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Effect of high pressure homogenization (HPH) on the physical stability of tomato juice

Mirian Tiaki Kaneiwa Kubo [a], Pedro E.D. Augusto [a,b,*], Marcelo Cristianini [a]

[a] Department of Food Technology (DTA), School of Food Engineering (FEA), University of Campinas (UNICAMP), Brazil
[b] Technical School of Campinas (COTUCA), University of Campinas (UNICAMP), Brazil

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ABSTRACT

High pressure homogenization (HPH) is a non-thermal technology that has been widely studied as a partial or total substitute for thermal food processing. Although the aspect of microbial inactivation has been widely studied, there are only a few works in the literature dealing with the physical–chemical changes in fruit products due to HPH, especially regarding its rheological properties. The present work evaluated the effect of HPH (up to 100 MPa) on the physical stability of tomato juice. HPH changed the tomato juice particle size distribution (PSD), pulp sedimentation behavior, serum cloudiness (turbidity), color and microstructure, by disrupting the suspended pulp particles. It therefore increased juice stability to sedimentation and changed its color due to leakage of lycopene from the disrupted cells. The effect of homogenization pressure on the physical properties of the juice followed an asymptotic behavior. The results indicated that the HPH could be used as a valuable tool to promote desirable physical property changes in food products, such as increasing the consistency and reducing particle sedimentation and serum separation, hence improving sensory acceptance.

1. Introduction

High pressure homogenization (HPH) technology consists of pumping a fluid through a narrow gap valve using high pressure intensifiers, which greatly increases its velocity resulting in depressurization with consequent cavitation and high shear stress. Thus the particles, cells and macromolecules suspended in the fluid are subjected to high mechanical stress, becoming twisted and deformed (Floury, Bellettre, Legrand, & Desrumaux, 2004; Pinho, Franchi, Augusto, & Cristianini, 2011). Several studies have evaluated the use of HPH for microbial inactivation in fruit products. The use of HPH as a partial or total substitute for thermal food processing has been studied for tomato (Corbo, Bevilacqua, Campaniello, Ciccarone, & Sinigaglia, 2010), apple (Donsì, Esposito, Lenza, Senatore, & Ferrari, 2009; Pathanibul, Taylor, Davidson, & Harte, 2009; Saldo, Suárez-Jacobo, Gervilla, Guamis, & Roig-Sagués, 2009), mango (Tribst, Franchi, Cristianini, & Massaguer, 2009, 2011), açaí (Aliberti, 2009), orange (Campos & Cristianini, 2007; Tahiri, Makhlouf, Paquin, & Fliss, 2006), carrot (Pathanibul et al., 2009; Patrignani, Vannini, Kamdem, Lanciotti, & Guerzoni, 2009, 2010) and apricot (Patrignani et al., 2009, 2010) juices.

However, although microbial inactivation has been widely studied, there are only a few works in the literature dealing with the physical–chemical changes in fruit products due to HPH processing. Moreover, further than a preservation technique, the HPH technology has been recently proposed as an interesting unit operation in order to improve food and food component properties.

The effect of HPH on the color of banana juice (Calligaris, Foschia, Bastolomeoli, Maifreni, & Manzocco, 2012), serum cloudiness (turbidity), pulp sedimentation and the microstructure of pineapple pulp (Silva et al., 2010), and carrot, broccoli and tomato dispersions and emulsions (Lopez-Sanchez, Nijsse, et al., 2011; Lopez-Sanchez, Svelander, Bialek, Schummm, & Langton, 2011) has been studied. Although tomato juice plays an important role in food consumption nowadays, to date the effect of HPH on its physical stability has not been published.

Tomato is one of the most popular and widely grown vegetables in the world (Nisha, Singhal, & Pandit, 2011). It is one of the most important vegetables in the food industry and is widely included in the human diet. Homogenization is a commonly used unit operation in tomato processing, and moreover HPH has been proposed for use as a valuable tool to promote desirable changes in the physical properties of food products (Augusto, Ibarz, & Cristianini, 2012b).

The effect of HPH on the rheological properties of tomato juice was recently studied. HPH processing decreased the viscosity of the juice serum (Augusto, Ibarz, & Cristianini, 2012a) and increased the consistency, thixotropy, viscous and elastic behavior of the tomato juice (Augusto et al., 2012b; Augusto, Ibarz, & Cristianini, 2013). A rheological analysis indicated that this technology could be used to increase the consistency of tomato juice, improving its sensory

* Corresponding author at: COTUCA, UNICAMP, R. Culto à Ciência, 177, Botafogo, CEP: 13020-060, Campinas, SP, Brazil. Tel.: +55 1935219950.
E-mail address: pedro@cotuca.unicamp.br (P.E.D. Augusto).

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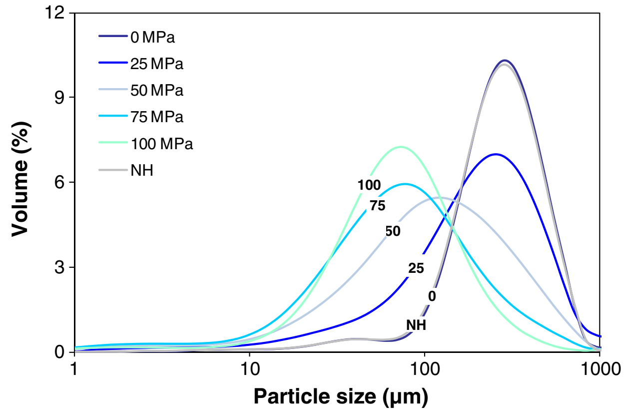

Fig. 1. Effect of HPH (0–100 MPa; NH = non-homogenized) on the tomato juice particle size distribution (PSD).

acceptance, reducing the need for adding hydrocolloids and reducing particle sedimentation and serum separation.

Therefore, the present work evaluated the effect of high pressure homogenization (HPH) on the physical stability of tomato juice, evaluating the particle size distribution (PSD), pulp sedimentation, serum cloudiness, juice color and microstructure.

2. Materials and Methods

2.1. Tomato juice

As described by Augusto et al. (2012b), a 4.9 °Bx tomato juice was obtained by diluting a commercial 30 °Bx pulp in distilled water. The commercial pulp was used to guarantee standardization and repeatability. It was obtained using the hot break process, concentrated by evaporation at 65 °C, thermally processed by the UHT method and aseptically packaged.

