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Metabolomic studies after high pressure homogenization processed low pulp mandarin juice with trehalose addition. Functional and technological properties

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ABSTRACT

This work aimed to determine the effect of homogenization pressures (HPH) and addition of trehalose on the functional and technological properties of low pulp mandarin juice (LPJ). A set of experiments was designed, combining a non-targeted metabolomic ^{1}H NMR based approach together with suspended pulp and transmittance, hesperidin, vitamin C and antioxidant activity analysis. Suspended pulp increased with HPH and trehalose addition. Flavonoid hesperidin initially decreased with HPH but trehalose addition resulted in less flavonoid degradation during storage, increasing the effect with the HPH. Vitamin C was not affected by trehalose and pressure treatment but more Vitamin C degradation was observed in trehalose samples during storage. Antiradical activity improvement by trehalose was conditioned by homogenization pressures and specific bioactive compounds. ^{1}H NMR based approach highlighted the HPH effect on the microbiological aspects of low pulp mandarin juice by the identification of key molecules responsible of the microorganism profile evolution during storage.

1. Introduction

High pressure homogenization (HPH) process is a non-thermal technology applied in the food industry, mainly used to disrupt pathogens and spoilage microorganisms, inactivate enzymes and improve the nutritional and technological quality of food products. HPH has been demonstrated, in comparison with other technologies such as thermal treatments, to be less destructive of food compounds when related to sensory and nutritional properties. HPH can be used in the citrus industry for increasing the yield of citrus juices (Lortkipanidze et al., 1972) and for improving quality factors such as viscosity (Crandall and Davis, 1991; Patrignani et al., 2009), shelf-life (Maresca et al., 2011) and colour (Lee and Coates, 2004). The application of HPH to mandarin juices has been demonstrated to increase the stability of suspension and therefore improve the availability of bioactive compounds with antioxidant activity (Betoret et al., 2012). However, the degradation of those compounds during processing and storage is important. Previous studies have demonstrated that management of processing technologies can have influence on the functional properties of the final products obtained (Betoret et al., 2015; Barba et al., 2015a; Barba et al., 2012, 2015b; Zinoviadou et al., 2015). The addition of ingredients able to interact with food matrix can have a significant influence on bioactive compounds activity, degradation or release. Trehalose is a disaccharide able to maintain and preserve a wide group of biologically active molecules. This effect is due to the establishment of interactions that can contribute to the formation of a barrier able to maintain the integrity of the cellular structures and to prevent the decay during processing operations and/or storage (Colaço and Roser, 1994).

Juices are complex mixtures of macro- and micro- components. In most cases, the process treatment can modulate the entire molecular profile of the juices, beyond the few molecules at the center of attention, with possible unexpected consequences on the overall quality and acceptance. This is particularly important when the studied treatment is known to influence simultaneously several quality aspects, such as microbial spoilage, enzymatic activity or bioactivity. When possible unknown consequences of a treatment are looked for, a non-targeted screening exploration, analysing tens of compounds simultaneously, is highly desirable. In this respect, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy has

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recently gained interest in food and nutritional sectors, due to its ability to give intrinsically quantitative information about the metabolic profile of foodstuff. Being non-destructive and highly reproducible over a wide range of metabolites concentration, ^{1}H NMR is able to analyze hundreds of compounds simultaneously within minutes and with minimal sample preparation (Laghi et al., 2014).

The aim of this work is to study the effect of high pressure homogenization (20 and 100 MPa) and trehalose (10 and 30%) addition, on technological and functional properties of Ortanique citrus fruit low pulp juices (LPJ). With the goal of obtaining a combination of information both on aspects of known interest and on the overall molecular profile of juices, a set of experiments was designed, combining a non-targeted metabolomic investigation based ^{1}H NMR together with suspended pulp and transmittance, hesperidin, vitamin C and antioxidant activity analysis.

2. Material and methods

2.1. Sample preparation and processing

Ortanique fruit, a hybrid of tangerine and sweet orange (*Citrus sinensis* x *Citrus reticulata*) was provided by Rural S. Vicent Ferrer cooperative located in Benaguacil (Valencia), Spain. The preparation of the juices was carried out according to the patent WO/2007/042593 titled "Method of obtaining refrigerated pasteurized citrus juices" (Izquierdo et al., 2007). The fruits were washed by immersing them in tap water, drained, and squeezed in an extractor ("GAM" MOD.SPA 1400 rpm, Cesena, Italy). Raw juice was centrifuged at 3645 g during 5 min at 4 °C (Beckman Coulter AvantiTM J-25, Milan, Italy), homogenized with a Panda Plus pilot homogenizer (Niro Soavi, Parma, Italy) 20 and 100 MPa and no homogenized, pasteurized at 63 °C for 15 s (Roboqbo, Bologna, Italy), collected in sterile jars, and quickly frozen at −18 °C until analyzed. In juice samples with trehalose, an amount of 10 and 30% (w/w) was added before homogenization.

