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Preservation of Food With High Oil Content by Supercritical Impregnation Techniques

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Nuts are very important foods for human nutrition due to their high content of mono- and polyunsaturated fatty acids. However, they are substrates very sensitive to oxidation, appearing rancidity that affects both the taste and the smell, that led to the consumer's rejection. To avoid this, the food industry uses some strategies that sometimes substantially alter the composition and nutritional value of nuts. A promising possibility for the conservation and stability against its oxidation is to impregnate a natural antioxidant such as methyl gallate using CO_2 in supercritical conditions. This technology is being used recently for the impregnation of different natural substances on polymers for its use in the food and biomedicine field. However, the direct supercritical impregnation of compounds into food has been scarcely investigated.
In the present work, it is studied the effect of pressure and temperature in the impregnation process of methyl gallate on peeled peanuts. Additionally, the effect of the depressurization rate and impregnation time was studied at the most convenient conditions. The results obtained indicate the best conditions for impregnation were reached at 150 bar and 45 ºC. These conditions improved the antioxidant capacity of the nuts while preserving the quality by avoiding breakages during the process.

1. Introduction

Lipid oxidation and microbial growth are the main causes of most foodstuff spoilage, being particularly important for fresh food, fourth range products, or peeled nuts, causing losses of both sensory and nutritional qualities. Lipid oxidation is of primary importance on food rich in mono- and polyunsaturated fatty acids since the rancidity are sensorial perceived yet in early states (B. Weinreich, 2010). Peanuts and other shelled nuts have a longer shelf life since they are protected from oxygen. However, those marketed in the peeled or ground format are especially sensitive to oxidation reactions (Grüner-Richter et al., 2012), so one of the challenges in nut production is to avoid oxidation during the manufacture and storage. Apart from the use of modified atmosphere packaging, there are some commercial strategies to fight the oxidation of nuts, such as adding sugar or chocolate coatings, drying, or toasting. However, these processes severely affect the sensory properties and the nutritional composition by increasing calories or reducing the nutritional value (Weidner, 2018).
New technological treatments as coating with encapsulated antioxidant agents are being explored to stabilized the lipid fraction of nuts (Dodorni et al., 2017). Another suitable technology is direct-food impregnation with active compounds, such as enzymes, minerals, vitamins, and antioxidants, among others, into the edible parts of the food product. Some of the techniques traditionally used are osmosis impregnation, vacuum impregnation, ultrasound, or edible coating (Radziejewska-Kubzdela et al., 2014). However, these processes possess certain limitations. On one side, the mass transfer mechanisms are restricted by diffusion and capillary and are dependent on some food properties such as humidity, geometry, or food composition. On the other hand, generally, subsequent drying stages are needed to eliminate remained liquids on the food's surface, which produce Maillard Reactions and causes changes both in taste and texture (Yang et al., 2019).
The use of supercritical fluids has arisen great interest in recent decades for improving these industrial processes as it solves the problems presented by traditional impregnation methods. Active packaging is one

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of the most innovative applications aimed at increasing the shelf life of these products with minimal modification of its formulation. These packages contain compounds with antioxidant and antimicrobial activity that aim to extend the shelf life of the food while maintaining its composition intact. For instance, Cejudo Bastante et al. (2018) demonstrated that a PET/PP food package impregnated with bioactive compounds from olive leaf extracts significantly reduced the peroxide index of peeled, packaged sunflower seeds compared to not impregnated packaging. Another alternative is the direct supercritical impregnation of products, which is based on the facility of CO_2 in solving active compounds and then incorporate them into different matrices thanks to its high diffusivity, while providing a solvent-free product once the process ends. The supercritical impregnation of rosemary extract and carnosic acid into different peeled nuts has been previously reported in the literature with promising results attending to its preservation, but previous steps of decoloration and deodorization of the extract were required to reduce the sensorial impact of the aromatic terpenes (Grüner-Richter et al., 2012). Considering this, the present study aims to incorporate a lower sensorial impact compound as methyl gallate (MG) and evaluate the structural modification and the antioxidant capacity of the peanuts after impregnation treatment. MG is a phenolic compound already present in nuts (Fuente-Maqueda et al., 2020) with a strong antioxidant capacity that intervene in hinders lipid oxidation (Mansouri et al., 2020). In this sense, their incorporation into peanuts would help in maintaining longer their functional compounds and sensory properties by adding a compound naturally contained among their constituents.

