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Production of Shelf-Stable Annurca Apple Juice with Pulp by High Pressure Homogenization

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Abstract

Annurca apple juice was processed by high pressure homogenization (HPH) to inactivate the endogenous microbial flora, with the aim of maintaining the organoleptic properties and the polyphenolic content of the fresh juice. In particular, it was shown that the shelf life of clear juice can be prolonged for many weeks of storage both at 4°C and 37°C upon HPH treatment at 250 MPa. Instead, the juice with pulp required higher pressure levels (300 MPa) for microbial stabilization both at 4°C and 37°C. The sample stored at 37°C exhibited a physical instability, with deviation in color and pH, which reduced the shelf life, but the samples stored at 4°C exhibited an excellent microbial stability and no observable variation of pH and color, suggesting that HPH can represent a good option for non thermal pasteurization of Annurca apple juice.

Interestingly, the HPH treatment delivered also significant changes to the distribution of suspended particles, whose comminution had a measurable effect on viscosity.

KEYWORDS: high pressure homogenization, mild pasteurization technologies, Annurca apple juice

1. INTRODUCTION

In the recent years, high pressure homogenization (HPH) was proposed as novel technology of non-thermal sanitization of liquid foods, due to its ability to mechanically disrupt microbial cells. The core of the HPH unit is its specifically designed adjustable nozzle where the fluid, pressurized up to 350 MPa, is subjected to intense fluid-mechanical stresses (cavitation, turbulence, elongational and shear stresses) upon pressure drop.

Many studies were conducted on microbial suspensions in water and buffers, which are excellently reviewed by Diels and Michiels (2006), even though the mechanisms of inactivation are not yet completely clear (Kleinig and Middelberg, 1996 and 1996). Recently we also focused on this aspects, trying to correlate the kinetics of inactivation with the fluid dynamic stress of the microorganisms under homogenization (Donsì et al., 2009).

On the other side, real food applications mainly focused on milk and milk-based products, due to the possibility of attaining at the same time the reduction of the microbial flora and the disruption of the fat globules, and hence conjugating in a single machine the traditional homogenization and pasteurization units (Paquin, 1999; Bury et al., 2001; Geciova et al., 2002; Vachon et al., 2002; Thiebaud et al., 2003).

Recently, a growing interest towards natural and minimally processed products stimulated some research also towards fruit juices, where HPH can contribute to the pasteurization process under mild conditions, to obtain a shelf-stable product, resembling in taste and appearance the freshly squeezed juice.

Already in 1993, a first patent was issued for processing of fresh squeezed juices by HPH at pressure levels around 100 MPa, claiming an increase of shelf life and a decrease of microbiological activity in the homogenized juice, and stability of flavor and palatability for 40 days at a storage temperature of 4°C, compared to juice homogenized under conventional pressures, while untreated juice deteriorated in 20 days (Clark et al., 1993).

Some recent works were devoted to investigate the inactivation of specific microorganisms inoculated into orange juice. For instance, Briñez *et al*. (Briñez et al., 2006a, 2006b and 2007) investigated the inactivation of *Escherichia coli*, *Listeria innocua* and *Staphylococcus* spp., after a two-stage HPH treatment in a dual valve Stansted Power fluids machine (2008) at a pressure of 300 MPa in first valve and 30 MPa in second valve (inlet temperature of 6°C and 20°C). The authors also followed the evolution of the residual population during storage at 4°C. The treatment reduced the population of *E. coli* from 7 log cfu/ml to ~4 log cfu/ml (Briñez et al., 2006b), while *L. innocua* was reduced by ~3 log units (Briñez et al., 2006a), and *S. aureus* and *S. carnosus* by ~3 log units (Briñez et al., 2007). The survival population from all microorganisms constantly decreased during storage at 4°C due to the acid conditions of the juice.

Campos and Cristianini studied the inactivation by HPH in a Stansted Power fluids machine (2008) of common contaminants of acid juices, such as *Saccharomyces cerevisiae* and *Lactobacillus plantarum*, inoculated in orange juice. They reported complete inactivation of the two microorganisms already at a pressure level of 250 MPa, hence suggesting the HPH technique as a viable option for juice pasteurization (Campos and Cristianini, 2007).

