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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). 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, Betoret, Rocculi & Dalla Rosa, 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 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 x g during 5 min at 4 °C (Beckman Coulter AvantiTM J-25, California, United States), pasteurized at 63 ºC for 15 s (Roboqbo Qb8-3, Bologna, Italy), collected in sterile jars, and quickly frozen at -18 ºC 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 ºC, 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 ºBrix 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 x g during 10 minutes at 27 ºC (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 ºC 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 13000 x g during 3 min at 4 ºC (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 ºC. 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 ºC. 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 & 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.

## 3. Results and discussion

3.1. Characterization of *L. salivarius* spp. *salivarius*

The strain *L. salivarius* spp. *salivarius* CECT 4063 was chosen due to its demonstrated activity against *Helicobacter pylori* infection and because of the results obtained previously (Betoret *et al*.,

2012). To characterize the strain, both antagonist activity and bacteriocin production were evaluated.

Antagonist activity of *L. salivarius* spp. *salivarius* was evaluated against the most common pathogens responsible of food toxic infections or food degradation (Table 1). *L. salivarius* spp. *salivarius* showed a positive effect against all food pathogens. Antagonist activity was always high with 6-10 mm or >10 mm inhibition halo for most of pathogens with low levels for *L. plantarum*, *E. faecalis* and *S. enteritidis* in which the inhibition halo was 1-6 mm. In order to see if antagonist activity was a result of bacteriocin production, this one was evaluated. No inhibitory activity was detected in the cell supernatant boiled and neutralized, boiled and not neutralized, filtered and neutralized.

3.2 Mandarin juice with *L. salivarius* spp. *salivarius*, physicochemical and structural characterization

Mandarin juices homogenized at 0, 20 and 100 MPa with 0, 100, 300 g/kg of trehalose were characterized by measuring brix, pH, and acidity (Table 2). Trehalose addition had a significant effect ($p \leq 0.05$) on brix, pH and acidity values obtained. Density and viscosity in mandarin juice homogenized at 0, 20, 100 MPa and with trehalose addition in 0, 100, 300 g/kg were determined (Table 2). Trehalose addition had a significant effect ($p \leq 0.05$) on both density and viscosity measurements. It was possible to observe an increase of both parameters with trehalose addition.

Fruit juices suspension contains cellular organelles and membranes, oil droplets, chromoplasts, fragments of cellular wall such as pectin, cellulose and hemicellulose, and functional compounds (Baker & Cameron, 1999). As observed in previous studies, homogenization operation associated to the juices production can have influence on the stability of suspended pulp and thus the functional compounds present in the cloud (Betoret, Betoret, Carbonell & Fito, 2009). Separated pulp by centrifugation at 365 x g and supernatant transmittance were measured in mandarin juice with

probiotic microorganism homogenized at 0, 20, 100 MPa with 0, 100, 300 g/kg trehalose addition (Table 2). As expected, and according to previous studies (Betoret, Betoret, Carbonell & Fito, 2009), separated pulp and transmittance levels decreased as homogenization pressures increased. This effect can be explained taking into account that homogenization pressures decrease particle size of pulp compounds transforming the suspended pulp that tends to precipitate into more stable background pulp (Betoret, Betoret, Carbonell & Fito, 2009) and thus stabilizing cloud particles. In those juices with 100 g/kg trehalose addition it was possible to observe a decrease of separated pulp and transmittance values that it was even bigger in juices with 300 g/kg trehalose addition. This effect could be due to three main reasons: on one hand, samples with trehalose had less quantity of juice and on the other hand trehalose could interact with cloud compounds stabilizing the suspension and maintaining juice cloudiness. Also trehalose addition leads to increased viscosity and therefore an increased resistance against sedimentation and resistance to movement.

### 3.3 Mandarin juice with *L. salivarius* spp. *salivarius*, quantity and stability of probiotic microorganisms

*L. salivarius* spp. *salivarius* growth was determined in juices homogenized at 0, 20, 100 MPa with 0, 100, 300 g/kg trehalose addition after 1, 2, 3, 7 and 10 storage days (Table 3).

