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Effect of high pressure processing on carotenoid and phenolic compounds, antioxidant capacity, and microbial counts of bee-pollen paste and bee-pollen-based beverage

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ABSTRACT

The optimal high pressure processing treatments (200–400 MPa, 5–15 min) of a pasty matrix of bee-pollen mixed with peptone water (1.5 g/mL) and bee-pollen added to a pineapple juice-based beverage matrix (0–10% (w/v)) were studied in order to guarantee food safety and maximum retention of bioactive compounds. *Salmonella* and yeasts were used as target microorganisms, while total carotenoid content (TCC), total phenolic content (TPC), and antioxidant capacity (FRAP) were studied from the food quality point of view. For the pasty matrix of bee-pollen, the results showed a significant influence of pressure and time, increasing the levels of TPC, FRAP, and TCC, in comparison with a control sample. A treatment of 395 MPa for 15 min was found as the optimal. For the pineapple juice-based beverage matrix, the factors pressure and bee-pollen concentration increased the levels of TPC, FRAP and TCC. Optimal conditions were found at 315 MPa for 14.5 min with 8% (w/v) of bee-pollen.

Industrial relevance: This investigation demonstrated the efficacy of the application of HPP on bee-pollen to inactivate microorganisms, both pathogenic and spoilage, up to 5-log 10 cycles. Additionally, a structural modification of the grain was achieved, with a consequent extractability of bioactive compounds and an increasing in the antioxidant activity, higher than 60% in comparison to fresh bee-pollen. The inclusion of bee-pollen in a fruit juice-based beverage matrix had a positive effect on the contribution of bioactive compounds that the fruit juice itself does not contain, such as carotenoids, for which bee-pollen can be considered as a natural additive that enhances the product functional characteristics.

1. Introduction

Increased consumer awareness of natural food products has modified current diets, encouraging consumers to demand food that not only provides the essential nutrients for life but also contains substances with potential health benefits. Moreover, and perhaps in response to consumer demands, current trends in the food preservation industry include avoiding the utilization of intense thermal treatments and chemical additives to promote foods rich in secondary metabolites that act as natural preservatives, achieving a minimum impact on the nutritional and physicochemical properties of foods.

Bee-pollen is the main source of protein for bee colonies. Worker bees transport the pollen from flowers into the hive by carrying it on their hind legs as pellets that they form with movements of their front legs, using combs, hairs, and salivary secretions (Almeida-Muradian, Pamplona, Coimbra, & Barth, 2005). Proper hive management promotes additional pollen collection aimed at human consumption, since it can be considered as a food or food additive owing to its content of a wide range of nutrients (Human & Nicolson, 2006). Pollen consumption and marketing have recently achieved some diffusion; however, this product went practically unrecognized as a food product for a long time, except by vegetarian or naturist consumers (Fuenmayor et al., 2014). At present there are only a few countries (e.g. Spain, China, Hungary, Argentina, and Brazil) where pollen production is economically attractive; even so, the pollen consumer market has strengthened during the last couple of decades (Campos et al., 2008). Countries such as Brazil, Argentina, Switzerland, Spain, and Mexico have established official quality standards and recognized pollen as a food product (Bogdanov, 2011).

Bee-pollen can be considered as a functional dietary supplement, especially because of its antioxidant properties (Kaškonienė, Ruočkuvienė, Kaškonas, Akuneca, & Maruška, 2014), its micronutrient composition (Somerville & Nicol, 2002), its fatty acid profile (Markowicz et al., 2004), and its therapeutic or disease-preventing functions (Pinto et al., 2010).

The most important bioactive substances in bee-pollen are phenolic compounds and carotenoids. Phenolic compounds are the most

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abundant secondary metabolite source in bee-pollen, responsible for the color of the grain (yellow, brown, red, purple, etc.), and its characteristic bitter taste (Bogdanov, 2011). Some phenolic compounds present in pollen are: *p*-hydroxybenzoic, *p*-coumaric, vanillic, gallic, and ferulic acid, quercetin, isorhamnetin, galangin, chrysin, and pinocembrin (LeBlanc, Davis, Boue, DeLucca, & Deeby, 2009). Carotenoids are also important for color and for other biological functions, such as antioxidant activity, provitamin A activity, and enhancement of the immune system (Fernández-García et al., 2012). In particular, the following carotenoids have been identified in pollen: β-carotene, cryptoxanthin, β-carotene-5,6,5,6-diepoxide, zeaxanthin, antheraxanthin, violaxanthin, neoxanthin, flavoxanthin, lutein, 9/9-(Z)-lutein, and luteoxanthin (Schulte, Mäder, Kroh, Panne, & Kneipp, 2009).

In spite of these properties, previous research suggests that the availability for humans of the beneficial components present in bee-pollen is limited (Cook, Awmack, Murray, & Williams, 2003). There have been doubts about the ability of the human digestive system to break the outer layer of pollen and to absorb substances found inside. Various *in vitro* simulations of human digestion suggest that pollen is partially digested – between 48% and 59% – (Franchi, Corti, & Pompella, 1997). The outer layer is known as exine, a very strong, firm compound made of sporopollenin, which preserves the substances that are in the interior of the grain from oxidation, radiation, and chemical degradation due to UV light (Rowley & Skvarla, 2000). Sporopollenin structure has been extensively studied: it is made primarily of carbon, hydrogen, and oxygen, with an empirical formula $C_{90}H_{144}O_{27}$ (Atkin et al., 2011). It has also been proposed that its structure consists of a lipid copolymer of *p*-hydroxycinnamic acids (ferulic and *p*-coumaric acid) and fatty acids, cross-linked with ethers and esters, and some types of carotenoids, tocopherols, pro-vitamin A, and vitamin D (Thomasson et al., 2010).