2. Material and methods

2.1 Raw materials and reagents

Carbon dioxide (99.99%) was purchased from Abello-Linde S.A. (Barcelona, Spain). Peeled penuts were obtained from a local market (Cadiz, Spain). 2,2-Diphenyl-1-pricrylhydrazyl reagent (DPPH) and methyl galate were obtained in Sigma-Aldrich (Steinheim, Germany). Organic solvents (hexane, ethyl acetate, acetonitrile, methane and formic acid) were purchased in Panreac (Barcelona, Spain).

2.2 Supercritical impregnation procedure

Experiments were developed in a lab-scale high-pressure equipment provided by Thar Technologies (Pittsburgh, PA, USA), which includes a condenser, a P50 high-pressure pump, a pre-heater, a 104 mL vessel with a thermostatic jacket, and a back-pressure regulator (BPR) (Figure 1). All units are monitored by a controller.

The impregnation process was developed in batch mode. A stir bar was placed at the bottom of the vessel to ensure the homogenization of the headspace with constant stirring. Then, 86 mg of methyl gallate (MG) and ~30 g of raw entire peeled peanuts were introduced into the vessel, separated by a metallic mesh to avoid physical contact. CO_2 was pumped at a flow rate of 10 g/min until reaching the pressure value set in each experiment. Once passed 5 h of impregnation time, the system was depressurized at 2 bar/min. Samples were collected and stored at 4 ºC in darkness until analysis. The impregnation flowchart is depicted in Figure 1.

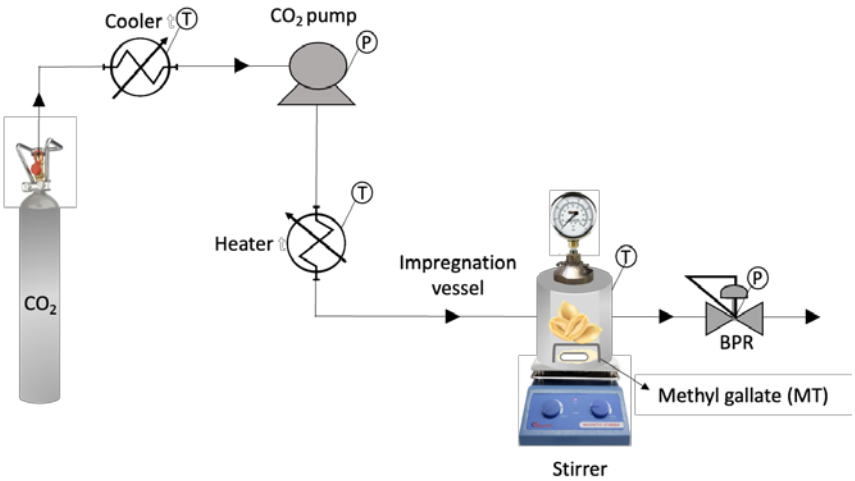

Figure 1. Impregnation process flowchart

A 2^3 screening experimental design was analysed varying pressure (100, 150, and 200 bar), and temperature (35, 45, and 55 □C). Then, the depressurization rate and impregnation time were also studied at the most convenient pressure-temperature conditions selected in the experimental design.

2.3 Quantification of MG impregnated

Impregnated nuts were extracted aid with ultrasounds for 60 min with hexane in order to obtain the peanut oil that contains the MG deposited. Then, 300 µL of the oil were pre-concentrated by solid-phase extraction (SPE) using a C18 column (40 µm of particle size) previously pre-conditioned using water, methanol, and hexane. 1 mL of methanol was used as the elution solvent. The fraction was recovered and analysed in an HPLC Agilent 1100 (Agilent, Germany) coupled with a UV/Vis detector and a column SynergiHydro-RP C18 (4 µm, 150 mm x 3 mm i.d.) (Phenomenex, US.). The mobile phase consists of a solvent A of 0.1% formic acid in water, and a solvent B of 0.1 %formic acid in acetonitrile. The amount of MG was quantified at 278 nm using the following calibration curve

$$\text{Area} = 101962\ C_{MG} + 4.0023;\ R^2 = 0.999 \quad (1)$$

Then, the impregnation yield (I%) was calculated as the amount of MG quantified respecting the amount of the oil recovered in the extraction. To evaluate possible interference of the measured, the initial amount of MG on the samples was analysed and quantified in trace levels, so it was not considered a variation factor. Quantification of the MG impregnated was done in duplicate.