The pulp was divided into small portions in the laboratory, packaged in high density polyethylene bottles and frozen at −18 °C. This procedure allowed for the use of the same product throughout the whole project. Before use, the samples were thawed at 4 °C and diluted using distilled water at 50 °C to ensure better hydration (Tehrani & Ghandi, 2007).

Potassium sorbate (0.8% m/m) was then added to the juice in order to allow for microbial stability during the 60-day storage evaluation at 25 °C, and it was allowed to rest for 24 h at 5 °C to ensure complete hydration and the release of incorporated air. The juice pH was 5.6.

2.2. High pressure homogenization (HPH) process

The juice was homogenized at pressures ranging from 0 MPa (control) to 100 MPa using a high pressure homogenizer (Panda Plus, GEA Niro Soavi, Italy). The maximum homogenization pressure was set at 100 MPa as the main rheological changes take places at this range, and an asymptotic behavior, with minor changes, was observed in previous studies at pressures above 100 MPa (Augusto et al., 2012a,b, 2013).

Samples were introduced into the equipment suction section at 5 °C and quickly cooled using an ice bath just after the homogenization valve. The maximum temperature reached was ~27 °C (for the sample processed at 100 MPa just before the ice bath). The experiments were carried out with three replicates.

2.3. Effect of HPH on the physical stability of the juice

The effect of HPH on the physical stability of the juice was evaluated from its particle size distribution (PSD), pulp sedimentation, serum cloudiness, juice color and microstructure (optical and scanning electron microscopy), comparing the non-homogenized sample (NH) with the HPH processed (0, 25, 50, 75 and 100 MPa) samples just after processing. These analyses were also carried out with three replicates.

Moreover, the samples were evaluated during 60 days in order to understand the effect of HPH on the physical stability of the juice. For this purpose, the samples were stored at 25 °C (BOD TE391, Tecnal, Brazil) in graduated cylinders (pulp sedimentation analysis) or in high density polyethylene bottles covered with aluminum sheets. The evaluation was carried out five times during the first 15 days and three times each during the next three 15-day periods.

2.3.1. Particle size distribution (PSD)

The sample particle size distribution (PSD) was measured by light scattering (Malvern Mastersizer 2000 with Hydro 2000s, Malvern Instruments Ltd., UK). In addition the volume-based mean diameter (D[4,3] according to Eq. (1), where n_i is the number of particles with diameter d_i) and the area-based mean diameter (D[3,2], according to Eq. (2)) were also evaluated. Both equivalent diameters were evaluated since the D[4,3] is highly influenced by large particles whereas D[3,2] is more influenced by the smaller ones (Bengtsson & Tornberg, 2011; Lopez-Sanchez, Nijsse, et al., 2011).

$$D[4,3] = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \quad (1)$$

$$D[3,2] = \frac{\sum_i n_i d_i^3}{\sum_i n_i d_i^2} \quad (2)$$

2.3.2. Microstructure — optical (OM) and scanning electron microscopy (SEM)

The juice microstructure was evaluated using both optical (OM) and scanning electron (SEM) microscopy.

The OM analyses were carried out by carefully placing the sample (20 μL) onto a glass slide, covering with a coverslip (Mert, 2012) and carefully rotating the coverslip at 45° to guarantee the same orientation

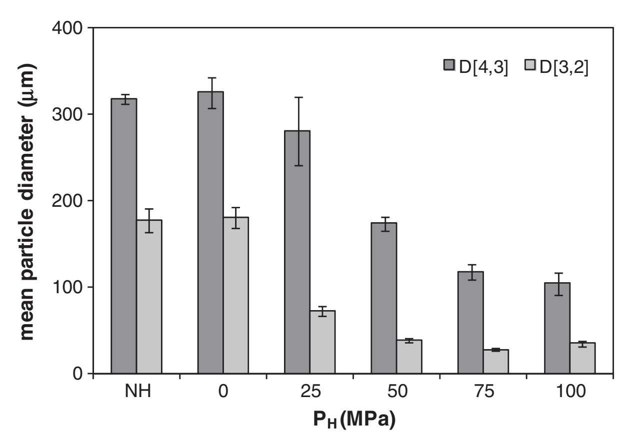

Fig. 2. Effect of HPH (0–100 MPa) on the tomato juice mean particle diameter (D[4,3] and D[3,2]). The vertical bars represent the standard deviation for each value.