Tahiri et al. (2006) showed that at least 5 passes of treatment at 200 MPa and at 25°C were required to inactivate pathogenic and spoilage microflora in orange juice, by HPH in an Emulsiflex machine (Avestin, 2008), achieving a reduction of 2.3 log units for *Lactobacillus plantarum*, of 1.6 log units for *Leuconostoc mesenteroides*, of 2.5 log units for *Saccharomyces cerevisiae*, of 4 log units for *Penicillium ssp*., and of 6 log units for *Escherichia coli*.

We investigated the effect of multi-pass homogenization in a bench-scale Stansted Fluid Power machine (2008), up to 250 MPa on *E. coli* and *Lactobacillus delbrueckii*, and on the basis of the attained results, a pasteurization treatment (150 MPa and 3 passes) was proposed for Annurca apple juice, which maintained a satisfactory microbial stability and the organoleptic properties over 14 days of refrigerated storage (4°C), with negligible changes of pH, Brix%, Vitamin C values and color attributes (Donsì et al., 2006).

The aim of the present work is the deeper investigation of HPH as a technique for fruit juices pasteurization in a pilot scale homogenizer, for the production of a shelf-stable juice. In fact, together with the interest in minimally processed juices, there is an increasing trends towards juices containing pulp, which are perceived by consumers as more natural. We tried to apply HPH to the pasteurization of juice with pulp of Annurca apple, an apple grown in Campania Region and very rich in polyphenolic compounds, with recognized antioxidant activity (Fini et al., 2007; D'Abrosca et al., 2006; D’Angelo et al., 2007).

2. MATERIALS AND METHODS

2.1. Annurca apple juice

The juice was obtained from biological apples of Annurca cultivar from Campania Region. The apples were sorted, washed thoroughly, cut in 4 pieces, and immerged in a chemical bath with 0.2%wt ascorbic acid and 1%wt citric acid to inhibit browning reactions.

The apples were then reduced to pulp, blanched and sieved (1 mm mesh) to remove fibrous material.

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To produce the apple juice, the puree was mixed with water (50:50), sugar was added to a final concentration of 15 g/l.

Clarified juice was produced by centrifugation at 9000 rpm for 20 min.

2.2. High Pressure Homogenization

The juice, either clarified or with pulp, was processed by HPH at different pressure levels (between 150 and 300 MPa) in a Stansted Power Fluids (2008) pilot plant with a flow rate of 120 l/h. The plant consists of two intensifiers, for generating an almost continuous flow rate of 120 l/h through the homogenizer stage. The homogenizer stage is made of two disruption valves, where the pressure drop takes place. In the primary valve, the main pressure drop occurs, from the operating pressure down to 40 MPa, while in the secondary valve, the residual drop from 40 MPa to atmospheric pressure takes place. In a previous work, we showed that splitting the pressure drop among the two valves, rather than concentrating it in the primary one, significantly enhanced the disruption of *Escherichia coli* suspensions (Donsì et al., 2009). After the second valve, the fluid goes through a water-cooled heat exchanger, where the process medium, heated up to high temperatures during the homogenization (with a rate of 0.18°C/MPa), is rapidly cooled below 30°C. Batches of at least 2 liters were treated for each condition.

2.3. Characterization

2.3.1. Microbial analysis

Total microbial count in unprocessed and processed samples was conducted by plating on PCA from Oxoid and incubating at 32°C for 48 h or 72 h.

2.3.2. Physical properties

Color evolution was studied by colorimetric analysis, evaluating lightness L* and color coordinates a* and b* (+a* is the red direction, -a* is the green direction, +b* is the yellow direction, -b* is the blue direction). pH and %Brix were also used to characterize the juice.

Particle size distribution by static light scattering (Mastersizer 2000) and viscosity as a function of temperature (AR2000 rheometer) were used to characterize the physical changes delivered to the juice by HPH treatment.

2.3.3. Shelf life

The shelf life of the product was evaluated on control and processed samples stored in 50 ml sterile tubes at 4°C (refrigerated shelf life) or at 37°C (accelerated shelf life). Together with microbial stability, the evolution of pH, %Brix and color was monitored over time.

All experiments were conducted in duplicate.