2.4 Antioxidant capacity of impregnated nuts

The antioxidant activity of the impregnated nuts was determined by analysing the reaction of the extracted oil in the presence of the 2,2-Diphenyl-1-pricrylhydrazyl reagent (DPPH). To do so, 100 µL of extracted oil were introduced in 3.9 mL of $6 \cdot 10^{-5}$ M DPPH solution prepared in ethyl acetate. The reaction was monitored at 515 nm until the steady state was achieved. The efficient concentration (EC_{50}) of extracted oil was determined in each impregnation condition in order to define the oil concentration required to reduce the DPPH reagent by 50%. Analyses were done in duplicate.

2.5 Scanning Electron Microscopy SEM

Nuts impregnated under the most convenient conditions were analysed by SEM (Quanta 200) in order to observe the deposition of MG on the nuts' surface and evaluate possible structural damages.

3. Results and discussion

3.1 Study of the impregnation conditions

The impregnation parameters were optimized according to the I% of MG. The CO_2 density at each condition has to be taking into consideration since it would determine both the solubility of MG and the diffusivity of the compound in the matrix to be impregnated (Champeau et al., 2015).

The density of supercritical CO_2 increases with an isothermal increase of pressure, with the consequent increase in solubility of the MG in the CO_2 (Murga et al., 2002). According to the results of Murga and researchers, the solubility of MG at 40 ºC increase with pressure, reaching values from $1.2 \cdot 10^7$ to $5.56 \cdot 10^7$ M in the range of 100 to 200 bar. When increasing temperature until 50 ºC, the solubility drastically increments until *ca.* $10 \cdot 10^7$ M when pressure is above 200 bar. These values are in concordance with the I% obtained (Figure 2A), since pressures over 150 bar turn into a decrease in the impregnation efficiency. At those conditions, the partition coefficient of the MG deviates to the supercritical phase instead of to the peanut surface, favouring the dragging of the compound during the depressurization. Besides, the decrease in the diffusivity hinders yet the penetration of the MG into the peanut matrix. When analysing the experimental design, the pressure was pointed as the only significant parameter, being a positive variable of the impregnation process (Figure 2B). However, it should be mention that pressures of 200 bar cause a high impact on the nuts integrity so that conditions were discarded. Attending to the I% values (Figure 1a), a Gauss-Bell behaviour seems to be observed in the data obtained, in which low and very high levels of solubility of MG lead to low impregnation yields, being the most optimal levels those obtained at 150 bar, where the solubility of MG in the supercritical phase is *ca.* 5 to $6 \cdot 10^7$ M. At 150 bar, the increase from 45 to 55 ºC did not cause a significant change in the impregnation yield, maintaining adequate yields above 2 mg_{MG}/g_{oil}. In this sense, 45 ºC was selected as the most convenient temperature as this would entail energy savings in the process.