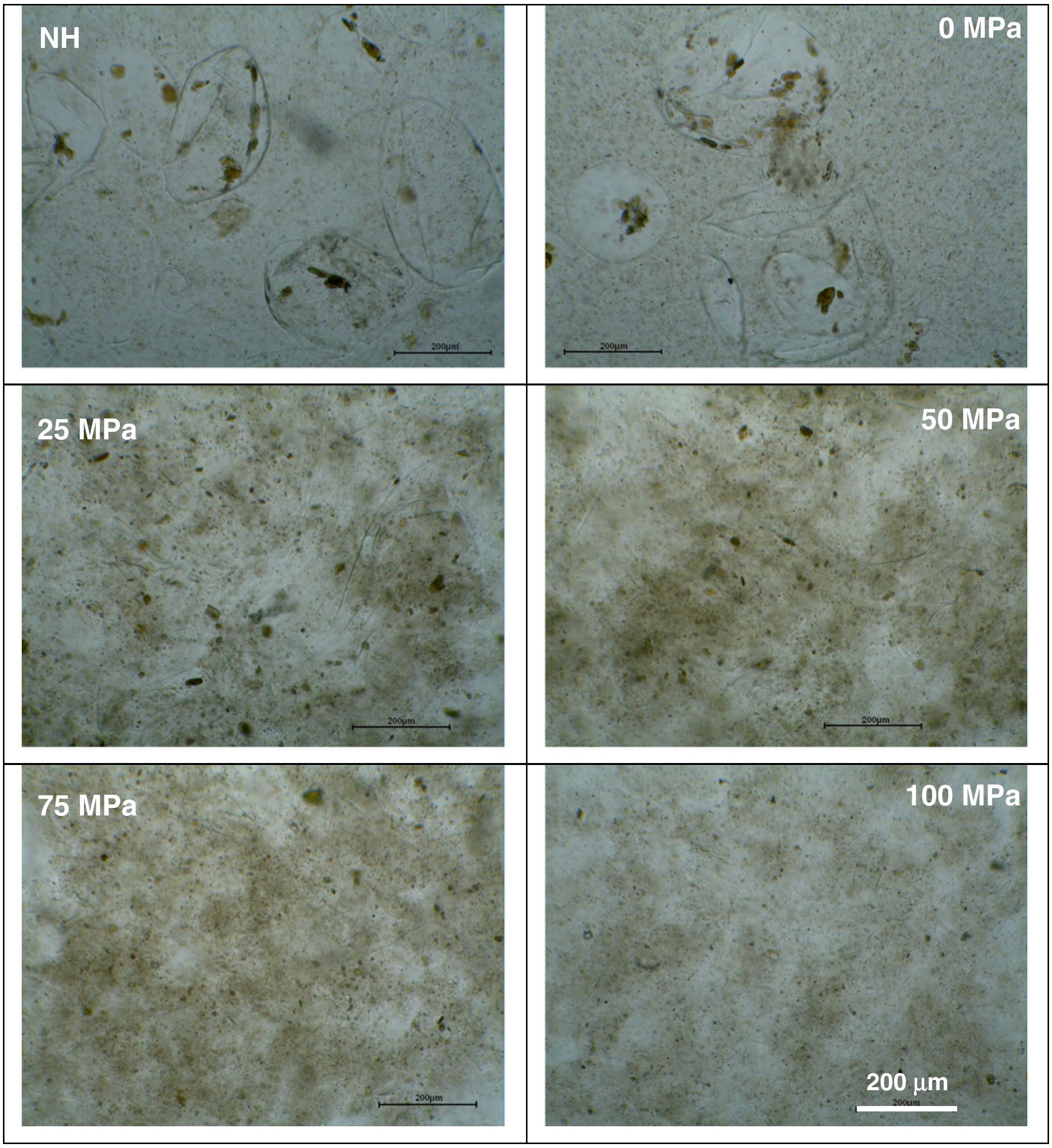

Fig. 3. Effect of HPH on the tomato juice microstructure: optical microscopy (OM) using a 12.5× objective. The scale bar shows 200 μm.

for the samples (Bayod & Tornberg, 2011), before observing in an optical microscope with a 12.5× objective (Carl Zeiss Jenaval, Carl Zeiss MicroImaging GmbH, Deutschland), a digital camera and the EDN2 Microscopy Image Processing System software.

The SEM analyses were carried out using a scanning electron microscope (TM3000 Tabletop Microscope, Hitachi High-Technologies Corporation, Japan) at 15 kV, observing the sample at 50× magnification. For this analysis, the juice was previously frozen using liquid-N_2 and freeze-dried (Edwards Super Modulyo, Edwards Vacuum, UK) as described by Sato and Cunha (2009).

2.3.3. Pulp sedimentation

Pulp sedimentation was evaluated using 25 mL graduated cylinders filled with the samples, and stored at 25 °C (BOD TE391, Tecnal, Brazil) for 24 h (early evaluation) and for a total of 60 days (simulating a shelf life evaluation). As described by Silva et al. (2010) and Vendrúsculo and Quadri (2008), the sedimentation index (IS) was obtained using Eq. (3).

$$IS = \text{sedimentation volume}/\text{total sample volume} \quad (3)$$

2.3.4. Serum cloudiness (turbidity)

The serum cloudiness was evaluated after centrifuging the juice samples at 10,000 *g* for 10 min at 20 °C (Mikro 200R, Andreas Hettich GmbH & Co.KG, Germany). The optical density (absorbance) of the supernatant (i.e., the juice serum) was then measured at 660 nm using a Beckman DU800 spectrophotometer (Beckman Coulter, Corona, CA, USA) with distilled water as the reference, and directly related to the turbidity (Kincal et al., 2006; Silva et al., 2010).

2.3.5. Instrumental color

The instrumental color of the samples was obtained using a Color Quest XE colorimeter (Hunter Associates Laboratory, USA), with illuminant D65, angle of 10° previously calibrated with a RSIN white reference ($L^*=92.03$, $a^*=-0.88$, $b^*=0.63$), as described by Sánchez-Moreno, Plaza, de Ancos, and Cano (2006) and Rodrigo, van Loey, and Hendrickx (2007). The samples were placed in glass cuvettes, and three readings were obtained for each replicate.

The CIELab technique was used for the evaluation, where the values for L^* (lightness), a^* (redness: green to red) and b^* (yellowness: blue to yellow) were first obtained and then used to express the color changes according to the hue value (h, color angle,