The highest growth of probiotic microorganisms was obtained in juices homogenized at 20 MPa. Trehalose addition in 300 g/kg resulted in low levels of microorganism growth, probably due to the osmotic pressure created in the media. However, 100 g/kg of trehalose addition did not show significant differences when compared with juices without trehalose. When considering storage days, juices with 300 g/kg of trehalose addition presented lower microorganism content that remain stable during ten storage days between 5.5 – 3.5 log CFU/ml values. In samples with 100 g/kg of trehalose addition, levels of probiotic microorganism remained constant until third storage day, from which they start decreasing. In no homogenized samples, high levels of probiotic

microorganisms quickly started decreasing with storage days. In samples homogenized at 20 and 100 MPa, there was an increase in probiotic microorganism at second storage day, probably due to the nutrients availability favored by the small sizes of cloud particles, decreasing from third storage day in samples homogenized at 100 MPa. Samples homogenized at 20 MPa had the highest *L. salivarius* spp. *salivarius* content, stable until seventh storage day.

It seems that smaller size cloud particles together with sub-lethal homogenization pressures creates an optimal environment for *L. salivarius* spp. *salivarius* growth. In the same way, cloud stability created by compounds interaction made possible constant preservation of microorganism until seventh storage day. Addition of trehalose without creating an osmotic stress for the microorganism, reinforced juice cloud stability and maintained high microorganism levels until the tenth day of storage.

3.4 Functional properties of *L. salivarius* spp. *salivarius*. Determination of cell hydrophobicity and effect of processing technology

In probiotic microorganisms, cellular hydrophobicity has been related with the strain capacity to adhere and interact with intestine wall (Basson, Craig & Zhang, 2007; Burns et al., 2008). Hydrophobic strains have been described as more cellular and tissue invasive, being able to adhere to the intestine wall thus making possible a successive colonization. Surface hydrophobicity in *L. salivarius* spp. *salivarius* cells was calculated in MRS Broth medium homogenized at 0, 20, 100 MPa with 0, 100, 300 g/kg of trehalose addition (Table 4).

Cell hydrophobicity of *L. salivarius* spp. *salivarius* in MRS Broth showed high results in all samples, with values in range 67 – 98 %. Cell hydrophobicity increased with homogenization pressures. Highest cell hydrophobicity was obtained in samples homogenized at 100 MPa. As observed in previous studies (Patrignani et al., 2009), sub-lethal homogenization pressures can change cellular structure of microorganism facilitating their adhesion to the digestive system. The

effect of trehalose addition was different depending on levels of homogenization pressures applied. In samples no homogenized, the addition of trehalose increased cell hydrophobicity. However, in homogenized samples, trehalose addition decreased cell hydrophobicity. Specifically, in those samples homogenized at 20 MPa, trehalose addition of 100 and 300 g/kg resulted in lower hydrophobicity values, while in samples at 100 MPa there were not significant differences between hydrophobicity values calculated in 100 and 300 g/kg trehalose addition samples.

Cell hydrophobicity of *L. salivarius* spp. *salivarius* incubated in mandarin juices with 0, 100, 300 g/kg trehalose addition and then homogenized at 0, 20 and 100 MPa was calculated (Table 5). Both homogenization pressures and trehalose addition had a significant effect ($p \leq 0.05$) on cell hydrophobicity. Analyzed samples showed values in a range 30 – 84 %. In samples no homogenized, addition of trehalose resulted in an increased values of hydrophobicity, with higher levels in those samples with 100 g/kg of trehalose addition. The tendency of the results was the same in samples homogenized at 20 and 100 MPa, with slightly higher values obtained for 100 MPa homogenization pressures. There were not significant differences in hydrophobicity values obtained in samples homogenized and 100 g/kg of trehalose addition. However, 300 g/kg of trehalose addition resulted in higher levels of cell hydrophobicity.

