have favourable fatty acid and nutrient profiles, there is growing interest in evaluating their use on an industrial scale. In particular, the availability of stable walnut derivatives in form of powder, would allow to spread their use by increasing the convenience. In the present study, an optimal walnut paste was obtained by milling kelners roasted at 110 °C for 10 min. In order to limit the oxidation process and to stabilize the fat matrix, new solid products have been created by adding biopolymers to the paste. The developed freeze-drying technique, can be considered as a valid method to safeguard the sensory and nutritional properties of walnuts, ensuring the slowdown of the degradation reactions. In particular, the formulation containing maltodextrins DE12, tragacanth gum, and betaglucans (at a ratio of 1:100 with the walnut paste) originated a stable polysaccharide network giving the lyophilized product a compact structure without altering the original chemical-physical parameters. Based on these results, it is conceivable to expand the market of walnuts proposing innovative freeze-dried products as high quality semi-finished products for the confectionery industry and/or for direct consumption.

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