3. RESULTS

3.1. Microbial inactivation

The effect of HPH of different intensity on the endogenous microbial population of clear juice and of juice with pulp (whose properties are reported in Table 1) was evaluated by varying the level of homogenization pressure and number of passes through the homogenizer.

Table 1. Properties of the Annurca apple juice (clear juice and juice with pulp).

	Clear Juice	Juice with pulp
Ph	3.5	3.5
%Brix	7.5	6.7
Solid fraction (g/g)	0	0.07
Initial total microbial count (cfu/ml)	$4.0 \cdot 10^2$	$4.5 \cdot 10^3$

The initial microbial population of the clear juice and of the juice with pulp is different due to the centrifugation process, that separates a significant microbial fraction ($\sim 4.1 \cdot 10^3$). For this reason, pressure levels required for complete inactivation in clear juice are lower than in juice with pulp (Figure 1).

Indeed, already after a 200 MPa treatment, the survival population in clear juice attains values of concentration that are proximate to the detection limit of the plate-count method (1 cfu/ml). In accordance, the increase of pressure level to 250 MPa does not further increase the lethality in the clear juice, as shown in Figure 1.

Differently, for the juice with pulp an almost linear dependence of the log units of inactivation with respect to process pressure can be observed, with the lowest value (10 cfu/ml), attained at the highest applied pressure, that did not reach the detection limit (Figure 1).

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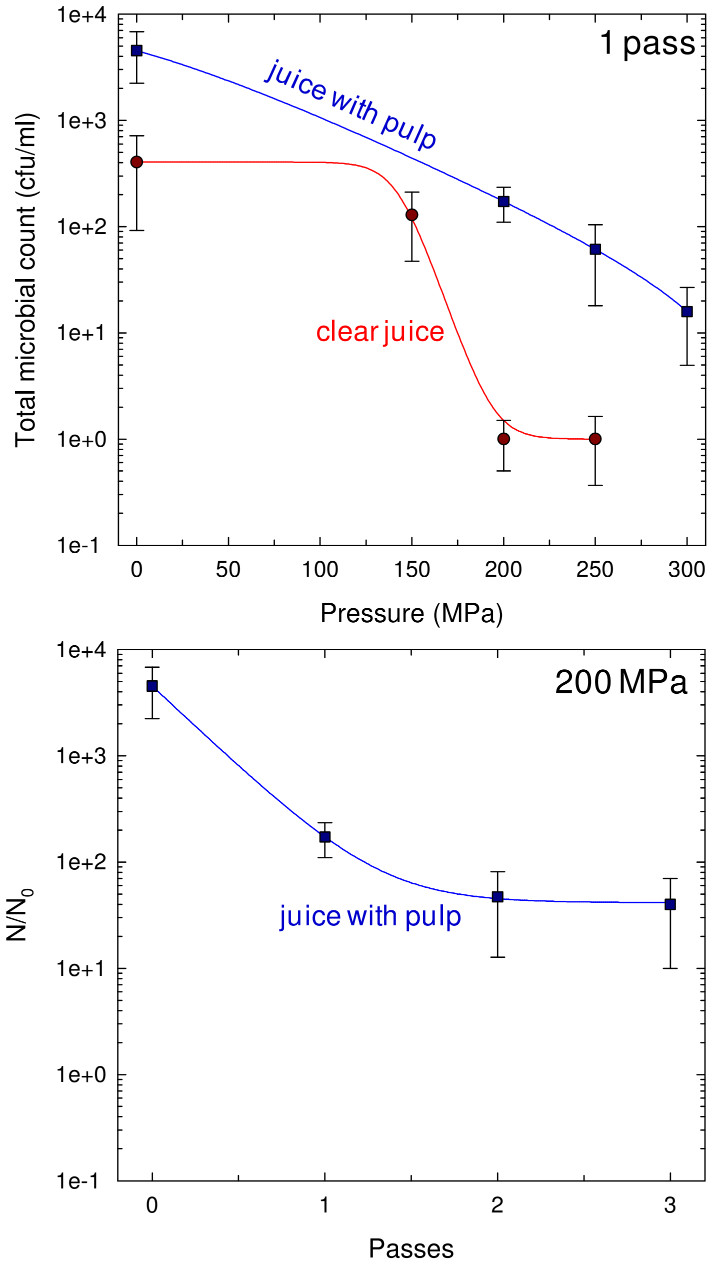

Figure 1. Effect of pressure and number of passes on inactivation of total microbial flora in juice with pulp and in clarified juice.

