



**HAL**  
open science

## Concentration and purification of lycopene from watermelon juice by integrated microfiltration-based processes

Luis Chaparro, Claudie Dhuique-Mayer, Soraya Castillo, Fabrice Vaillant, Adrien Servent, Manuel Dornier

### ► To cite this version:

Luis Chaparro, Claudie Dhuique-Mayer, Soraya Castillo, Fabrice Vaillant, Adrien Servent, et al.. Concentration and purification of lycopene from watermelon juice by integrated microfiltration-based processes. *Innovative Food Science & Emerging Technologies / Innovative Food Science and Emerging Technologies* , 2016, 37, pp.153-160. 10.1016/j.ifset.2016.08.001 . hal-01517191

**HAL Id: hal-01517191**

**<https://hal.science/hal-01517191v1>**

Submitted on 2 May 2017

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## **Concentration and purification of lycopene from watermelon by crossflow microfiltration**

Luis CHAPARRO<sup>a</sup>, Claudie DHUIQUE-MAYER<sup>b</sup>, Soraya CASTILLO<sup>a</sup>,  
Fabrice VAILLANT<sup>b\*</sup>, Adrien SERVENT<sup>b</sup>, Manuel DORNIER<sup>c</sup>

<sup>a</sup>: UCLA. Departamento de Procesos Agroindustriales, Programa de Ingeniería Agroindustrial, Avenida las Industrias. Barquisimeto 3001, Venezuela.

<sup>b</sup>: CIRAD, UMR95 QualiSud, TA B-95/16, 73 rue J.F. Breton, F-34398 Montpellier cedex 05, France.

<sup>c</sup>: Montpellier SupAgro, UMR95 QualiSud, 1101 av. Agropolis, B.P. 5098, F-34093 Montpellier cedex 05, France.

\*: Corresponding author: <fabrice.vaillant@cirad.fr>.

### **Abstract**

The aim of the study was to evaluate a new process based on crossflow microfiltration for concentration and partial purification of lycopene from watermelon. The process included sequentially juice extraction, enzymatic liquefaction, crossflow microfiltration and centrifugation. Different technological schemes were compared including or not a diafiltration step in order to remove soluble solids and increase lycopene purity. Microfiltration was performed at 50-60 °C using alumina tubular membranes with average pore diameter from 0.2 to 1.4 µm and varying the transmembrane pressure from 0.5 to 2.0 bar. Using the best operating conditions at the crossflow microfiltration step, permeate flux was close to 110 L.h<sup>-1</sup>.m<sup>-2</sup> and lycopene concentration was increased 11 times in the retentate. Centrifugation at 10,000 g for 10 min, enabled to enhance lycopene concentration up to 4 times. Diafiltration allowed to decrease total soluble solids from 100 to 20 g.kg<sup>-1</sup> and so to purify lycopene further. No cis-isomerization was highlighted that proved that the process maintained carotenoid integrity. Finally, the optimal processing scheme allowed to obtain a natural extract of lycopene 42 times more concentrated and 34 times purer than the

initial juice. The results looked very promising for industrial application even if economic viability has still to be assessed.

**Keywords:** microfiltration, carotenoids, diafiltration, antioxidant.

## **Introduction**

Lycopene is a carotenoid considered as an important fat-soluble food component with health benefits. Numerous epidemiologic studies provide strong evidence that lycopene and its metabolites are active in several biological activities (Wang, 2007). Its high biological activity and enhancing immune function has been published by Bramley (2000), Waliszewski *et al.* (2010), Cruz *et al.* (2013). Anese *et al.* (2013) reported that the importance of lycopene as a bioactive component is due to a number of epidemiological studies have concluded that this carotene-rich foods is associated with a lower risk of some degenerative diseases. Also, cell culture assays and dietary intervention have provided additional evidence for that lycopene may play a role specifically in preventing prostate cancer (Wertz *et al.* , 2004).

The Major diet intake of lycopene comes from consumption of tomato and tomato products. Another dietary source from south countries was watermelon, one of the most popular fruits in the world whose pink or red flesh color is the most attractive feature. Lycopene with  $\beta$ -carotene were carotenoids responsible for its color and being the main bioactive component (Wen *et al.*, 2013).

According to Tarazona *et al.* (2013) watermelon juice is naturally rich in lycopene and L-citrulline, an amino-acid very efficient hydroxyl radical scavenger and thus a strong antioxidant. These compounds make watermelon an excellent option in the application in the design of functional foods. However watermelon extract has to be adapted to increase their availability for effective incorporation in foodstuffs. Perkins and Collins (2006) reported that the concentration of lycopene in watermelon was around  $50 \text{ mg.kg}^{-1}$ . However these values may drastically vary depending on the variety, maturity and according to the harvest time and climatic factors.

Lycopene (C<sub>40</sub>H<sub>56</sub>) is an open-polyene chain lacking the β-ionone ring structure with 13 double bonds. Literature reported lycopene as a powerful scavenger of singlet oxygen (a reactive oxygen free radical precursor) (Shi *et al.*, 2011). According to Bonn *et al.* (2010) in fresh vegetable products, about 95% of lycopene was in all-*trans* form which is thermodynamically the most stable form and was in food matrices trapped in the pigment-protein complex localized in chromoplasts. However, lycopene isomers such as 5 *cis*, 9 *cis*, 13 *cis* or 15 *cis* were reported in processed foods due to the effect of the application of high temperatures, exposure to light or oxygen incorporation. Although these form were found in plasma, it was advisable to avoid isomerization, to use soft processing technologies (Van Buggenhout *et al.*, 2010).