Some preservation treatments (osmotic dehydration, modified atmospheres, frying, microwave, freezing, and pasteurization) cause microstructural modifications in treated foods, facilitating the liberation of compounds from the food matrix, which would contribute to increasing the fraction that is absorbed during digestion (Guardeño, Sanz, Fiszman, Quiles, & Hernando, 2011). High pressure processing (HPP) is one of the most economically viable of what are known as non-thermal treatments (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). The effects of HPP on the nutritional and bioactive compounds and the microstructure of food have been studied (Barba, Criado, Belda-Galbis, Esteve, & Rodrigo, 2014; Vázquez-Gutiérrez et al., 2013), showing that this treatment causes structural changes that favor the structural compaction and extractability of bioactive compounds.

Consequently, it would be interesting to study the effect of HPP on microbial inactivation, as well as the extractability and availability of bioactive compounds of bee-pollen. The objective of this study was to assess the extraction capability and effects of HPP on bee-pollen components. Two HPP treatments, consisting of a pasty matrix of bee-pollen mixed with peptone water and, in addition, bee-pollen added to a pineapple juice-based beverage matrix, were optimized in terms of the maximum amount of bioactive compounds and the maximum reduction in the microbial load (more than 5 log reductions). Such processes would make it possible to select the conditions and bee-pollen concentration with the highest bioactive compound content in order to develop new ingredients of interest for formulating special foods.

2. Materials and methods

2.1. Samples

Collected bee-pollen was provided by manufacturers from the Colombian central region known as Cundiboyacense Highland. The bee-pollen was subjected to convection drying at 60 °C for 6 h. The pineapple juice-based commercial beverage contained in a Tetra-Pak® carton package was selected for bee-pollen inclusion. It was purchased from a local supermarket and then stored at room temperature previous to experimental studies.

2.2. Microorganisms

Two microorganisms were selected to assess the effects of HPP and bee-pollen on reducing the microorganisms' concentration and growth. *Salmonella typhimurium* (CECT 443) and *Zygosaccharomyces rouxii* (CECT 1229) were obtained from lyophilized pure cultures provided by the Spanish Type Culture Collection. *S. typhimurium* represents a widely recognized foodborne pathogen, and *Z. rouxii*, a known spoilage yeast, mainly of sweet foods and beverages, and resistant to many of the common food preservation methods (Leandro, Sychrova, Prista, & Loureiro-Dias, 2011). The stock vials containing *S. typhimurium* and *Z. rouxii* were generated following the methods described by Saucedo-Reyes, Marco-Celdrán, Pina-Pérez, Rodrigo, and Martínez-López (2009). The average cell concentrations were ca. 5.0×10^6 cfu/mL for *S. typhimurium* and ca. 5.1×10^6 cfu/mL for *Z. rouxii*. Values were established by viable plate count, using Tryptic Soy Agar (TSA; Scharlau Chemie S. A., Spain) and Potato Dextrose Agar acidified with tartaric acid (1% (v/v)) (PDA; Scharlau Chemie S. A., Spain) for the spreading of samples.

2.3. Sample preparation and HPP treatments

Two studies were carried out; first, bee-pollen was moistened with peptone water as a neutral reference medium (1.5 g/mL) and the product resulted in a bee-pollen paste. Inoculated and uninoculated paste samples were then poured into polyethylene bags and heat-sealed (MULTIVAC Thermosealer, Switzerland) before undergoing HPP treatment.

A second study was performed, considering a food matrix as a carrier of bee-pollen grains. For this purpose, different concentrations of bee-pollen (2.5 and 5 g) were added to pineapple juice samples (50 mL) to obtain final bee-pollen concentrations of 5% and 10% (w/v), respectively. The higher bee-pollen concentration (10% (w/v)) was selected taking into account the reported average β-carotene daily intake required by an average person (8.1 mg) (Souverein et al., 2015); previous assays allowed evaluation of the bee-pollen's total carotenoid content (454.05 ± 4.10 mg β-carotene/kg). A blank sample was formulated with 50 mL of pineapple juice. Then the inoculated and uninoculated samples were packed in polyethylene bags that were heat-sealed (MULTIVAC Thermosealer, Switzerland) before being inserted in the pressure vessel.

HPP treatments were performed in a unit with a 2.35 L vessel volume with a maximum operating pressure of 600 MPa (High-Pressure Food Processor, EPSI NV, Belgium). The samples were pressurized at 200, 300, and 400 MPa, at room temperature (18–22 °C), for 5, 10, and 15 min, using a compression rate of 300 MPa/min and a decompression time < 1 min not including come-up and come-down times. All other parameters such as pressure level, pressurization time, and temperature were automatically controlled. Once the treatment had been completed, the samples were taken from the vessel, immersed in an ice-water bath and then stored under refrigeration (3 ± 1 °C) until use.

2.4. Total carotenoid content

The total carotenoid content was measured as suggested by Hornero-Méndez and Mínguez-Mosquera (2001), with modifications. The sample (5 g) was extracted with 25 mL of cooled acetone using a homogenizer (IKA T25 Basic Ultra-Turrax) and vacuum filtered. This process was performed three more times. The extract was added gradually to 50 mL of ethyl ether in a decanting funnel. With each addition of extract, enough NaCl solution (100 g/L) was added to separate the phases and transfer the pigments to the ether phase. Then the aqueous phase was removed. The ether phase was treated several times with