Noticeably, if the survival fraction is considered (the ratio N/N_0 between the survival and the initial populations), in both juices, at most 2 log cycles of reduction can be achieved, even though this value is attained already at 200 MPa for the clear juice, while a significant higher pressure (300 MPa) is required for the juice with pulp. The differences can be ascribed to the protective role exerted by the pulp on microorganisms, which prevent the main disruptive effects, such as elongational and shear stresses, responsible for bacterial inactivation.

In the case of multiple passes through the homogenizer, Figure 1 shows that the effect of any additional pass is not additive, but a reduction of efficiency can be observed. Indeed, the inactivation curve as a function of the number of passes exhibits a clear asymptote at about 10 cfu/ml. The second pass has a very low impact on residual microorganisms, with a reduction of 0.5 log cycles, while the additive disruption due to the third pass is almost negligible. In accordance with the results reported in a previous work (Donsì et al., 2009), the reduced efficiency of incremental passes can be attributed to the distribution of individual cell resistance to HPH treatment, with a selection of the more resistant cells after each pass.

3.2. Clear juice

3.2.1. Shelf life

Clear juice was produced by centrifugation of the juice with pulp, but this treatment was not sufficient to completely remove the microbial load. The residual microbial population after centrifugation is indeed of about $4.0 \cdot 10^2$ cfu/ml (Table 1), which cannot warrantee a very long shelf life of the product. As shown in Figure 2, the microbial population in the control increase very rapidly and after 2 weeks of storage at 4°C reaches already 10^4 cfu/ml. The control samples stored at 37°C exhibited a significantly faster microbial growth rate, making the juice stable for less than a week.

Figure 1 showed a dependence of the attained inactivation on the severity of the treatment, that can be also found in the shelf life of the product. Results of shelf life shows that, while 150 MPa cannot reduce the population below what was considered the spoilage threshold of the product (100 cfu/ml), there is an evident difference between treatment at 200 and at 250 MPa, even though the measured level of inactivation is the same (1 cfu/ml). The difference is particularly evident in the accelerated shelf life (storage at 37°C), where the product treated at 250 MPa is stable for more than 6 weeks. The refrigerated storage slowed down significantly the growth rate of the microorganisms, but also in this case the shelf life of product treated at 250 MPa is prolonged for more than 60 days, while 200 MPa can assure less than 4 weeks of stability.

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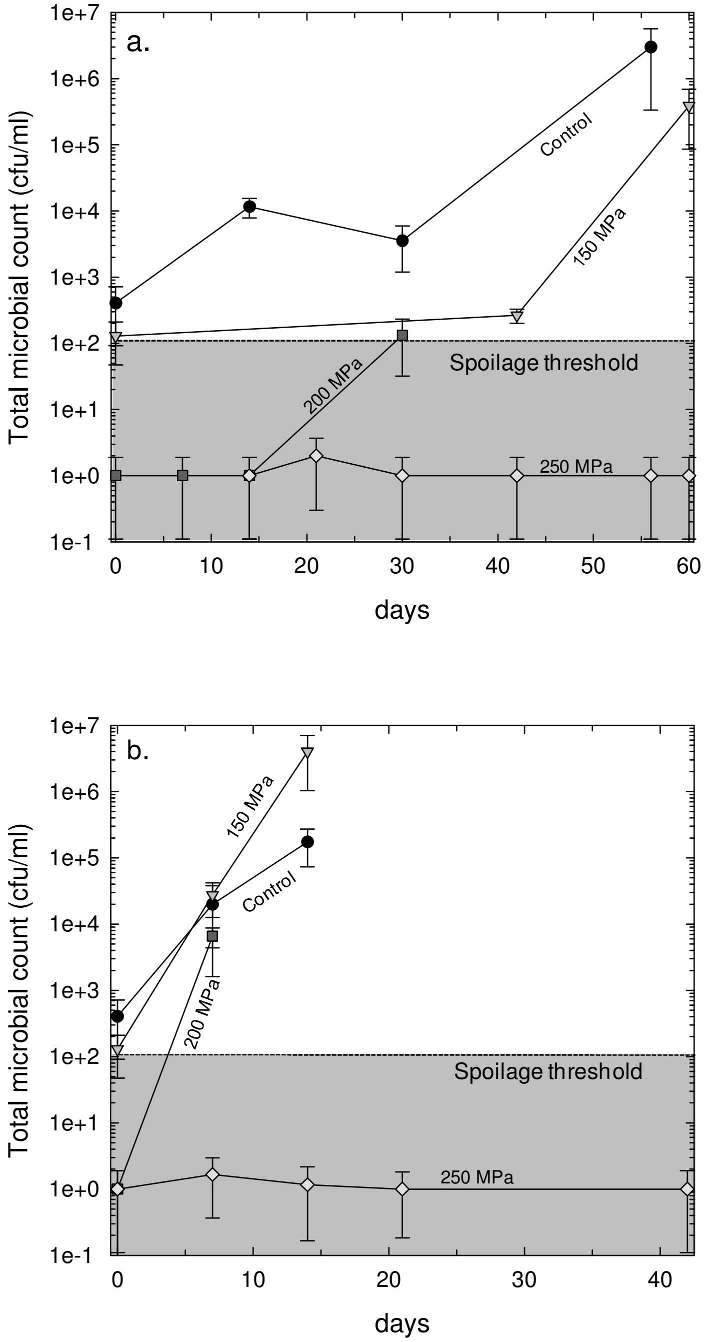