Crossflow microfiltration is a membrane process that is largely used to clarify or to stabilize without heating fruit juices like for example tomato juice (Razi *et al.*, 2012) or even watermelon juice and other plant extracts like roselle extract (Cissé *et al.*, 2011). During microfiltration of these products, hydrophobic compounds are generally retained by the membrane because they are associated with the insoluble solids. Using this property Abreu *et al.* (2013) applied microfiltration for the concentration of carotenoids from an aqueous extract of cashew apple. This process allowed to increase carotenoid concentration in the retentate by 10 obtaining a final product with high amount of carotenoids with a great potential as yellow natural colorant. In such a case, microfiltration used in diafiltration mode could be interesting in order to purify the compounds retained by the membrane through removing the soluble fraction.

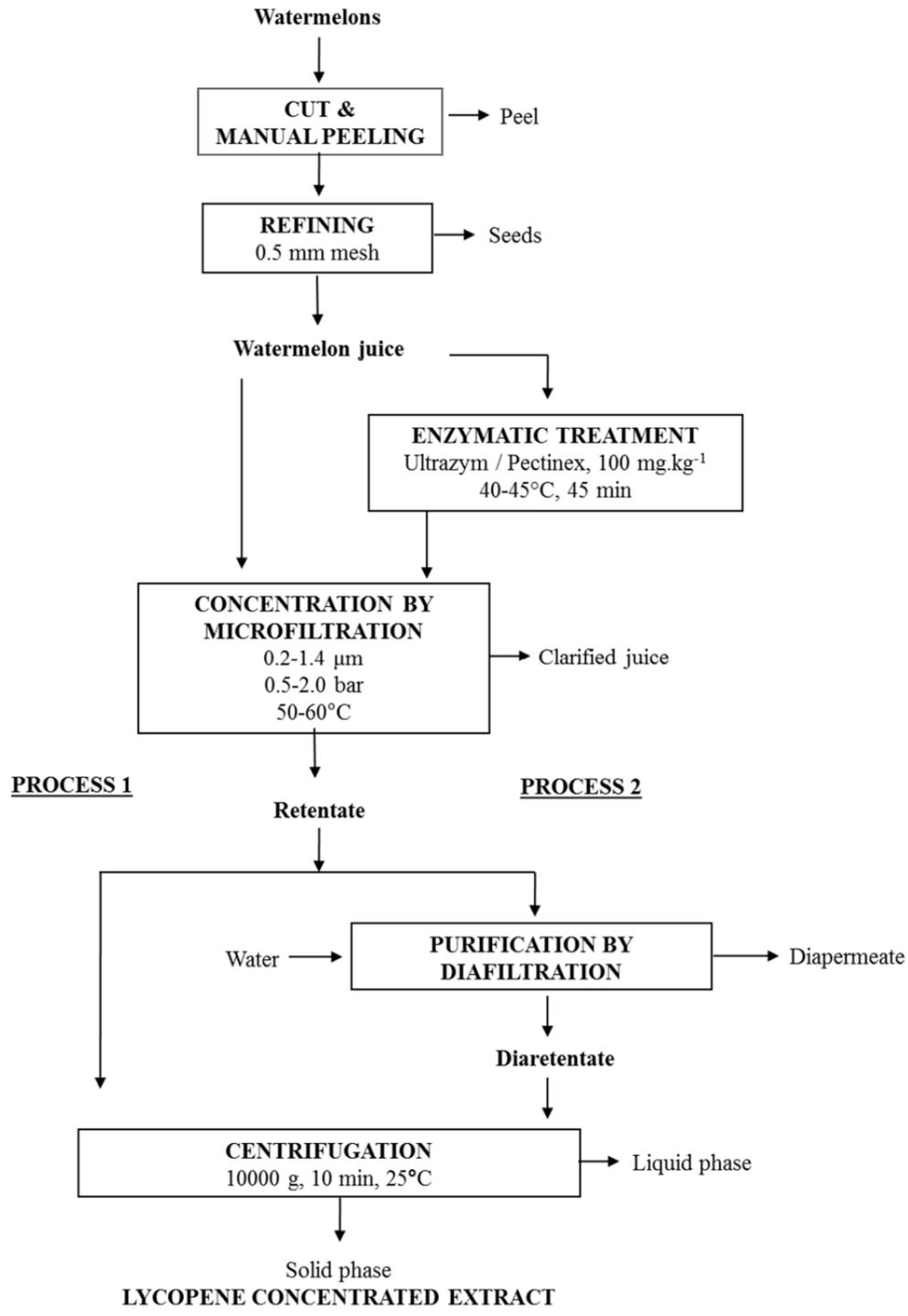
The aim of this paper was to propose and evaluate an environmentally friendly process (without organic solvent) of concentration and purification of lycopene from watermelon juice. This process was based on a microfiltration step for obtaining extracts of lycopene without modifying its native structure.