As explained by Iaconelli et al., (2015) the measurement of bacterial hydrophobicity remains a good indicator to evaluate variation in bacterial surface properties, especially for an identical strain treated with different processes. The hydrophobicity of *L. salivarius* spp. *salivarius* incubated in mandarin juices was lower than that obtained in MRS Broth. The change in cell wall hydrophobicity could be a result of bacterial stress to certain culture conditions, such as low pH, high temperature and hyperosmotic stress (Lopez et al., 2000; Remeta et al., 2002). It seems that in those samples with 300 g/kg of trehalose addition, slightly higher pH and created interactions between trehalose, probiotic microorganisms and juices cloud, protected *L. salivarius* spp. *salivarius* and decreased its stress suffered during growing. Decreasing cloud particles size by homogenization, although could contribute to suspension stability, seemed not improve their

hydrophobicity when they were growth in mandarin juices. High values obtained in samples no homogenized with 100 g/kg trehalose addition could be only explained taking into account the variability in cloud juice particles. Bigger particle sizes and less reactive points, could change the interactions created in the media, protecting better *L. salivarius* spp. *salivarius* from stress conditions. Trehalose is a disaccharide able to interact with various compounds, forming a glassy amorphous matrix around the tertiary structure of the proteins and phospholipids exerting a protective effect on various technological processes (Colaço & Roser, 1994). There are a lot of studies demonstrating the ability of trehalose to interact with probiotic cell surface and showing its protecting effect during drying processes (Crowe, Carpenter, Crowe, & Anchordoguy, 1990). However, there is lack of studies in literature that evaluate the effect of trehalose and juices cloud interactions on hydrophobicity changes in probiotic microorganisms. This effect should be further investigated.

In order to avoid the stress suffered by probiotic microorganisms during incubation in mandarin juices that resulted in microorganism cellular surface changes, cell hydrophobicity of *L. salivarius* spp. *salivarius* incubated in MRS Broth with 0, 100, 300 g/kg of trehalose addition, homogenized at 0, 20 and 100 MPa and then transferred to mandarin juices, was calculated (Table 6). All obtained values were in range 40 – 80 %, higher than those obtained with *L. salivarius* spp. *salivarius* incubated in mandarin juices and lower than those obtained in MRS Broth. It was possible to eliminate partially the microorganism growth stress created by low pH in juices that resulted in microorganism cellular surface hydrophobicity changes, and improve the values obtained. In this case, as in MRS Broth results, homogenization pressures and trehalose addition improved cell hydrophobicity.

## 4. Conclusions

Both food matrix and processing affected functional properties of *L. salivarius* spp. *salivarius*. In an optimal growth media, both homogenization pressures and trehalose addition improved cell

hydrophobicity. Under stress growth conditions, trehalose addition improved cell hydrophobicity and influenced the effects promoted by homogenization pressures. It is necessary to further study trehalose interactions with juices cloud compounds together with microorganism cell surface to understand the mechanisms of action.

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Table 1. Antagonist activity of *L. salivarius* spp. *salivarius* against most common food pathogenic and spoilage bacteria (Siroli et al., 2015; Pisano et al., 2011). 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 | ++ |

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

Table 2. Physicochemical characterization of mandarin juices with *L. salivarius* spp. *salivarius*. Values expressed as mean ± standard deviation. The values provided are the average of three replicates. In the case of separated pulp and transmittance the values provided are the average of six replicates.

