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Effect of high pressure processing and trehalose addition on functional properties of mandarin juice enriched with probiotic microorganisms

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ABSTRACT

This work aimed to determine the effect of homogenization pressures and addition of trehalose on the functional properties of mandarin juice enriched with *Lactobacillus salivarius* spp. *salivarius*. Physicochemical and structural properties of mandarin juice were evaluated and related with quantity and stability of probiotic microorganism as well as with its hydrophobicity. Both food matrix and processing, affected functional properties of *L. salivarius* spp. *salivarius*. Homogenization pressures and trehalose addition affected quantity and stability of probiotic microorganisms during storage. 20 MPa and 20 MPa with 100 g/kg of trehalose allowed obtaining 10^6 colony forming units (CFU)/ml mandarin juice after ten storage days. In MRS growth, cell hydrophobicity increased with homogenization pressures, with values in range 67–98%. Highest cell hydrophobicity was obtained in samples homogenized at 100 MPa. Under stress growth conditions, cell hydrophobicity values were in a range 30–84%. In samples no homogenized, addition of trehalose resulted in an increased values of hydrophobicity, with highest levels in those samples with 100 g/kg of trehalose addition.

1. Introduction

Sustainable food production stands at the intersection of several growing needs. Firstly, the needs of consumers for improved food security and safety, as well as healthy needs. Secondly, the quest for economic sustainability of food production, based on cost reduction and increased product differentiation. Third, the growing concern for reversing the over exploitation of natural resources, waste generation, and the contribution to climate change (Fava et al., 2013).

Functional foods can help to prevent or improve some diseases thus contributing directly to the public health and global sustainability. Specifically, probiotic foods can help to prevent or improve the treatment of digestive system diseases and can suppose an alternative strategy to fight antibiotic excessive uses which produce antibiotic resistances and result in a high cost for the Health European System, waste generation and effluent contamination (Betoret et al., 2016, pp. 149–165). In the development of a probiotic functional food, it is necessary to consider the effect of processing operations on the final product. Foods are mostly complex mixtures of macro and micro components organized in a structure that can trap active compounds, modulating their release or inhibiting their activity (Betoret, Betoret, Rocculi, & Dalla Rosa, 2015). Food matrix, in its raw state or transformed during processing, can have a significant effect on the functionality of bioactive compounds. To choose an appropriate food matrix and technological process, as the key step for the success of a specific functional food, it is necessary to understand the establishment of some interactions between bioactive compounds, cellular structures and technological ingredients that contributing to a "barrier" formation can help to maintain the integrity of bioactive compounds preventing the action of some deterioration factors during processing or storage and ensuring the active compound gaining access to the functional target site in the organism. Structure – property – process relationships approach can help developing probiotic functional foods allowing detecting strengths and weaknesses of the system in order to generate technologically feasible strategies that contribute to the success of a functional food (Betoret et al., 2015).

The objective of this research was to determine the effect of homogenization pressures and addition of trehalose on the functional properties of mandarin juice enriched with *Lactobacillus*

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salivarius spp. *salivarius,* a probiotic microorganism with potential effect against *Helicobacter pylori* infection.

2. Material and methods

2.1. Sample preparation

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, Carbonell, Navarro, & Sendra, 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, California, United States), pasteurized at 63 °C for 15 s (Roboqbo Qb8-3, Bologna, Italy), collected in sterile jars, and quickly frozen at −18 °C until analyzed.

2.2. Mandarin juices with L. salivarius spp. salivarius

To obtain mandarin probiotic juices, 2 ml/l of de Man, Rogosa & Sharpe (MRS) Broth (VWR, Milan, Italy) with 9 log colony forming units (CFU)/ml *L. salivarius* spp. *salivarius* CECT 4063 (Spanish Type Culture Collection, Valencia, Spain) were transferred to mandarin juices following the procedure described by Betoret et al., 2012. After incubation for 24 h at 37 °C, the juices were homogenized with a Panda Plus pilot homogenizer (GEA Niro Soavi Panda PLUS, Parma, Italy) at 0, 20 and 100 MPa. In juice samples with trehalose (Cargill, Milan, Italy), an amount of 100 and 300 g/kg was added before homogenization and incubation steps.