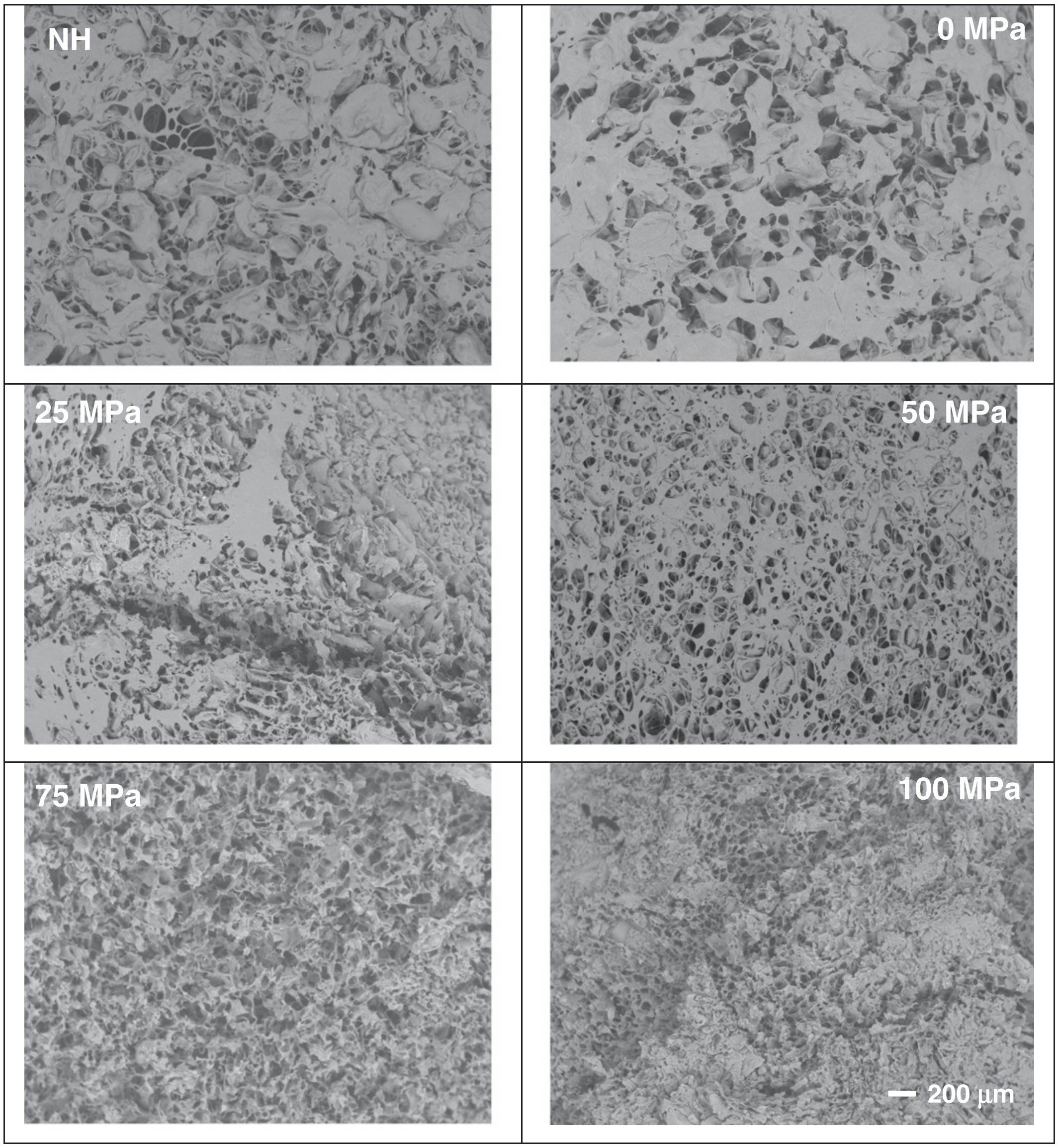

Fig. 4. Effect of HPH on the tomato juice microstructure: scanning electron microscopy (SEM) at 50× magnification. The scale bar shows 200 μm.

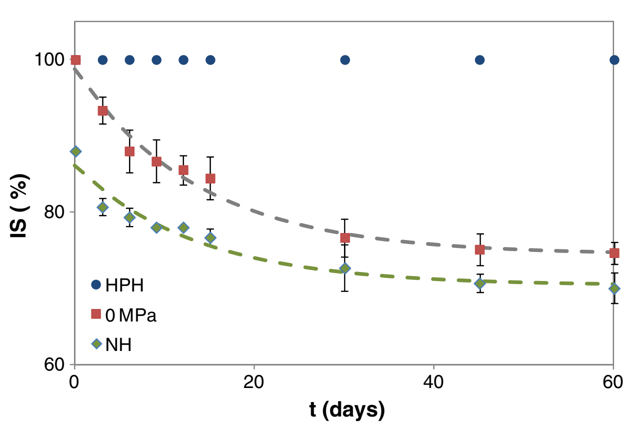

Fig. 5. Effect of HPH on tomato juice sedimentation. Vertical bars are the standard deviation in each value and the dashed curves are the models described in Table 1. As there was no sedimentation in the homogenized samples (25, 50, 75 and 100 MPa), they are symbolized together as "HPH".

Eq. (4)), chroma value (C*, color saturation, Eq. (5)) and the total color change (ΔE, Eq. (6)).

$$h = \operatorname{arctg}\left(\frac{b*}{a*}\right) \tag{4}$$

$$C* = \sqrt{(a*)^2 + (b*)^2} \tag{5}$$

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{6}$$

Table 1
Mathematical modeling of the sedimentation of tomato juice during 60 days of storage (25 °C): NH and 0 MPa samples. (IS in % and t in days).

Model	$IS = IS_{equilibrium} + (IS_{initial} - IS_{equilibrium}) \cdot e^{-k \cdot t}$	
Homogenization	NH	0 MPa
$IS_{equilibrium}$	70.36	74.39
$IS_{initial}$	86.07	98.79
k	0.074	0.073
R^2	0.95	0.98

2.4. Mathematical modeling

When relevant, some of the properties evaluated were modeled as a function of the homogenization pressure (P_H) using non-linear regression and the software CurveExpert Professional v.1.2.3, with a significant probability level of 95%.

3. Results and Discussion

3.1. Particle size distribution (PSD)

Fig. 1 shows the effect of HPH (0–100 MPa) on the particle size distribution in tomato juice (PSD). As expected, the homogenization processing reduced the mean particle diameter, as previously observed for tomato juice (Augusto et al., 2012b), other tomato products (up to 9 MPa, Bayod & Tornberg, 2011; Bayod, Willers, & Tornberg, 2008; Bengtsson & Tornberg, 2011; up to 60 MPa, Lopez-Sanchez, Nijsse, et al., 2011) and other vegetable products, such as passion fruit juice (up to 28 MPa, Okoth, Kaahwa, & Imungi, 2000), citrus juices (up to 30 MPa, Betoret, Betoret, Carbonell, & Fito, 2009; Sentandreu, Gurrea, Betoret, & Navarro, 2011; up to 170 MPa, Lacroix, Fliss, & Makhloue, 2005), apple juice (up to 200 MPa, Donsì et al., 2009), and apple, broccoli, carrot and potato sauces and emulsions (up to 9 MPa, Bengtsson & Tornberg, 2011; up to 60 MPa, Lopez-Sanchez, Nijsse, et al., 2011).

Moreover, the changes in particle diameter were less pronounced between 75 MPa and 100 MPa than between 0 MPa and 75 MPa. The effect of the homogenization pressure (P_H) on the disruption of suspended particles seems to follow an asymptotic behavior, i.e., at higher pressures an increase in P_H caused smaller changes in particle size distribution. In fact this can even be observed in the D[4,3] and D[3,2] values (Fig. 2) and, consequently, in the rheological behavior of the product (Augusto et al., 2012b, 2013).

Fig. 2 shows the reduction in the volume-based mean diameter (D[4,3], Eq. (1)) and in the area-based mean diameter (D[3,2], Eq. (2)) due to the homogenization pressure. Although both equivalent diameters were reduced during HPH, the reduction in D[3,2] of the samples between 0 MPa and 50 MPa (~80%) was higher than the reduction in D[4,3] (~50%).

Since the D[4,3] is highly influenced by large particles and the D[3,2] more influenced by smaller ones (Bengtsson & Tornberg, 2011; Lopez-Sanchez, Nijsse, et al., 2011), this result indicates a considerable increase in the number of small particles when the juice is processed at 50 MPa. Moreover, the reduction in D[3,2] between 50 MPa and 100 MPa (~10%) was smaller than the reduction in D[4,3] (~40%), which indicates that the subsequent disruptions are preferentially of the larger suspended particles, corroborating the values obtained for PSD in Fig. 1.