| **Pressure MPa** | **Trehalose (g/kg)** | **ºBrix ($g_{soluble\ solids}/g_{liquid\ phase}$)** | **pH** | **Acidity (g/l)** | **Density ($\cdot 10^3$) (g/l)** | **Viscosity ($\cdot 10^{-3}$) (Pa·s)** | **Separated pulp (ml/l)** | **Transmittance (%)** |
|---|---|---|---|---|---|---|---|---|
| **0** | **0** | 13.53 ± 0.06 | 3.87 ± 0.06 | 1.59 ± 0.05 | $1.06 \pm 0.02^{c}$ | $1.57 \pm 0.06^{e}$ | $93.33 \pm 0.06^{ab}$ | $20.1 \pm 1.2^{a}$ |
| **0** | **100** | 21.40 ± 0.12 | 3.97 ± 0.06 | 1.37 ± 0.06 | $1.09 \pm 0.03^{b}$ | $2.23 \pm 0.06^{d}$ | $83.33 \pm 0.06^{c}$ | $17.8 \pm 2.2^{ab}$ |
| **0** | **300** | 36.70 ± 0.12 | 4.30 ± 0.12 | 0.90 ± 0.08 | $1.17 \pm 0.02^{a}$ | $6.10 \pm 0.12^{a}$ | $46.66 \pm 0.06^{d}$ | $7.5 \pm 1.6^{c}$ |
| **20** | **0** | 13.03 ± 0.06 | 3.87 ± 0.06 | 1.53 ± 0.03 | $1.06 \pm 0.02^{c}$ | $1.7 \pm 0.2^{e}$ | $86.66 \pm 0.06^{bc}$ | $16.6 \pm 1.3^{b}$ |
| **20** | **100** | 20.70 ± 0.12 | 3.93 ± 0.06 | 1.39 ± 0.05 | $1.09 \pm 0.02^{b}$ | $2.23 \pm 0.06^{d}$ | $83.33 \pm 0.12^{c}$ | $7.1 \pm 0.8^{c}$ |
| **20** | **300** | 35.8 ± 0.2 | 4.17 ± 0.06 | 0.9 ± 0.2 | $1.16 \pm 0.04^{a}$ | $4.7 \pm 0.2^{c}$ | $2.00 \pm 0.02^{e}$ | $6.7 \pm 0.3^{c}$ |
| **100** | **0** | 13.2 ± 0.2 | 3.8 ± 0.02 | 1.5 ± 0.2 | $1.05 \pm 0.03^{c}$ | $1.67 \pm 0.06^{e}$ | $10.00 \pm 0.02^{a}$ | $9.0 \pm 2.7^{c}$ |
| **100** | **100** | 20.90 ± 0.12 | 3.9 ± 0.02 | 1.4 ± 0.2 | $1.084 \pm 0.013^{b}$ | $2.2 \pm 0.2^{d}$ | $8.00 \pm 0.02^{c}$ | $5.37 \pm 0.08^{d}$ |
| **100** | **300** | 34.6 ± 0.4 | 4.2 ± 0.02 | 0.9 ± 0.2 | $1.16 \pm 0.02^{a}$ | $5.0 \pm 0.2^{b}$ | $2.00 \pm 0.02^{e}$ | $1.8 \pm 0.2^{e}$ |

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 3. *L. salivarius* spp. *salivarius* content in mandarin juices during storage (log CFU/ml). Values expressed as mean ± standard deviation. The values provided are the average of three replicates.

| **Pressure** | **Trehalose** | **Storage days** | | | | |
|---|---|---|---|---|---|---|
| **MPa** | **g/kg** | **1** | **2** | **3** | **7** | **10** |
| **0** | **0** | $8.10 \pm 0.02^{abc}$ | $7.8 \pm 0.2^{b}$ | $7.09 \pm 0.06^{bcd}$ | $5.84 \pm 0.09^{d}$ | $5.2 \pm 0.2^{d}$ |
| **0** | **100** | $7.7 \pm 0.4^{cd}$ | $8.54 \pm 0.09^{a}$ | $7.5 \pm 0.09^{ac}$ | $7.6 \pm 0.02^{b}$ | $6.29 \pm 0.02^{b}$ |
| **0** | **300** | $4.7 \pm 0.2^{f}$ | $4.0 \pm 0.2^{c}$ | $4.5 \pm 0.02^{e}$ | $3.5 \pm 0.2^{f}$ | $3.54 \pm 0.09^{f}$ |
| **20** | **0** | $8.39 \pm 0.04^{ab}$ | $8.58 \pm 0.04^{a}$ | $8.5 \pm 0.9^{a}$ | $8.45 \pm 0.05^{a}$ | $6.77 \pm 0.12^{a}$ |
| **20** | **100** | $8.19 \pm 0.02^{b}$ | $8.22 \pm 0.12^{ab}$ | $8.25 \pm 0.12^{ad}$ | $7.8 \pm 0.3^{b}$ | $6.8 \pm 0.3^{a}$ |
| **20** | **300** | $5.3 \pm 0.2^{e}$ | $4.3 \pm 0.5^{c}$ | $4.6 \pm 0.8^{e}$ | $4.8 \pm 0.2^{e}$ | $4.98 \pm 0.03^{d}$ |
| **100** | **0** | $7.46 \pm 0.12^{d}$ | $8.6 \pm 0.2^{a}$ | $8.24 \pm 0.02^{a}$ | $7.6 \pm 0.2^{b}$ | $5.80 \pm 0.14^{c}$ |
| **100** | **100** | $7.8 \pm 0.2^{bd}$ | $7.8 \pm 0.02^{b}$ | $7.8 \pm 0.3^{ab}$ | $6.81 \pm 0.05^{c}$ | $5.5 \pm 0.3^{cd}$ |
| **100** | **300** | $4.7 \pm 0.2^{f}$ | $4.5 \pm 0.02^{c}$ | $4.39 \pm 0.12^{e}$ | $4.7 \pm 0.3^{e}$ | $4.2 \pm 0.2^{e}$ |