2.2. Physicochemical characterization

Total soluble solids were measured as Brix with a digital refractometer (Pal-1; Atago Co., Ltd., Tokyo, Japan). Total titratable acidity was assessed by titration with 0.1 N NaOH and expressed as the percentage of citric acid. pH was measured with a potentiometer (micropH Crison GLP21). The values provided are the average of three replicates.

2.3. Suspended pulp and transmittance

Suspended pulp was evaluated reading the separated pulp (%) by centrifugation at 3500g during 10 min at 27 °C (FMC FoodTech, 2005). The supernatant was collected and evaluated its transmittance at 650 nm in spectrophotometer (Shimadzu UV-1601). The values provided are the average of six replicates.

2.4. Flavonoid hesperidin

The content of the main flavonoid hesperidin was determined using HPLC LC-1500 (Jasco, Carpi, MO, Italy) with a diode array detector (DAD) and filled with a C18 reversed-phase column (150 × 4.60 mm, Phenomenex Kinetex® 5U C18 100°) following the method described in Betoret et al., 2009. The juice samples were measured after 0, 3 and 10 days of storage. The values provided are the average of three replicates.

2.5. Vitamin C

Vitamin C content was measured by HPLC LC-1500 (Jasco, Carpi, MO, Italy) equipped with thermostat autosampler and diode array detector (DAD).

Fresh juice samples were centrifuged at 15,000 g (4 °C, 5 min) and aliquots (1 mL) of supernatant were filtered with nylon filter 0.45 μm and then 10 μL were injected into the HPLC C18 reverse phase column (150 × 4.60 mm, Phenomenex Kinetex® 5U C18 100°). System conditions were established according to Odriozola-Serrano et al. (2007).

The juice samples were measured after 0, 3 and 10 days of storage. The values provided are the average of three replicates.

2.6. Antiradical activity

The antiradical activity was determined by ABTS and DPPH tests. The ABTS test was based on the method proposed by Polydera et al. (2005). A volume of 15.3 μL juice was added to ABTS solution. The absorbance was measured with a spectrophotometer Beckman Coulter DU 730 Life Science model every 30 s for a total time of 30 min. The results were expressed as TEAC (Trolox Equivalent Antiradical Capacity). The values provided are the average of twelve replicates. The DPPH test was based on the method proposed by Brand-Williams et al. (1995). A volume of 30 μL of juice was added to DPPH solution. The absorbance was measured with a spectrophotometer (Beckman Coulter model DU 730 Life Science) at 515 nm every 2 min for a total time of 70 min. The results were expressed as $mmol \cdot L^{-1}$ equivalents of ascorbic acid. The values provided are the average of twelve replicates.

2.7. Untargeted metabolomics approach

Samples were prepared for analysis, and ^{1}H NMR spectra were registered and processed, according to Dellarosa et al., 2016. Spectra were manually integrated giving rise to 89 protons signals in the typical regions of sugars, amino acids, organic acids, alcohols, polyphenols and nucleotides. At least five replicates were analyzed for each sample group. The obtained 102 × 89 (samples × signals) matrix, scaled and centred, underwent signals assignments and multivariate analysis.

NMR signals assignment was performed by comparison with works performed on similar food matrices at comparable pH (Capitani et al., 2012; de Oliveira et al., 2014; Le Gall et al., 2001; Spraul et al., 2009), assignment through Chenomx software (Chenomx, Alberta, CA) and comparison with HMDB and Madison public databases. In case of unresolved ambiguity, suitable 2D experiments were performed.

To study the changes occurring during the storage period and upon the tested treatments, sparse Partial Least Square Regression (sPLSR) (Lê Cao et al., 2008) and its discriminant analysis counterpart (sPLSDA) (Lê Cao et al., 2011), were performed, as implemented in mixOmics package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Train and test sets accounted for 70% and 30% of the samples respectively. The sPLSR and sPLSDA models were trained by 10-fold validation based on minimal root mean square error (RMSEP) and error rate, respectively. The maximum parsimony of the models was looked for by building and testing 1000 models, and by retaining only the molecules with average VIP value (Variable Importance in Projection) (Eriksson et al., 2001) above one and accepted by sparsity algorithm more than 500 times. The key metabolites arisen from the sPLS models were employed for linear regression and linear discriminant analysis (Ripley, 1996), in order to describe changes during storage and upon HPH treatments. This approach led to