Becker, Miers, Nutting, Dietrich, and Wagner (1972) studied the dimensions of tomato cells and found values between 400 μm × 600 μm and 600 μm × 1000 μm. Thus, it is to be expected that the control juice, with particle diameters ranging between ~100 μm and ~1000 μm, would be constituted of some whole cells and their fragments, obtained during processing of the tomato pulp. As explained by Augusto et al. (2012b), the homogenization process disrupts the remaining cells and breaks their fragments into small suspended particles. It is to be expected that the smaller fragments would be less susceptible to being broken during processing when compared to the bigger ones or even to the whole cells, which explains the observed effect of P_H on the suspended particle disruption behavior, which was further confirmed by the microstructure analyses.

Finally, no differences in PSD, D[4,3] and D[3,2] were observed between the two control samples, i.e., the non-homogenized (NH) sample and that homogenized at 0 MPa (just pumped through the equipment). This shows that just passing the juice through the equipment causes no particle disruption.

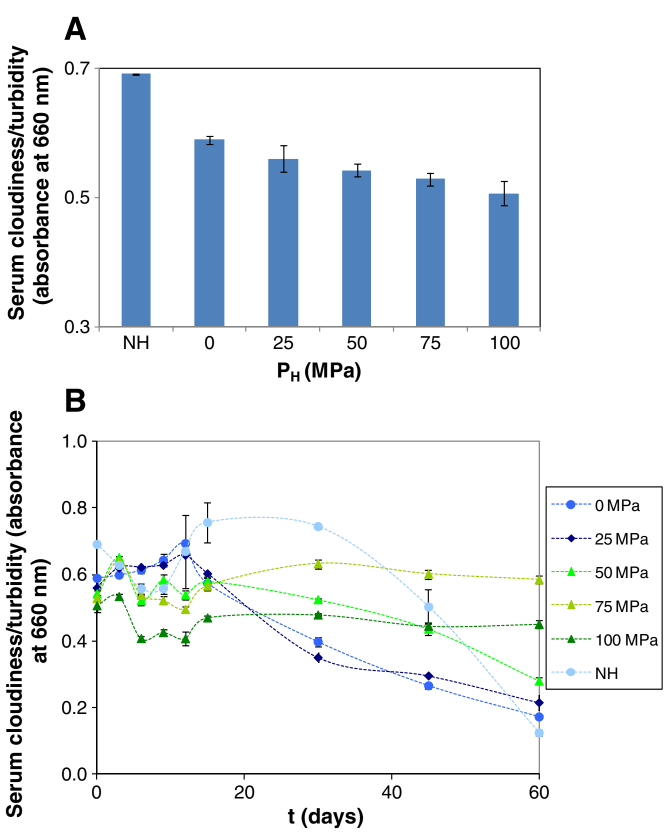

Fig. 6. Effect of HPH on the tomato juice serum cloudiness: (A) just after the homogenization process and (B) throughout the 60 days at 25 °C. Vertical bars are the standard deviation for each value.

3.2. Microstructure

Figs. 3 and 4 show the microstructures of tomato juice by optical (OM) and scanning electron (SEM) microscopy, respectively. Firstly, the non-homogenized (NH) sample and that homogenized at 0 MPa showed similar structures. Furthermore, there was a clear difference between the control samples (NH and 0 MPa) and the homogenized samples (25, 50, 75 and 100 MPa).

Whereas the control samples showed whole cells, with intact membranes and the characteristic lycopene crystals, the homogenized samples just showed a large amount of small particles, composed of cell walls and internal constituents suspended in the juice serum (Fig. 3). As expected and confirmed by the PSD analysis, the suspended particles were smaller at higher P_H values, highlighting the effect of HPH in disrupting the fruit pulp particles.

Moreover, the small particles resulted from the higher P_H values tended to form aggregates, as can be seen in Fig. 3. As explained by Augusto et al. (2012b), cell disruption and subsequent fragmentation not only increased the surface area of the suspended particles, but also changed the properties of the particles and serum. Cell fragmentation exposed and released wall constituents such as pectins and proteins, improving the particle–particle interactions and resulting in aggregates. In fact, the authors observed an important increase in thixotropy of the tomato juice due to the HPH, which directly describe the changes on juice microstructure.

Similar and complementary results can be seen in the SEM images (Fig. 4), where a network microstructure with a progressively smaller grid in relation to the P_H can be seen.

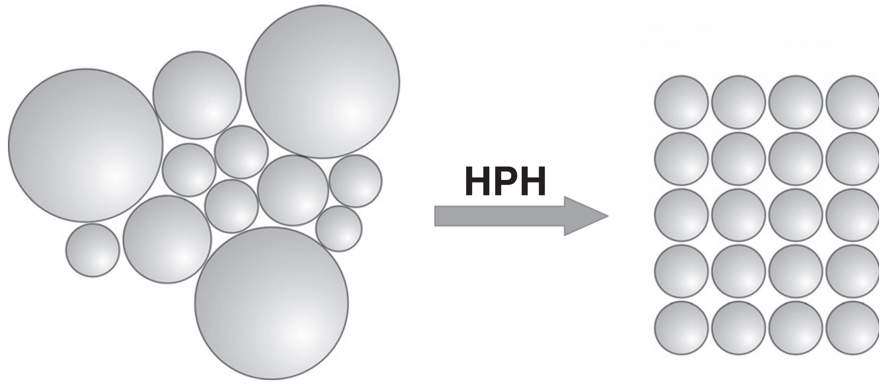

Fig. 7. Effect of HPH on the suspended particles of tomato juice: schematic representation of the particles and aggregates.

Therefore, the results obtained for the microstructure confirm the changes in PSD and rheological behavior (Augusto et al., 2012b, 2013) occurring in the tomato juice as a result of HPH, and suggest other changes in the physical-stability of the product.

3.3. Pulp sedimentation

Fig. 5 shows the sedimentation index (IS) for the control and homogenized (up to 100 MPa) samples.