Figure 2. Evolution of total microbial population in clear juice before and after HPH treatment at different pressure levels and number of passes, upon storage at 4°C (a) and 37°C (b)**.**

3.3. Juice with pulp

3.3.1. Shelf life

Independently on pressure level and number of passes, the only treatment, among those investigated for juice with pulp, capable to stabilize the product for long times is the treatment at 300 MPa (single pass), as clearly shown in Figure 3. In fact, even though multiple passes (> 2) at 200 MPa and a single pass at 250 MPa can reduce the initial microbial load below the spoilage threshold, after only one week the microbial population of the samples increased over 10^4 cfu/ml in most of the cases. In particular, independently on the severity of the treatment, all samples exhibited a rapid microbial growth, with the exception of the sample treated at 300 MPa, where the juice was physically and microbiologically stable for 3 weeks at 37°C and for 9 weeks under refrigerated conditions. Moreover, the sample stored at 37°C exhibited some browning after 3 weeks of storage, even though it was microbiologically stable, while the refrigerated sample did not exhibit any browning.

This is evident in Figure 4, where the color analysis highlights that the samples stored at 37°C all exhibited a strong deviation of the color, with browning after only 3 weeks, as shown by the reduction in lightness (L*) and the increase of red (a*) and yellow (b*) proportion.

The color deviation can be attributed to the intense enzymatic activity at 37°C, that had consequences on the color parameters of the juice.

Figure 4 also shows that pH did not change over storage and with respect to the fresh juice (control at day 0) under refrigerated conditions, while a small decrease (from 3.5 to 3.2) can be observed for the HPH processed juice when stored at 37°C. Conversely, in the control juice, when stored at 37°C, due to intense microbial activity, the pH level rapidly decreased to 2.6 in less than a week.

Interestingly for samples stored at 4°C, no significant color deviation was observed with respect to control and over storage time, suggesting that no reduction occurred in the polyphenol content after the HPH treatment.