## **Materials and methods**

### Raw material and processing

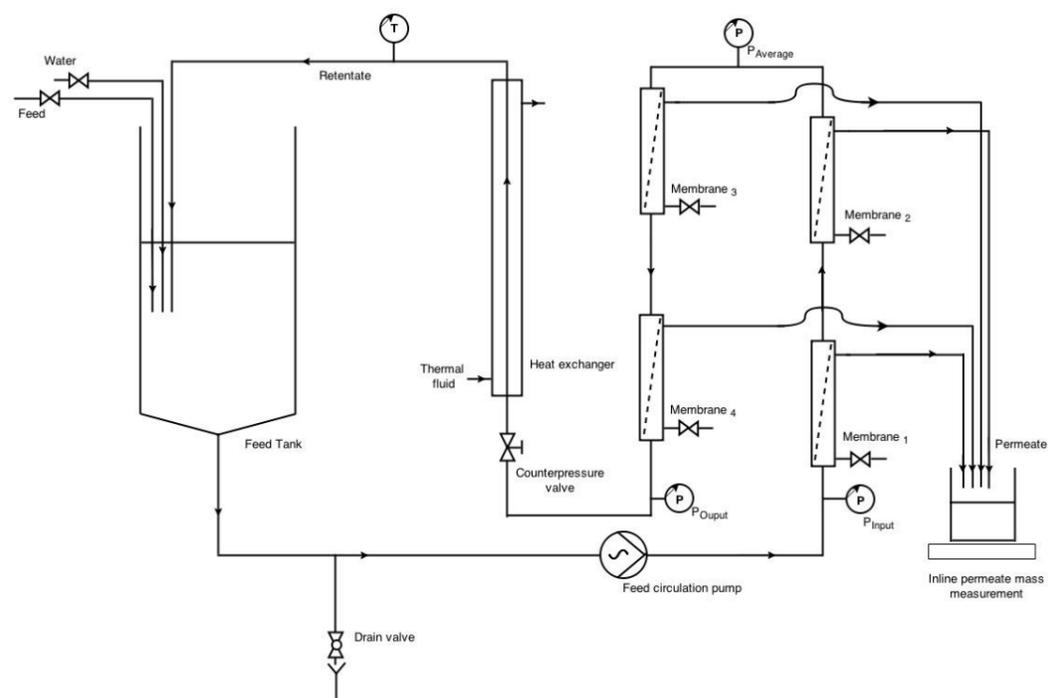
Watermelon fruits (*Citrullus lanatus* L.) were purchased from the local market in Clapiers (Hérault, France). After washing with tap water, they were disinfected with chlorinated water (200 mg·kg<sup>-1</sup> active chlorine), cut and peeled manually and, then subsequently refined in a horizontal pulper Auriol PH3 (Marmande, France) with 0.5 mm mesh. The average mass of watermelon juice extraction yield was 62%. Epicarp and seeds represented 30% and 8% of the final product respectively. The juice was stored at -18 °C until processing. Before each test, the juice was macerated at 40-45 °C for 45 min with 100 mg·kg<sup>-1</sup> of the commercial enzyme blends Ultrazym AFP-L or Pectinex Ultra SP-L (NOVOZYMES, Bagsvaerd, Denmark). According to the supplier, these preparations are obtained from selected strains of *Aspergillus niger* and *Aspergillus aculeatus*. They mainly consist of cellulase and pectinase activities. They have been already used successfully in the processing of a large variety of fruit pulps (Silva *et al.*, 2005 ; Laorko, 2010 ; Nattaporn and Pranee, 2011 ; Machado *et al.*, 2012).

Two processes for the purification and concentration of lycopene were evaluated from watermelon juice. In the first one, the juice was microfiltrated and subsequent centrifugation was performed with the retentate obtained. In the second process it was included a diafiltration step with pure water prior to centrifugation in order to increase the purity of the final extract (**Figure 1**).



**Figure 1.** Flow diagram for lycopene extraction, concentration and purification processes.

Microfiltration experiments were performed in a pilot equipment of laboratory (3 L feeding tank) manufactured by TIA (Bollène, France) with 4 tubular  $\alpha$ -alumina membranes in series Membralox T1-70 (PALL EXEKIA, Bazet, France) with 55 cm<sup>2</sup> effective area each. Temperature was between 50 and 60°C and crossflow velocity was 6 m·s<sup>-1</sup> (**Figure 2**). Selection of pore diameter and transmembrane pressure for microfiltration process were defined according to the results of evaluation of filterability of fresh watermelon juice using membranes with 0.2, 0.5, 0.8, 1.4  $\mu\text{m}$  of average pore diameter and by applying an average transmembrane pressure of 0.5 and 2.0 bar. For this optimization of operating conditions, the feed composition was maintained constant by recycling whole permeate in the feeding tank. The best operating conditions were selected based on the highest permeate flux ( $J_p$ ) and the best retention of lycopene. Concentration trials were then carried out by extracting continuously permeate and maintaining the retentate volume in the circulation loop with addition of fresh product. The process was run until a volumetric reduction ratio VRR (equation I) between 8 and 10.



**Figure 2.** Scheme of the crossflow microfiltration device.

$$\text{VRR} = 1 + \frac{V_p}{V_r} \quad (\text{I})$$

where  $V_p$  and  $V_r$ , permeate and retentate volumes (L), respectively.

Diafiltration process was carried out at constant volume until the soluble solids of the permeate decreased below  $20 \text{ g}\cdot\text{kg}^{-1}$ . The diavolume is defined according to equation II. The conditions of temperature, pressure and flow rate during diafiltration employed were the same than those applied during microfiltration.

$$\text{DV} = \frac{V_w}{V_r} \quad (\text{II})$$

Where  $V_r$  and  $V_w$ , retentate and added water volumes (L), respectively.

Centrifugation of the retentate was performed in a Beckman-Coulter brand J-E Avanti high speed centrifuge (California, USA) by applying an acceleration of  $10.000 \text{ g}$  for  $10 \text{ min}$  at  $25^\circ\text{C}$ . These conditions were established according to commonly used operating parameters in the juice industry.