Both the non-homogenized (NH) sample and the juice just passed through the equipment (0 MPa) showed an increase in the amount of sediment with time, with stabilization after ~30 days. The main changes took place in the first 20 days. Moreover, it is interesting to note that the non-homogenized (NH) sample showed quicker sedimentation than the 0 MPa sample. This difference seems to be due to the initial sedimentation of the NH juice, whose IS decreased from 100% to 88% in 24 h. Since no differences were shown between the samples with respect to their PSD, this divergence in the initial sedimentation rate could be associated with a possible disruption of large particle-aggregates during passage of the juice through the equipment. As the inter-particle forces are relatively weak in large aggregates, the shear stress due to pumping may be sufficient to separate the particles. Thus, the large aggregates still present in the native sample (i.e., the NH), would sediment quickly, showing this reduction in IS in just 24 h. Moreover, it is important to highlight that the PSD analysis itself dissociates these aggregates by mixing the solution in order to allow the laser to pass through the particles, but their presence can be observed by other techniques, such as time-dependent rheology (Augusto et al., 2012b). The IS of the NH and 0 MPa juices was then modeled as a function of storage time (at 25 °C) using an exponential decay function, showing good coefficient of determination ($R^2 > 0.95$). The model and parameters are shown in Table 1.

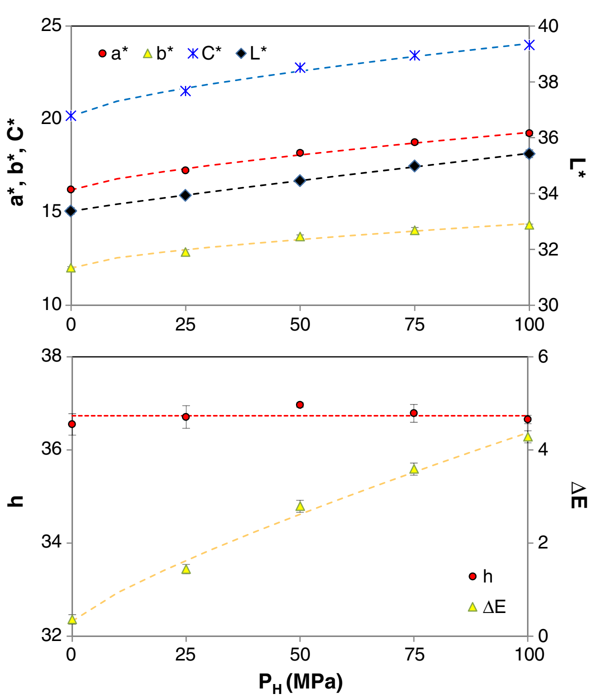

Fig. 8. Effect of HPH on the color of the tomato juice just after the homogenization process. Vertical bars are the standard deviation for each value; dashed curves are the models described in Table 2.

On the other hand, none of the homogenized samples (25, 50, 75 and 100 MPa) showed any pulp sedimentation even after 60 days of storage (25 °C) (Fig. 5).

Stokes law describes the sedimentation velocity of spheres as a function of the properties of the particles and the dispersed medium. According to Stokes law, the particle sedimentation velocity is proportional to the particle size (diameter) and the difference between the densities of the particles and the dispersed medium, and inversely proportional to the dispersed medium viscosity.

Therefore, the reduction in particle size during homogenization can be related to the greater stability of the homogenized samples, and HPH can be seen as an important tool in preventing pulp sedimentation.

Different behaviors were observed for pineapple pulp (Silva et al., 2010), where even the homogenized samples showed pulp sedimentation after 10 days of storage (25 °C). This difference can be attributed to the formation of particle aggregates, whose importance is different for each vegetable. In fact, Lopez-Sanchez, Svelander, et al. (2011) showed that each vegetable cell wall had a different behavior when processed by HPH. While carrot tissue requires higher shear values to be disrupted, the cell walls of tomato cells were broken even at moderate shear values.

3.4. Serum cloudiness (turbidity)

Fig. 6 shows the serum cloudiness of the tomato juice after centrifugation. As previously observed for pulp sedimentation, the NH sample showed different values for serum cloudiness as compared to the 0 MPa sample (Fig. 6A), showing once again that the product was submitted to shear stress even when just pumped through the equipment. This is similar to the results of Okoth et al. (2000) with passion fruit juice.