2.3. 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 mol/l NaOH (Sigma Aldrich, Milan, Italy) and expressed as the percentage of citric acid. A potentiometer was used to measure pH (micropH Crison GLP21, Barcelona, Spain). The viscosity was determined by using a portable viscometer (Hydramotion Viscolite 700, York, UK). The values provided are the average of three replicates.

2.4. Suspended pulp and transmittance

Suspended pulp was evaluated by sample centrifugation at 365 × g during 10 min at 27 °C (Amador, 2005). The supernatant was collected and evaluated its transmittance at 650 nm in spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). The values provided are the average of six replicates.

2.5. Characterization of L. salivarius spp. salivarius

Antagonist activity of *L. salivarius* spp. *salivarius* CECT 4063 was evaluated using the methodology described by Siroli et al., 2015. Concretely, 0.5 ml of specific pathogen was inoculated in 10 ml of Brain Heart Infusion (BHI) soft agar (VWR, Milan, Italy) and transferred to the *L. salivarius* spp. *salivarius* petri dish. The antagonist activity was evaluated by the inhibition area created by the probiotic microorganism after incubation at 37 °C for 24 h against pathogens associated with toxic infections or responsible of food degradation (Table 1). The target strain were chosen according to the literature Siroli et al., 2015 and Pisano et al., 2011. The values provided are the average of three replicates.

Bacteriocin production was evaluated by the inhibition area created by the supernatant after centrifugation at 13,000 × g during 3 min at 4 °C (Beckman Coulter AvantiTM J-25, California, United States) of *L. salivarius* spp. *salivarius* CECT 4063 boiled and neutralized, boiled but non-neutralized, filtered and non-neutralized against the food pathogens presented in Table 1. The values provided are the average of three replicates.

2.6. Microorganism counting

Juices homogenized at 0, 20, 100 MPa with 0, 100 and 300 g/kg of trehalose content, *L. salivarius* spp. *salivarius* CECT 4063 were stored during 0, 1, 2, 3, 7, 10 days at 4 °C. Each day, a juice sample was taken and the number of probiotic microorganisms were counted on double layer MRS agar (VWR, Milan, Italy) following incubation for 24 h at 37 °C. The values provided are the average of three replicates.

2.7. Hydrophobicity

L. salivarius spp. *salivarius* CECT 4063 hydrophobicity has been calculated following the methodology proposed by Vinderola and Reinheimer (2003) both in MRS Broth and in mandarin juices homogenized at 0, 20, 100 MPa with 0, 100 and 300 g/kg of trehalose content. Methodology was optimized to eliminate interferences in the measurement without affecting probiotic microorganism growth. The values provided are the average of six replicates.

2.8. Statistical analysis

A multi factorial ANOVA was carried out to determine the significant effect, with 95% confidence level, of the process variables with the software STATISTICA 10.

Table 1
Antagonist activity of *L. salivarius* spp. *salivarius* against most common food pathogenic and spoilage bacteria (Pisano et al., 2011; Siroli et al., 2015). The values provided are the average of three replicates.

		L. salivarius spp. *salivarius* CECT 4063 inhibition
L. monocytogenes	ATCC 13932	++++
L. monocytogenes	SCOTT A	++++
L. innocua	DSM 20649	++++
L. plantarum	V7B3	+
B. cereus	ATCC11966	+++
S. aureus	DSM 20231	+++
E. faecalis	ATCC29212	++
E. faecalis	EF37	+++
E. coli	DSM 18039	++++
E. coli	555	++++
S. enteritidis	E5	++

(no inhibition); + (inhibition 1–3 mm); ++ (inhibition 3–6 mm); +++ (inhibition 6–10 mm); ++++ (inhibition > 10 mm).