Concentration and purification factors have been defined as the ratios between final and initial concentrations and purities of lycopene for each operation and for the whole process. In our case, purity was expressed based on the total dry matter content of each product.

## Analyses

### *Physicochemical characterization*

Total soluble solids (TSS) were performed through a digital refractometer Pal3 Pocket Atago (Tokyo, Japan) and the total dry matter (TDM) in vacuum oven at  $30 \text{ mbar}$  according to AOAC (1990) procedures. Color was measured through the coordinates  $L^*$ ,  $a^*$ ,  $b^*$  CIE Lab with a manual chromameter CR-410 (Minolta, Tokyo Japan).

### *Extraction and carotenoid analysis*

Carotenoid extraction was carried out according to Dhuique-Mayer *et al.* (2007). Briefly, 2 g of watermelon juice (or 0.1 to 0.8 g for concentrate plus 10 mL water) were extracted two times by ethanol/hexane 4/3 (v/v). The residue was separated by filtration and re-extracted by ethanol and hexane. Organic phases were washed and were evaporated to dryness with a rotary evaporator at 37°C. Then, carotenoid extract was dissolved in 1 mL of 50/40/10 (v/v/v) mixture of dichloromethane, MTBE (methyltertbutyl-ether) and methanol before to be injected in HPLC.

An Agilent 1100 liquid chromatograph equipped with a photodiode array detector was used (Massy, France). The separation was carried out with a C<sub>30</sub> column (250 x 4.6 mm i.d., 5 µm, YMC, EUROP GmbH, Germany). The mobile phase consisted in H<sub>2</sub>O as eluent A, methanol as eluent B, and MTBE as eluent C. Flow rate was fixed at 1 mL·min<sup>-1</sup>. Column temperature was set at 25°C, and injection volume was 20 µL. A gradient program was performed according to the following A/B/C elution profile: 40/60/0 for 2 min, 20/80/0 for 5 min, 4/81/15 for 10 min, 4/11/85 for 60 min, 0/100/0 for 71 min and 40/60/0 for 72 min. Absorbance was measured at 470, 450 and 400 nm. The carotenoids were analyzed quantitatively by use of external calibration with lycopene and β-carotene. The calibration curves were constructed with 5 concentration levels, each in triplicate. Correlation coefficients ranged from 0.994 to 0.998.

#### *Antioxidant capacity measurement*

Antioxidant capacity of the lipophilic fraction of the process flow was measured according to the method applied by Gomes *et al.* (2013). The procedure is based on the determination with a spectrophotometer of the free radical ABTS discoloration (2,2'-azino-bis-3-ethylbenzotiazolin-6-sulfonic acid) when contacted with the oxidant fraction. For carrying out the reaction, 200 µL of the hexane extract obtained from each sample was mixed with 1.8 mL of 7 mM ABTS in ethanol solution, and then the absorbance was measured at 734 nm in the dark at 30°C after 15 min. The values were calculated using a standard calibration curve using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)

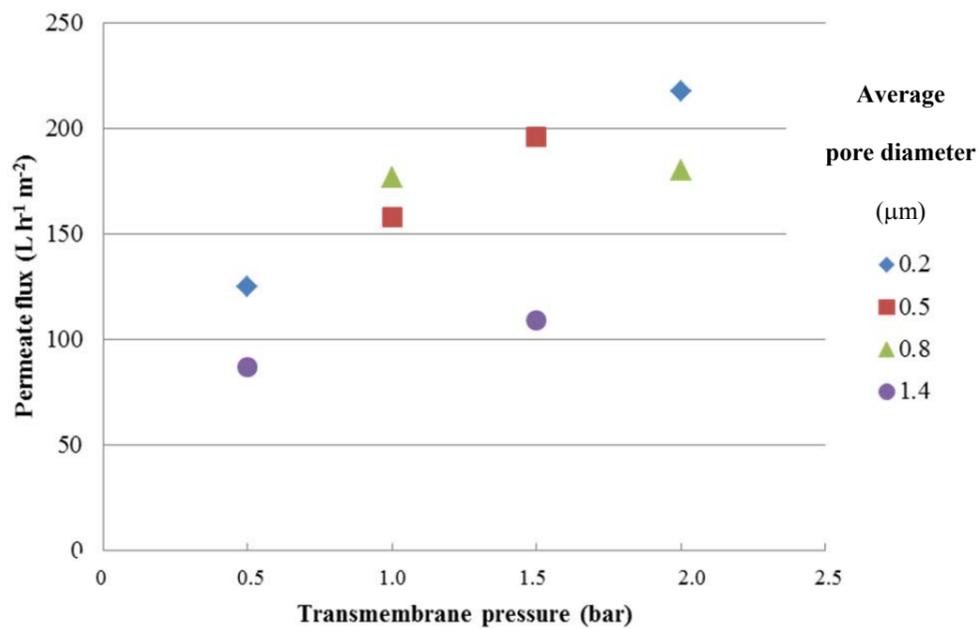
as reference antioxidant, and the results were expressed in  $\text{g eq}\cdot\text{Trolox}\cdot\text{kg}^{-1}$ . This procedure was used based on the results reported by Kuskoski *et al.* (2005) who concluded that the ABTS method is suitable to determine the antioxidant capacity of lipophilic compounds.