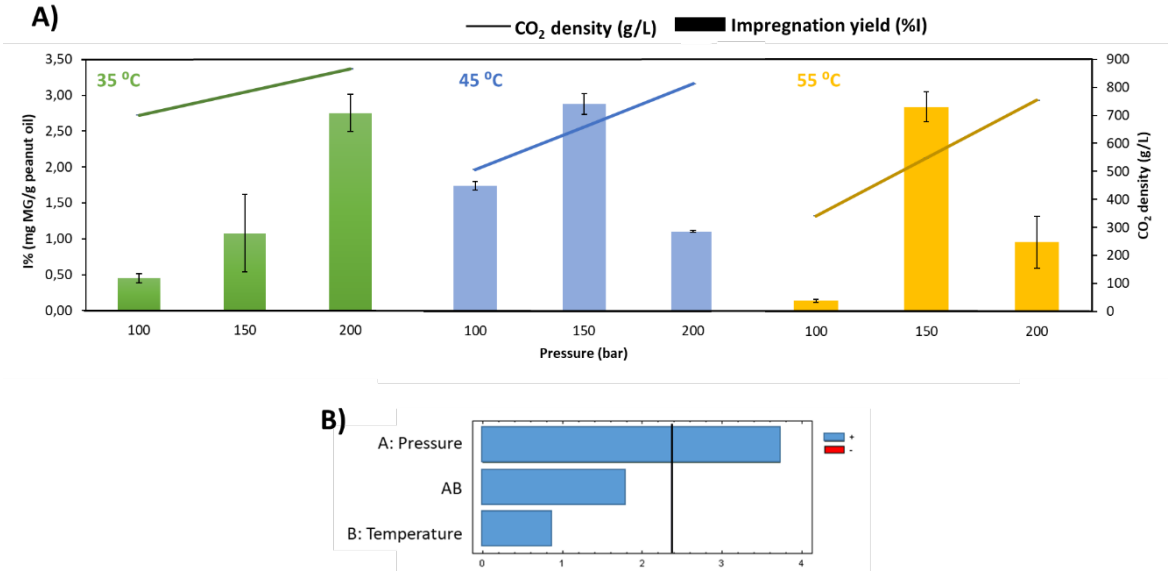

Figure 2. A) Impregnation yield (%I, mg MG/g peanut oil) and CO_2 density at each impregnation conditions; B) Pareto diagram of the impregnation yield

Both pressure and temperature are the determining factors in the impregnation processes, since they influence the solute's solubility in the supercritical solvent. Moreover, the impregnation time and the depressurization step are also critical variables. On one side, impregnation is a mass transfer process so knowing the kinetics of the reaction would aid to develop the process more efficiently. Moreover, extreme pressure changes during depressurization could improve the deposition of the compound but also affect the integrity of the product (Ameri et al., 2020). Therefore, both parameters have to be also defined to optimize impregnation conditions. As far as we know, it is the first time that both parameters have been studied in nut impregnation.

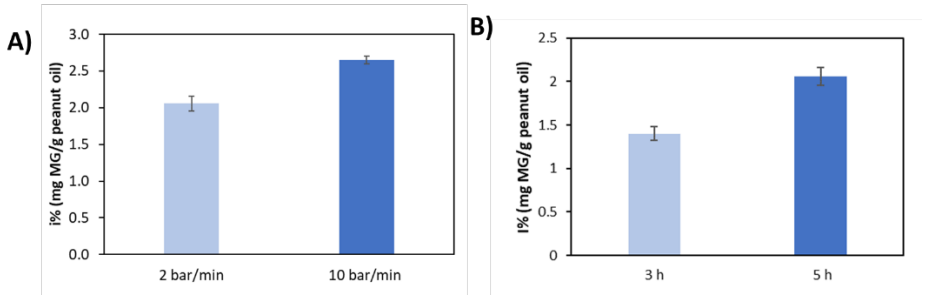

Figure 3. Effect of A) depressurization rate and B) impregnation time on the impregnation yield (I%) on samples impregnated at 150 bar and 45 ºC.

The impregnation yields obtained at a low depressurization rate of 2 bar/min were compared with a fast one of 10 bar/min. The operating conditions of pressure and temperature are those selected previously, *i.e.* 150 bar and 45 ºC. The impregnation time was kept constant at 5 hours in those experiments.

As can be seen in the Figure 3A, the I% is significantly higher when depressurization is fast. As the supercritical conditions are modified in a short period, the CO_2 rapidly reduced its density, decreasing the solubility of MG and favouring its deposition on the peanut's surface. However, that extreme pressure change in the vessels may cause the breakage of the nuts and therefore those conditions were not advisable for the impregnation of entire peanuts. The depressurization step is crucial in the impregnation of fragile products, where lower impregnation yield has to be sometimes assumed to obtain a product without mechanical damages still accepted by the consumer (Ongkasin et al., 2020).

Grüner-Richter et al. (2012) developed the impregnation of rosemary extract during 1 h on different nuts obtaining good impregnation efficiency and significant delay in its peroxidation. In these experiments, longer periods were studied to favour the solubilisation and subsequent impregnation into a complex matrix as nuts.

Time increments positively influenced the impregnation yield (Figure 3B), since 3 h were insufficient for achieving higher impregnation yields, obtaining better results at 5 h of treatment. Although longer periods should be explored, preliminary experiments with 24 h of impregnation time were discarded due to a co-extraction of peanut oil was observed (data not shown), which led to an early deterioration of the product and an adequate impregnation process.