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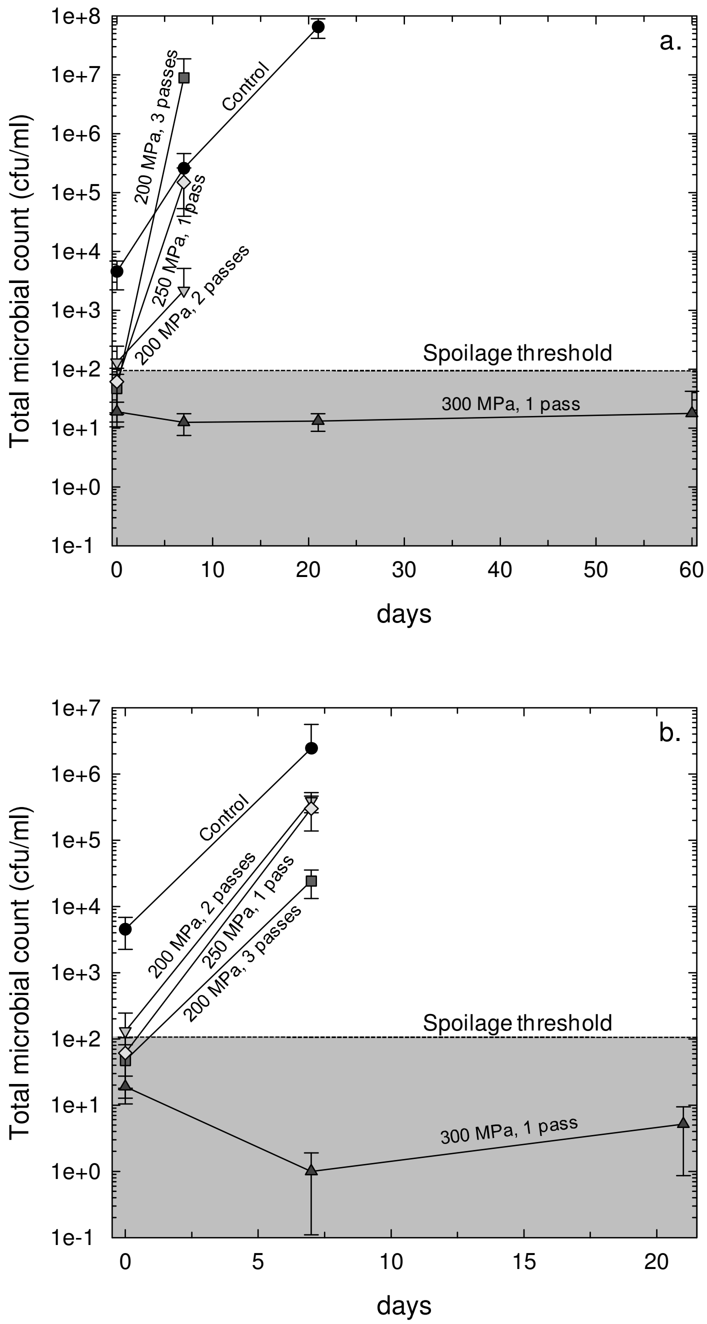

Figure 3. Evolution of total microbial population in juice with pulp before and after HPH treatment at different pressure levels and number of passes, upon storage at 4°C (a) and 37°C (b).