## **Results and discussions**

### Selecting operating conditions for microfiltration

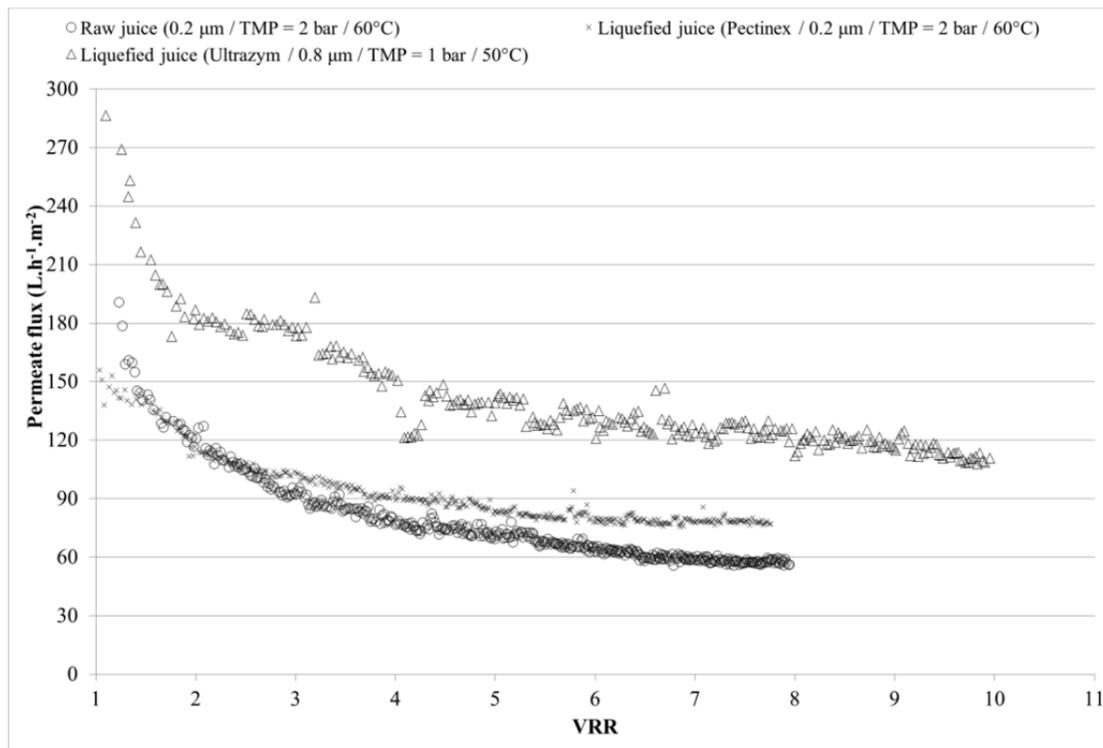
Permeate flux obtained at VRR 1 with membranes with different average pore diameters and at different transmembrane pressures were compared in **Figure 3**. Permeate flux were not correlated with the average pore diameter of the membrane. Except for the 1.4  $\mu\text{m}$  membrane that gave permeate flux clearly lower, all the membranes showed very similar performance. In the operating conditions tested, permeate flux were between 125 and 218  $\text{L h}^{-1} \text{m}^{-2}$  that is promising in an industrial point of view. Flux classically increased with transmembrane pressure except for the 0.8  $\mu\text{m}$  membrane. Whatever the operating conditions chosen no carotenoid was detected in permeate. Even with pore diameter of 1.4  $\mu\text{m}$ , the membrane completely retained carotenoids and an increase in transmembrane pressure did not affect the retention. This showed that carotenoids were retained with the insoluble solids of the juice. Indeed, carotenoids in fruits and vegetables were attached to the matrix of the pulp in different degrees of association (Ananthanarayan and Choudhari, 2007). Typically they were located in chromoplasts with size lower than 2  $\mu\text{m}$ , tightly bound to the cell and therefore can be separate by microfiltration processes together with insoluble compounds.

So, membranes with average pore diameter from 0.2 to 0.8  $\mu\text{m}$  at transmembrane pressure between 1 and 2 bar are well suited for the concentration of carotenoids from watermelon juice. The wider pores could be preferred in order to allow microorganism leakage limiting its concentration into the retentate during the treatment.



**Figure 3.** Stabilized permeate flux of microfiltration obtained with watermelon juice at FRV = 1 and 50°C using membranes with different pore diameters and at different transmembrane pressures.

As shown in **Figure 4** enzymatic liquefaction enhanced permeate flux in concentration mode considerably. For instance at VRR 7, fluxes were increased from 30 % to 100 % using commercial enzymatic preparations. Watermelon juice liquefied with Ultrazym and microfiltrated with the 0.8 μm membrane at 1 bar pressure gave the best performance. As largely described in literature (Vaillant *et al.*, 2000 ; Pinelo *et al.*, 2010 ; Machado *et al.*, 2012), the coupling of an enzymatic step with the microfiltration was clearly highlighted. Modifying the soluble and insoluble polysaccharide profile, this prior enzymatic treatment facilitates mass transfers through the porous media of the membrane by decreasing the viscosity and the fouling potential of the juice.



**Figure 4.** Permeate flux vs. volumetric reduction ratio (VRR) during clarification of watermelon juice by microfiltration: examples under different operating conditions.