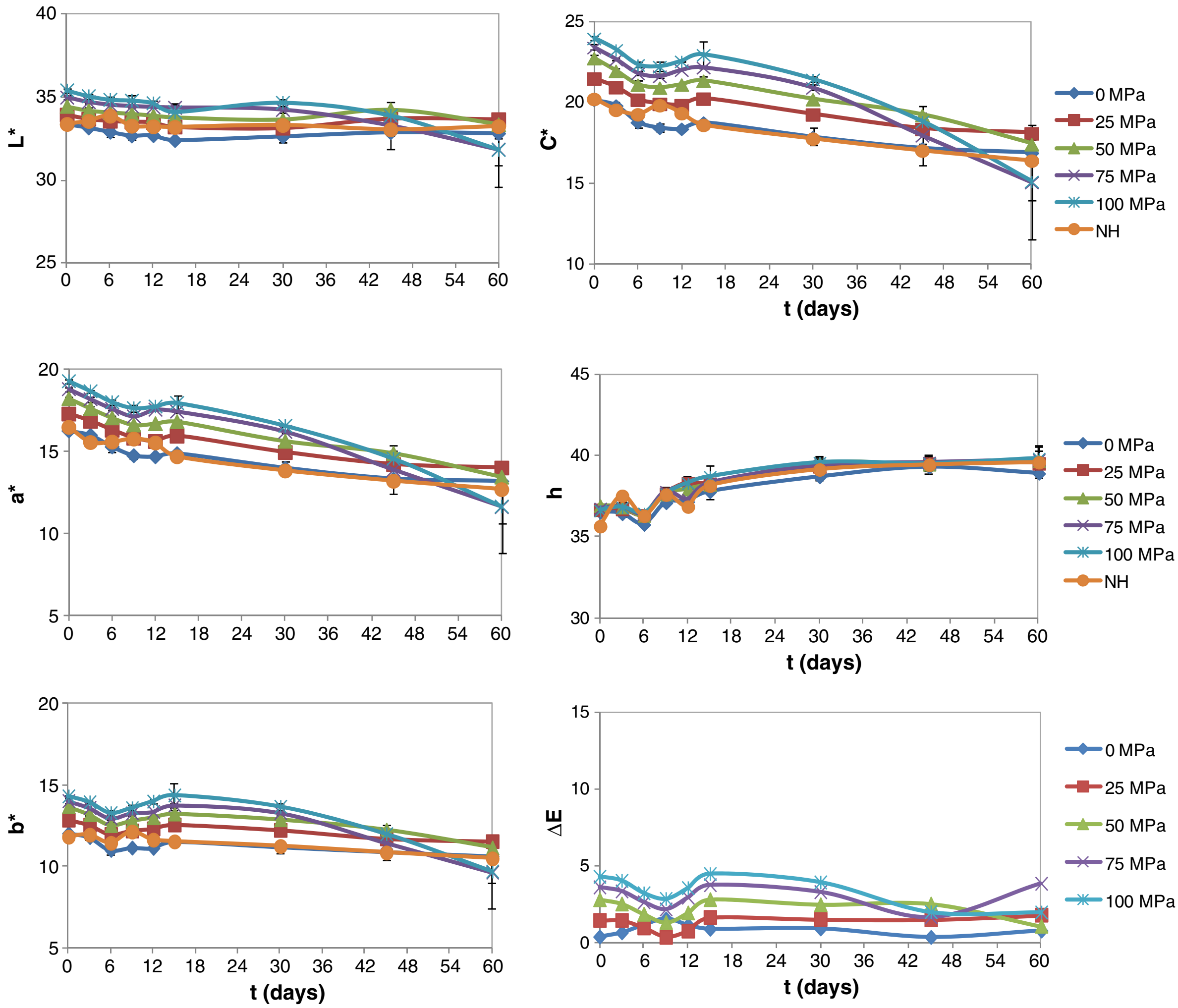

Fig. 9. Effect of HPH on the color of tomato juice throughout 60 days at 25 °C. Vertical bars are the standard deviation for each value.

Moreover, serum cloudiness decreased when the homogenization pressure increased (Fig. 6A). Since the smaller suspended particles allow more light to pass through the juice serum, with consequent lower absorbance values (Okoth et al., 2000; Silva et al., 2010), the obtained results can be attributed to the pulp disruption during the homogenization pressure. Moreover, it is in accordance with the previous observed PSD behavior.

Fig. 6B shows the serum cloudiness along the 60 days of storage. All the samples showed serum cloudiness variation along the time, reflecting the particle aggregation due to attractive forces. Moreover, the juices homogenized at higher P_H (75 and 100 MPa) showed lower variation than the others. The juices processed at 0 and 25 MPa showed similar behavior, with a high decrease after 12 days of storage.

Fruit juices are composed of an insoluble phase (the pulp) dispersed in a viscous solution (the serum). The dispersed phase, or pulp, is constituted of fruit tissue cells and their fragments, cell walls and insoluble polymer clusters and chains. The serum is an aqueous solution of soluble polysaccharides, sugars, salts and acids (Augusto et al., 2012a).

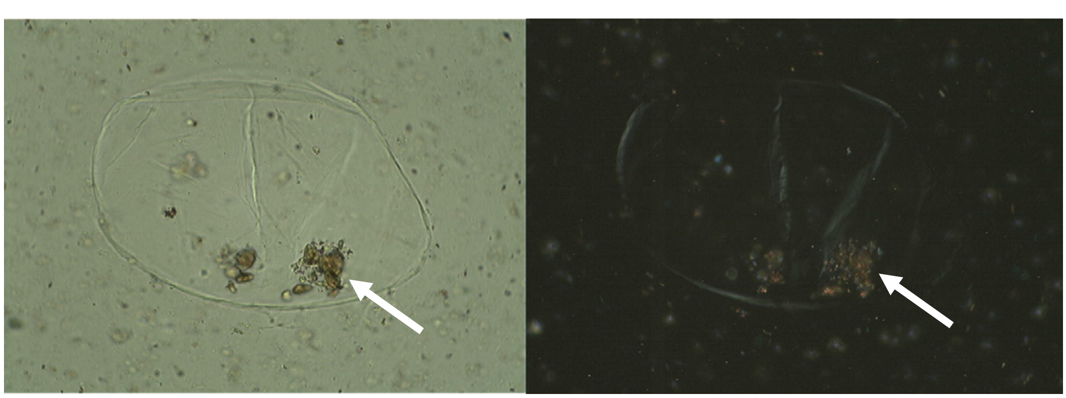

Fig. 10. Suspended cell and lycopene crystals in tomato juice. Optical microscopy using the 25× objective without polarized light (left) and under polarized light (right).

Table 2
Mathematical modeling of the color of tomato juice as a function of the homogenization pressure (P_H, in MPa). CP is the color parameter (L*, a*, b*, C*, h and ΔE).

Model	$CP = CP_{initial} + k_1 \cdot P_H^{k_2}$			
Color parameter	$CP_{initial}$	k_1	k_2	R^2
L*	33.37	0.03	0.92	0.99
a*	16.20	0.11	0.72	0.99
b*	12.00	0.13	0.64	0.99
C*	20.16	0.17	0.69	0.99
ΔE	0.32	0.09	0.82	0.99
h	36.73 (mean value)			0.99

In the serum cloudiness (turbidity) analysis, a parallel beam of radiation is passed through the sample and its absorbance is obtained. The absorbance is thus directly related to the sample cloudiness/turbidity, as the suspended particles are responsible for the absorption of radiation. In cloudy juices such as tomato, with a high concentration of suspended particles, a prior centrifugation is carried out, and the supernatant turbidity then measured.

Stokes law defines the sedimentation velocity of spherical and rigid particles, without interactions, in a Newtonian fluid. This velocity is directly proportional to the sizes of the suspended particles (diameter), the acceleration imposed and the difference between the particle and fluid densities, being inversely proportional to the fluid viscosity. Thus it describes sedimentation of the juice particles when only hydrodynamic forces are relevant.

Thus smaller particles tend to remain in suspension after centrifugation (which is always carried out with the same acceleration), increasing the absorbance values and turbidity.

However, Augusto et al. (2012b) described the importance of the non-hydrodynamic forces (electrostatic, Van der Waals) of juice processed by HPH, where the particle surface area is increased by the reduction in its size. As a result, aggregates are formed, whose behavior during centrifugation and the turbidity analysis is more complex. Precipitation of these aggregates is a function not only of their size but also of their density, which is directly related to their porosity.

As the P_H is increased, so the suspended particle sizes are reduced due to disruption, with a consequent increase in particle surface area and interactions, as well as aggregate formation. However, not only the particle diameters change, reducing its dimensions, but also their distribution, which is more uniform at higher P_H values (Fig. 1). Therefore, the particle aggregates of homogenized juices are more porous and consequently less dense than those of non-homogenized samples, since in less uniform structures the smaller particles fill the gaps among the bigger ones (Fig. 7).