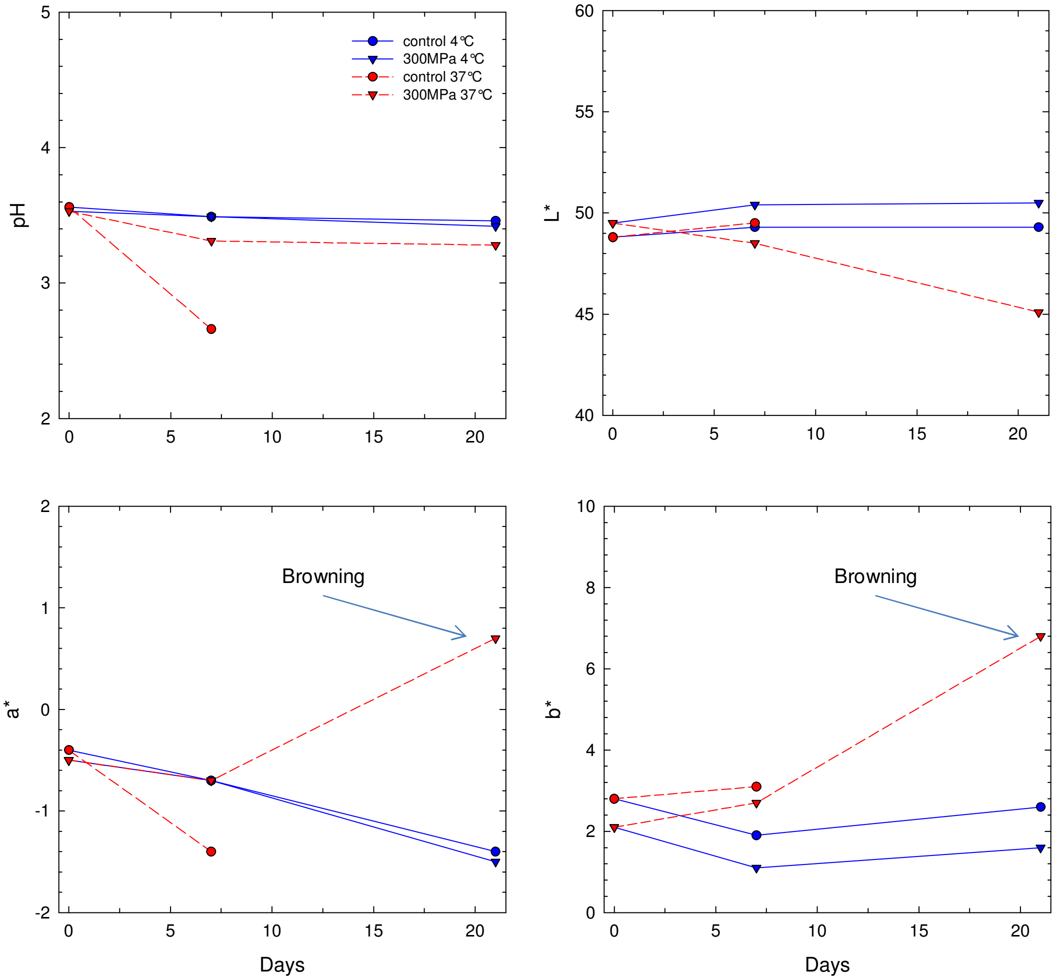

Figure 4. Evolution of pH and color, expressed as L*, a* and b*, for juice with pulp before and after HPH treatment at 300 MPa, upon storage at 4°C and 37°C.

3.3.2. Physical changes

Homogenization of orange juice to reduce the pulp mean particle size and the viscosity of orange juice concentrate is a well known technique (Powers et al., 1988; Grant, 1989), consisting of exposing the orange juice to high shear in a high pressure homogenizer, at pressures up to 50 MPa. The high pressure differential between the inlet of the homogenizer valve and the outlet results in high shear and cavitation, which may alter the size and the properties of the suspended and insoluble pulp in the juice.

Homogenization at higher pressure has a very strong effect on particle size distribution. Figure 5 shows that the first pass at 200 MPa generates a bimodal distribution, with 1 peak centered around 400 μm, as in the untreated juice, and the second peak centered around 40 μm. The second pass at 200 MPa is able to disrupt all residual big particles (above 100 μm), generating a distribution centered around 20 μm. An additional pass at 200 MPa does not cause any noticeable reduction in particle size. The HPH treatment carried out at 300 MPa is able to cause in a single pass the same distribution attained after 2 passes at 200 MPa.