Applying an enzymatic liquefaction with Ultradym and a pressure of 1 bar, permeate flux could be maintained above  $100 \text{ L h}^{-1} \text{ m}^{-2}$  until a VRR of 9 was reached. This result makes the concentration process of carotenoids very promising economically because high flux could be maintained at interesting concentration levels. The results were similar to those reported by Gomes *et al.* (2013), who evaluated the effect of microfiltration on lycopene content in fresh watermelon juice using tubular membranes of  $0.1 \mu\text{m}$ . The authors report permeate flux around  $90 \text{ L h}^{-1} \text{ m}^{-2}$  at  $\text{VRR} = 6$  and  $30 \text{ }^\circ\text{C}$  by applying a crossflow velocity of  $6.4 \text{ ms}^{-1}$  and a transmembrane pressure of 3 bar.

Carotenoid analysis showed that no losses occurred during microfiltration carried out at  $50^\circ\text{C}$ . On the contrary trials performed at  $60 \text{ }^\circ\text{C}$  led to significant damage of *all trans* lycopene. Losses reached between 31 and 42 % after concentrating the product up to a

VRR of 8 (around 8 h of filtration). The ratio *cis*-lycopene /(*cis*-lycopene + *trans*-lycopene) is increased from 3-4% to 15% probably due to the application of a temperature ten degrees higher and to longer process time.

Finally, the process chosen for lycopene concentration in watermelon juice combined an enzymatic liquefaction with Ultrazym preparation (100 mg kg<sup>-1</sup>, 40 °C, 45 min) with a microfiltration on a membrane with average pore diameter of 0.8 µm at 1 bar and 50 °C.

#### Biochemical and physico-chemical analysis of the process streams

##### *Process without diafiltration*

**Table 1** shows the evolution of the main characteristics of the product during the optimized process without diafiltration. Concentration factors obtained for *all trans* lycopene and β-carotene in the microfiltration step were close to 11. Because they were similar to VRR, it confirmed that no significant losses of carotenoids were found in the conditions studied. Similarly, Gomes *et al.* (2013) reported no losses during the production of lycopene from watermelon juice by microfiltration. The authors were able to obtain a concentration of lycopene in the retentate of 269 mg.kg<sup>-1</sup> at VRR 6. Whatever the processing step, the proportion of *cis*-isomer among the total lycopene forms was constant between 3 and 4 %. It confirmed that the treatment didn't generate significant isomerization. As expected, no trace of carotenoids was detected in the permeate. No significant differences in total soluble solids, acidity and pH were shown in permeate and retentate compared to the raw juice. As expected, the 0.8 µm membrane didn't retain any solutes and only insoluble fraction was concentrated.

The amount of lycopene in the process streams expressed in g.kgTDM<sup>-1</sup> is indicative of the degree of purity of the compound of interest. The purity in the retentate is nine times higher than in the feed juice because the amount of lycopene concentrated is directly proportional to the fraction of insoluble solids trapped by the membrane. This feature is particularly important because it shows that it is possible to make an effective separation of lycopene by

physical methods, allowing to concentrate and purify carotenoids without significant changes in their original structure.

The centrifugation of the retentate of the microfiltration step was performed in order to obtain a solid residue with a higher concentration. *Trans* lycopene was even concentrated 4 times allowing to reach final concentration of 2741 mg.kg<sup>-1</sup>. Likewise, the concentration of β-carotene increased up to 172 mg.kg<sup>-1</sup>, resulting in a product with a high intake of carotenoids, very promising for application in the design of products with nutritional value. Moreover centrifugation increased product purity 3 folds.

**Table 1.** Change in product composition during the process without diafiltration (operating conditions optimized, mean and standard deviation obtained at the end of the process with n = 3). Nd : not detected.

Product	TDM (g kg <sup>-1</sup> )	TSS (g kg <sup>-1</sup> )	TA (g kg <sup>-1</sup> )	pH	<i>All trans</i> lycopene (mg kg <sup>-1</sup> )	<i>Cis</i> lycopene (mg kg <sup>-1</sup> )	β- carotene (mg kg <sup>-1</sup> )	Purity of <i>all</i> <i>trans</i> lycopene (g kgTDM <sup>-1</sup> )	Antioxydant capacity (g eq. Trolox. kg <sup>-1</sup> )	Color (L*/a*/b*)
Raw juice	99 (2)	97 (2)	0.9 (0.05)	5.06 (0.03)	58 (5)	2.0 (0.1)	4.0 (0.4)	0.61	-	-
Liquefied juice	101 (1)	99 (2)	1.3 (0.02)	5.09 (0.05)	60 (6)	2.0 (0.3)	4.0 (0.6)	0.61	53 (1)	24.14 (0.31) / 21.41 (0.42) / 6.45 (0.39)
Microfiltration retentate	120 (1)	99 (5)	1.5 (0.1)	5.20 (0.06)	657 (32)	21 (1.6)	50 (1)	5.70	170 (16)	41.08 (0.29) / 28.37 (0.39) / 21.82 (0.03)
Microfiltration permeate	-	98 (6)	1.5 (0.1)	5.10 (0.10)	Nd	Nd	Nd	-	17.3 (0.2)	73.31 (0.53) / 1.41 (0.06) / 3.67 (0.17)
Centrifugated retentate (final solid phase)	179 (3)	123 (9)	-	5.20 (0.06)	2741 (143)	111 (7)	172 (7)	15.90	1267 (11)	-

*Process with diafiltration*

In that case, a diafiltration step was performed at the end of the concentration by microfiltration, in order to remove the solubilized fraction present and consequently increase the purity of the extract. This effect is observed in **Table 2**. Acidity, pH and soluble solids decreased in the retentate diafiltered (called diaretentate) at the end of the process, indicating that the water used allowed to remove solutes like sugars and organic acids from the retentate.