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 4. Cell hydrophobicity (%) of *L. salivarius* spp. *salivarius* incubated in MRS Broth. Values expressed as mean ± standard deviation. The values provided are the average of six replicates

| **Pressure** | **Trehalose** | **Cell hydrophobicity** |
|---|---|---|
| **MPa** | **g/kg** | **(%)** |
| **0** | **0** | $66.9 \pm 9.6^{gh}$ |
| **0** | **100** | $90.4 \pm 2.7^{ac}$ |
| **0** | **300** | $80.0 \pm 11.5^{de}$ |
| **20** | **0** | $94.3 \pm 0{,}8^{ab}$ |
| **20** | **100** | $84.8 \pm 6.9^{bcd}$ |
| **20** | **300** | $73.0 \pm 3.9^{efg}$ |
| **100** | **0** | $98.2 \pm 1.9^{efh}$ |
| **100** | **100** | $71.9 \pm 12.2^{df}$ |
| **100** | **300** | $79.40 \pm 1.12^{a}$ |

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 5. Cell hydrophobicity (%) of *L. salivarius* spp. *salivarius* incubated in mandarin juices. Values expressed as mean ± standard deviation. The values provided are the average of six replicates

| Pressure MPa | Trehalose g/kg | Cell hydrophobicity (%) |
|---|---|---|
| **0** | **0** | $32.5 \pm 7.9^{ef}$ |
| **0** | **100** | $86.3 \pm 4.6^{a}$ |
| **0** | **300** | $55.2 \pm 4.3^{d}$ |
| **20** | **0** | $27.3 \pm 1.5^{f}$ |
| **20** | **100** | $27.3 \pm 3.5^{f}$ |
| **20** | **300** | $63.4 \pm 5.8^{c}$ |
| **100** | **0** | $37.3 \pm 6.2^{e}$ |
| **100** | **100** | $35.3 \pm 6.2^{e}$ |
| **100** | **300** | $73.6 \pm 7.3^{b}$ |

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)

Table 6. Cell hydrophobicity (%) of *L. salivarius spp. salivarius* incubated in MRS Broth and transferred to mandarin juices. Values expressed as mean ± standard deviation. The values provided are the average of six replicates

| Pressure MPa | Trehalose g/kg | Cell hydrophobicity (%) |
|---|---|---|
| **0** | **0** | $40.1 \pm 6.9^{c}$ |
| **0** | **100** | $72.1 \pm 9.2^{a}$ |
| **0** | **300** | $55.3 \pm 3.4^{b}$ |
| **20** | **0** | $42.9 \pm 3.9^{c}$ |
| **20** | **100** | $72.1 \pm 0.3^{a}$ |
| **20** | **300** | $77.0 \pm 9.2^{a}$ |
| **100** | **0** | $57.2 \pm 11.8^{ab}$ |
| **100** | **100** | $52.9 \pm 7.6^{b}$ |
| **100** | **300** | $55.9 \pm 8.2^{b}$ |

* Values with different superscript letters in a column are significantly different ($p \leq 0.05$)