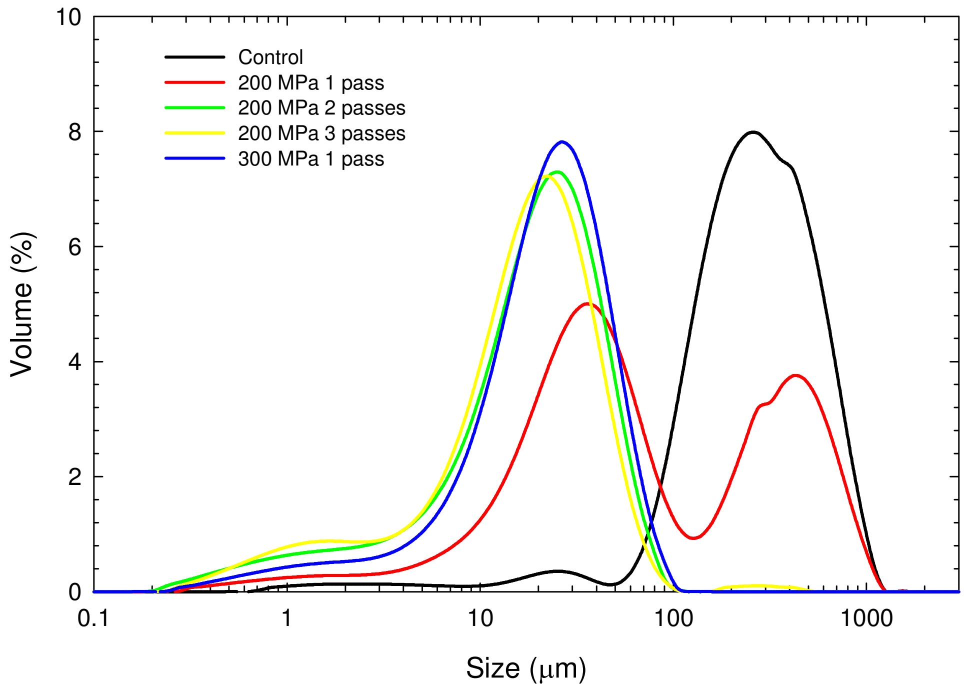

Figure 5. Effect of pressure and number of passes on particle size distribution of juice with pulp.

As a consequence of the reduction of particle size distribution, also the viscosity of the juice is influenced by the HPH treatment.

Shear stress vs shear rate plots exhibited predominant Newtonian behaviour for all the considered samples, over investigated temperatures. Hence, in Figure 6 the Newtonian viscosity of the treated and untreated juice is reported as a function of the temperature.

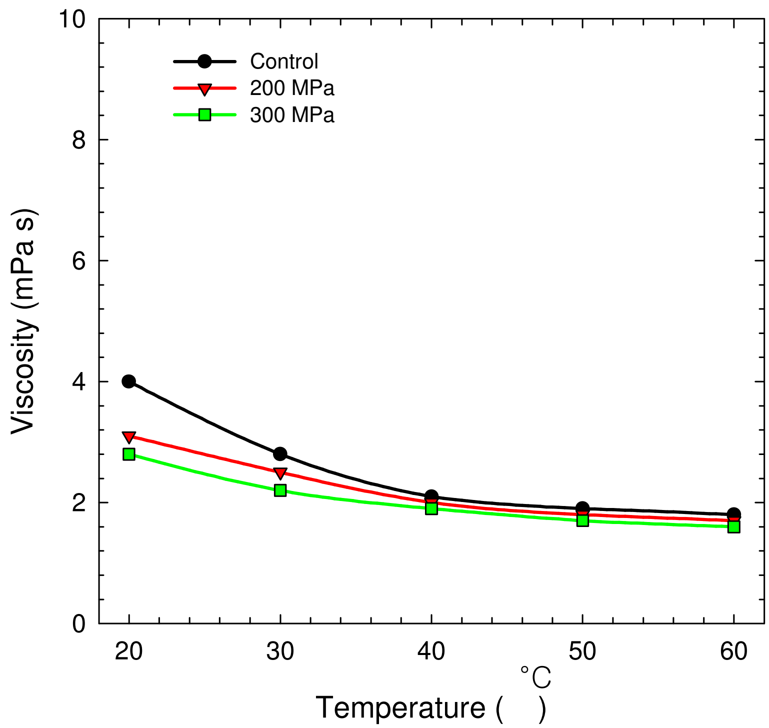

Figure 6. Effect of pressure level of the HPH treatment on viscosity of juice with pulp.

HPH processed samples (at 200 MPa and 300 MPa) are characterized by lower viscosity with respect to non processed ones, with lower viscosity at higher pressure of homogenization, and with the difference in viscosity becoming smaller at increasing temperature.

The application of HPH to the juice with pulp, in addition to the reduction of the microbial load can hence affect also the structure of the system, decreasing the viscosity while maintaining the pulp fraction through the decrease of the particle size of the suspended particles.

4. CONCLUSIONS

High pressure homogenization was implemented in the production line of Annurca apple juice, for the inactivation of endogenous microbial flora, maintaining the properties of the fresh juice, and improving the texture by comminution of the suspended particles. In particular, it was shown that the shelf life of clear juice can be prolonged for many weeks of storage both at 4 and 37°C

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upon HPH treatment at 250 MPa. Instead, the juice with pulp required higher pressure levels (300 MPa) for microbial stabilization both at 4°C and 37°C. It was observed that the sample stored at 37°C exhibited a physical instability, with deviation in color and pH, which reduced the shelf life under these conditions, and hence refrigerated storage is required to preserve the organoleptic characteristics and the nutritive properties, such as the polyphenolic content.

Interestingly, it was observed that the HPH treatment also delivered significant changes to the distribution of suspended particles, whose comminution has a measurable effect on viscosity.

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