As previously, diafiltration did not damage *all trans* lycopene because we didn't observed any formation of *cis* lycopene, that always represented 3 % of the whole lycopene present. Lycopene was concentrated around 10 times in the retentate, which coincided with the VRR reached (no significant loss of lycopene).

**Table 2.** Change in product composition during the process with diafiltration (operating conditions optimized, mean and standard deviation obtained at the end of the process, n = 3). Nd : not detected.

Product	TDM (g kg <sup>-1</sup> )	TSS (g kg <sup>-1</sup> )	TA (g kg <sup>-1</sup> )	pH	<i>All trans</i> lycopene (mg kg <sup>-1</sup> )	<i>Cis</i> lycopene (mg kg <sup>-1</sup> )	β-carotene (mg kg <sup>-1</sup> )	Purity of <i>all trans</i> lycopene (g kgTDM <sup>-1</sup> )	Antioxydant capacity (g eq.Trolox. kg <sup>-1</sup> )	Color (L*/a*/b*)
Raw juice	104 (0.2)	103 (1)	0.75 (0.02)	5.30 (0.01)	60 (2)	2 (0.5)	7 (0.3)	0.60	-	-
Liquefied juice	105 (0.3)	104 (1)	0.86 (0.02)	5.40 (0.01)	64 (2)	2 (0.1)	7 (0.1)	0.63	53 (1)	24.34 (0.21) / 21.51 (0.52) / 6.65 (0.49)
Diaretentate	39 (0.1)	28 (1)	0.20 (0.02)	5.80 (0.02)	580 (39)	18 (2)	47 (1)	15.3	204 (5)	47.26 (0.31) / 23.40 (0.12) / 16.69 (0.13)
Diapermeate	-	20 (1)	0.10 (0.02)	6.00 (0.02)	Nd	-	-	-	-	74.21 (0.43) / 1.39 ( 0.04) / 3.70 (0.2)
Centrifugated diaretentate (final solid phase)	127 (4)	28 (1)	0.20 (0.02)	5.80 (0.02)	2495 (181)	77 (8)	250 (23)	20.3	884 (6)	-

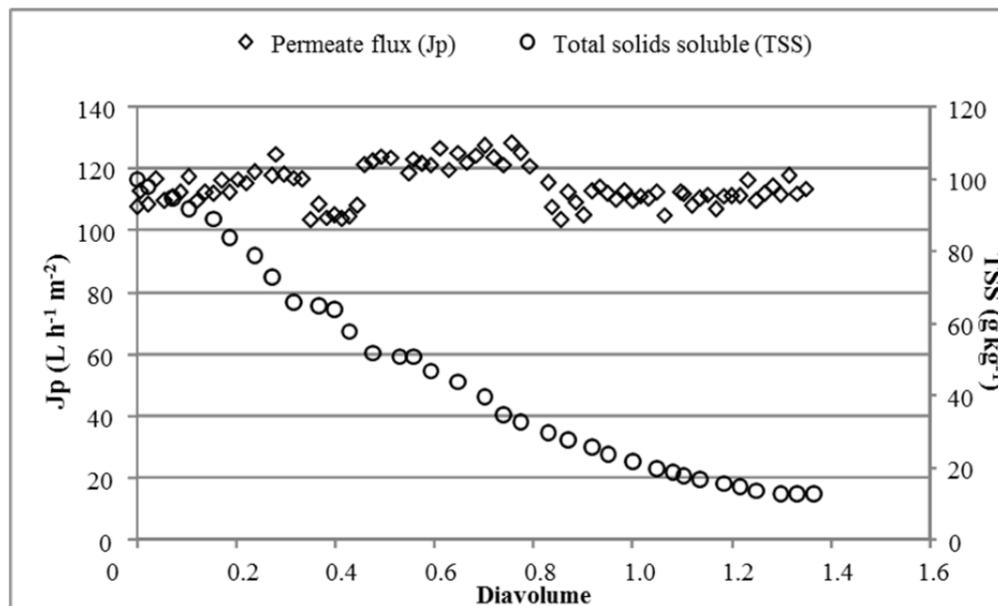
In summary, the integrated process without diafiltration allowed to obtain a final product 47 folds more concentrated in lycopene than the raw juice without damaging its structure (**Table 3**). This extract is 26 times purer relative to the feed stream. The main contribution of the proposed process is to obtain products with higher added value, rich in carotenoids without affecting the characteristics of the natural source.

**Table 3.** Concentration and purification factors (CF, PF) obtained for *all trans* lycopene during processing with and without diafiltration.

Processing step	Process without diafiltration (VRR = 10, DV = 0)		Process with diafiltration (VRR = 10, DV = 1.3)	
	Concentration factor	Purification factor	Concentration factor	Purification factor
Microfiltration or diafiltration	11.3	9.3	9.6	25.5
Centrifugation	4.2	2.8	4.3	1.3
<b>Whole process</b>	<b>47.5</b>	<b>26.1</b>	<b>41.3</b>	<b>33.2</b>

The most important contribution of the diafiltration step is the increase of the purity of the product. Diafiltered retentate purity was increased 33 folds (**Table 3**). It was much higher than in the previous process because of the reduction of soluble solids and with them the total dry matter. **Figure 4** shows the behavior of the permeate flux and total soluble solids during the diafiltration. Average permeate flux during diafiltration was  $114 \text{ L h}^{-1}\text{m}^{-2}$  similar to the flux obtained during microfiltration; meanwhile total soluble solids decreased exponentially in the permeate during processing until reaching  $20 \text{ g.kg}^{-1}$  at the end. This result is important to assess the profitability of the process depending on the amount of water used in the diafiltration and energy required to achieve a greater degree of removal of

solids. In that case, the water that was required to remove 72% of total soluble solids was 1.3 times the amount of the circulating retentate in the concentration loop.