The non-homogenized juices and juices homogenized at low P_H values (0, 25 and 50 MPa) have less uniform particle distributions, and this range in size can result in a more compact and less orderly network. However, due to possible particle rearrangements in the network, the structure takes longer to stabilize and thus variations in turbidity values occur during storage of the juice (Fig. 6B). Furthermore, the aggregate network can drag large particles and other aggregates during centrifugation, substantially reducing the supernatant absorbance (turbidity).

On the other hand, the juices homogenized at higher P_H values (75 to 100 MPa) have more uniform particles, which result in a porous network (as observed by microscopy, Figs. 3 and 4). The resulting structure is quickly stabilized due to the uniform particle size and larger surface area, their sedimentation being more difficult due to the dragging action of opposite forces and hence the turbidity values vary less during storage (Fig. 6B). Once again, these results are in accordance with those of PSD, sedimentation and microscopy.

3.5. Color

Fig. 8 shows the parameters of L* (lightness), a* (redness: green to red), b* (yellowness: blue to yellow), h (hue value, color angle, Eq. (4)), C* (chroma value, color saturation, Eq. (5)) and ΔE (total color change, Eq. (6)) as a function of the homogenization pressure. Fig. 9 shows the evolution of these parameters throughout the 60 days of storage at 25 °C.

The values for L*, a*, b*, C* and ΔE increased with increase in P_H, showing that the samples became clearer, more saturated in red and yellow and with a more intense color. On the other hand, the hue (h) parameter showed no significant difference. Similar results were observed by Betoret et al. (2009) with orange juice.

Lycopene is the most abundant carotenoid in tomato products, being responsible for their red color, and is located within the chromoplasts as a carotenoid–protein complex or as solid microcrystals (Colle et al., 2011). Fig. 10 shows the lycopene crystals within the cell, highlighted by the polarized light. Therefore, the effect of HPH on tomato juice color, especially on the increase in the red component (a*), can be explained by disruption of the cells and membranes and breakage of the chromoplast carotenoid–protein complexes during processing, allowing for the leakage of cellular material, including pigments such as lycopene. Thus HPH may improve the dispersion of lycopene, a fact which can be seen in the optical images of Fig. 3. Moreover, it is important to observe that this issue will influence the stability of the juice color during storage.

Cserhalmi, Sass-Kissb, Tóth-Markusb, and Lechnera (2006) reported that the difference in color could be classified in relation to the sample ΔE as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0) and great (6.0–12.0). Thus the juice homogenized at 50 MPa showed noticeable variation in color and those homogenized at 75 and 100 MPa showed a well visible difference in color as compared to the standard (Fig. 8; Table 2).

The values for L*, a*, b*, C* and ΔE were modeled as a function of the P_H. Due to the behavior observed (Fig. 8), a power-type function with an initial value (related to the original properties of the tomato juice, i.e., those processed at 0 MPa) was used to model the parameters. This is in agreement with the models obtained by Augusto et

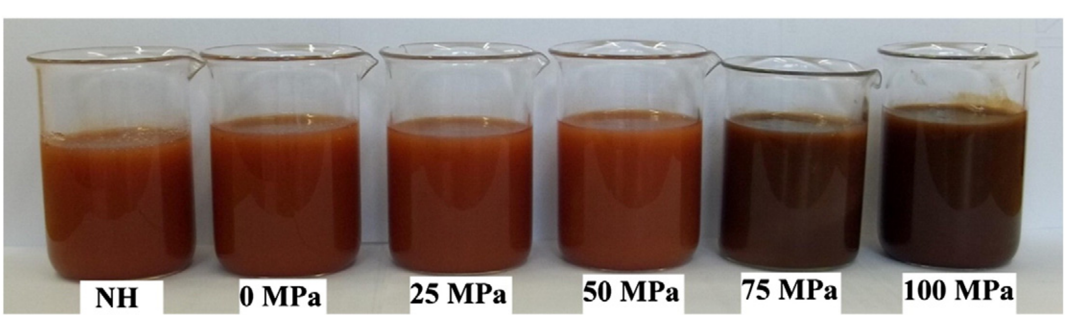

Fig. 11. Effect of HPH on the color of tomato juice: non-homogenized (NH) and homogenized samples at 0–100 MPa after 60 days at 25 °C.

al. (2012b) and the results obtained can be seen in Table 2. The function showed good coefficient of determination ($R^2 > 0.98$) with the experimental values and highlighted the asymptotic effect of the P_H, whose main effects are observed at lower P_H values. Moreover, the mean value of the hue parameter was also considered ($h = 36.73$).

The homogenization process disrupts the remaining cells and breaks the fragments up into small suspended particles, and it is to be expected that the small fragments would be less susceptible to breakage during processing than the larger ones or the whole cells, which explains the asymptotic effect observed by Augusto et al. (2012b).

Fig. 9 shows the changes in juice color throughout the 60 days of storage at 25 °C. The juice hue (h, a direct measure of the color) showed a small increase with time, indicating that the juice became slightly less red. Moreover, the chroma values (C*) showed a decrease during storage, especially the juices homogenized at 75 MPa and 100 MPa, indicating that the samples showed a less intense red color, since chroma values close to zero correspond to neutral colors (gray) while higher values correspond to brighter colors.

These changes may have resulted from oxidation of the lycopene by the dissolved oxygen in the juice, since this is the pigment responsible for the red color, and it is more dispersed in the processed samples. Thus, as observed in the optical microscopy images (Figs. 3 and 10), HPH increases the exposure of the lycopene, and this larger area makes it more susceptibility to oxidation. In fact, this is representative of the time of storage, even for a small amount of residual oxygen dissolved in the sample as can be observed in Fig. 11. Once again the results obtained are in agreement with the previous ones.

4. Conclusions

The present work evaluated the effect of high pressure homogenization (HPH) on the physical stability of tomato juice. HPH changed the particle size distribution (PSD), pulp sedimentation behavior, serum cloudiness (turbidity), color and microstructure of the tomato juice. The effect of homogenization pressure (P_H) on the disruption of suspended particles, and consequently on the other physical properties, followed an asymptotic behavior. The microstructure images confirmed the PSD analyses, and showed the leakage of lycopene after processing. HPH increased the juice stability to sedimentation, which could also be seen from the pulp sedimentation and serum cloudiness (turbidity) behaviors. The color of the juice also changed due to P_H, and was in accordance with disruption of the pulp and the leakage of lycopene. Moreover, the results obtained are in agreement with previously obtained rheological data, and deal with recent trends in process–structure–function relationship studies. These results indicated that HPH could be used to increase the consistency of tomato juice, improve its sensory acceptance, reduce the need for the addition of hydrocolloids and reduce particle sedimentation and serum separation.

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