3.2 Antioxidant capacity of the impregnated peanuts

The antioxidant capacity gained by the peanuts after the treatment is showed in Table 1 in comparison to the untreated peanuts. As can be seen, all conditions favoured the increment of the antioxidant capacity of the peanut oil extracted, which eventually intervene in delaying the lipid oxidation. The lower the EC_{50} value is, the higher is the antioxidant activity reached, since lower amounts of oil efficiently react with the 50% of the DPPH reagent. Nevertheless, there was no direct correlation between I% and EC_{50} values since the peanut oil contains other reactive compounds that could cause a synergistic effect in the reaction with the DPPH. Samples impregnated at 55 ºC seemed to achieve slightly higher antioxidant activity than the rest of conditions.

Table 1. Antioxidant capacity of impregnated peanuts.

Pressure (bar)	Temperature (□C)	EC_{50} (µg antioxidant/mL)
No impregnated peanuts		21.90 ± 1.99
100	35	12.20 ± 0.99
	45	12.50 ± 3.96
	55	9.95 ± 0.49
150	35	10.35 ± 3.18
	45	12.10 ± 0.99
	55	7.80 ± 2.55

4. Scanning electron microscopy

Impregnated and not impregnated peanuts were analysed by SEM. A comparison of both samples can be seen in Figure 4. The superficial homogeneity of the not impregnated peanut can be observed. Besides, there is no apparent damage of the impregnated peanut surface since any microscopic cracks were observed. However, certain superficial modifications can be perceived after the impregnation treatment (Figure 4B, 4C, and 4D) since some spherical particles, possibly attributed to the MG, were deposited in the peanut surface. The spherical morphology of this compound after supercritical impregnation has been previously described on impregnated food-grade films (Belizón et al., 2018). Although a partial internal diffusion of MG into the peanut matrix could not be discarded, the superficial deposition of the antioxidant is a good approach, since the outer surface of the peanut would be in contact with the oxygen that causes the lipid peroxidation in a greater extent, so the MG particles will be available for their rapid action.

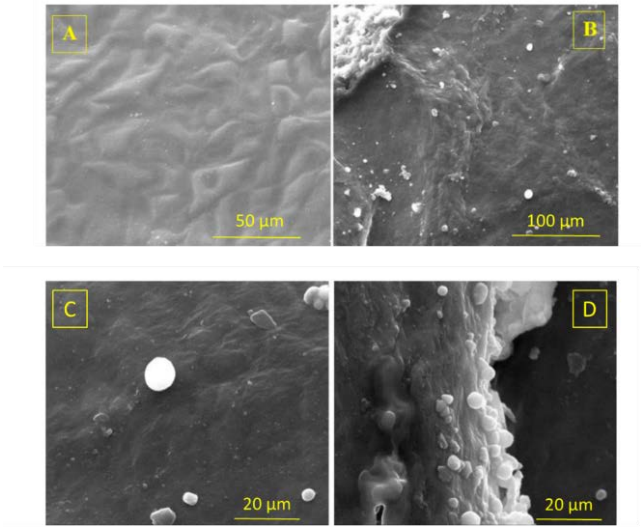

Figure 4. A) No impregnated peanut; B, C and D) Different images of the peanuts impregnated at 150 bar and 55 ºC.

5. Conclusions

Supercritical impregnation of nuts is presented as an alternative to prolong its shelf life. All conditions provide statistically higher antioxidant capacity respecting to the untreated samples, which will undoubtedly intervene in delaying lipid oxidation. Pressures above 150 bar and depressurization rates over 2 bar/min, although showing higher impregnation yields, were not recommended for the impregnation of entire nuts, since the breakage of the sample was observed. Nevertheless, those results have to be positively considered, since those conditions could be employed in crushed nuts, which in turn needs higher impregnation yields since the higher superficial area make them even more sensitive to oxidation.

All in all, the impregnation conditions of 150 bar 45 ºC and 5 h of impregnation, with a depressurization rate of 2 bar/min provide the lower physical modification of the product under study with the highest impregnation yield. Independently of the impregnation conditions, the antioxidant activity of the product improves significantly comparing with the untreated product. Even though further sensory and peroxide index analysis should be done in order to assess the suitability of the process, these results offer a good perspective of the use of this alternative treatment for nut's preservation.

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