**Figure 4.** Permeate flux (Jp) and total soluble solids (TSS) vs. diavolume during the diafiltration step.

In similar way to the first process, centrifugation allowed to increase 4 times the carotenoids concentration in the retentate that reached  $2500 \text{ mg kg}^{-1}$  for *all trans* lycopene. Moreover, a purification factor of only 1.3 was achieved in that case; this difference is attributed to the diafiltered retentate that behave like a partially purified extract resulting from the elimination of the soluble solids and therefore the retentate dry matter, whereby the centrifugation had a minor contribution to the purification thereof. The yield obtained in the centrifugation step was  $40 \pm 2 \text{ g Precipitated} \cdot 100\text{g retentate}^{-1}$ , which represent very interesting values for the application of this process on an industrial scale.

In summary, by applying the operations detailed in this second process (with diafiltration), it is possible to obtain a product with a concentration of lycopene 41 times higher and 33 purer than in the raw juice. An extract with a higher purity facilitates its application in the

design of products because there is less possibility that other components affecting the organoleptic properties like aroma and flavor of the matrix to which it will be incorporated.

*Lipophilic antioxidant capacity and color analysis of process streams*

The antioxidant capacity of hydrolyzed juice was 52.7 g Trolox eq. kg<sup>-1</sup>. These results are below than those reported by Tlili *et al.* (2011), but the authors admitted that these levels are highly influenced by genotype and sampling area in each material studied. Tirzitis and Bartosz (2010) showed that the antioxidant activity depends substantially on the method of extraction and measurement used. Nevertheless it can be consider the watermelon as a major source of bioactive compounds that could contribute significantly to health as part of antioxidant supplements.

The increase in antioxidant capacity according to total carotenoid content of the extracts obtained from the process products (hydrolyzate juice, permeate, retentate, retentate diafiltered, concentrated and diafiltered concentrate) was evaluated by linear regression. According to the results, the antioxidant activity of the lipophilic fraction of products is proportional to the increase in the total carotenoid content, showing a linear relationship ( $r^2 = 0.9575$ ), which shows that these compounds are those which constitute the increased antioxidant intake. Cuttriss *et al.* (2011) and Waliszewski and Blasco (2010) report that the main feature of bioactivity attributed to lycopene is its antioxidant activity acting as a blocker of free radical reactions.

Tarazona-Diaz and Aguayo (2013) concluded that the red reduction in watermelon juice is related to a decrease in suspended solids and lycopene content which are responsible for this feature. Carbonell *et al.* (2011) found similar changes in orange juices. Meanwhile, samples retained their coordinates have values that exceed twice the values of the juice with enzymatic treatment; these results represent an increase in the amount of pigment in the stream, however Perkins *et al.* (2001) indicate that the color coordinates no always correlate with the lycopene content, since it depends on the composition of the matrix as well as its fruit ripeness.

Moreover, retentate with and without diafiltration have different values for L\*, a\* and b\*. The final extract without diafiltration had higher values of Chroma, indicating a more intense red color. Accordingly diafiltration process had an effect on the color of the retentate, which could be associated with changes in pH of the product by reducing the percentage of acidity. Sharma *et al.* (2008), during the evaluation of the degradation kinetics of color in watermelon juice, observed a relationship between the change in pH and the changes of color. Color of the retentate stream suggest the possibility of applying these products as potential natural dyes in food.

## **Conclusion**

The processing scheme defined in this study allowed to concentrate lycopene from watermelon juice up to 48 times and to purify it up to 34 times without generating cis-isomerization and so keeping lycopene in its native form. Final extract contained around 2.5 g.kg<sup>-1</sup> of lycopene with a purity close to 20 g.kgTDM<sup>-1</sup>. Interest of diafiltration was clearly proved in order to purify carotenoids removing most of soluble solids. Permeate flux obtained were very promising to envisage industrial application (above 100 L.h<sup>-1</sup>.m<sup>-2</sup>). Antioxidant capacity shown by the products was highly correlated with the lycopene content in each sample. During the process, an important quantity of permeate is generated. This coproduct doesn't contain any carotenoids but contains the soluble solids especially sugars and organic acids of the initial juice. These features suggest the possibility of using it in developing clarified fruit drinks for instance.

These results are very promising for obtaining products with high concentration of lycopene and their application as bioactive ingredient in the design of products with added value. The next step will be the estimation of the economic viability of the process, the study of the stabilization of the extracts obtained, and application to other fruits with a higher carotenoid content and other carotenoid profiles.

Acknowledgements

This work was performed within the project "Use of novel processing methods for production optimization and quality and safety of fruits juices and drinks, and investigation of nonconventional sources for their nutritional fortification and improvement of functional properties" of the cooperation program of postgraduate (PCP) France-Venezuela agreement.

We thank the Center for International Cooperation in Agronomic Research for Development (CIRAD), the University Lisandro Alvarado (UCLA-VENEZUELA) and University Central of Venezuela (UCV-VENEZUELA) for support.

## References

- Abreu, F. A., Dornier, M., Dionisio, A. P., Carail, M., Caris-Veyrat, C., & Dhuique-Mayer, C. (2013). Cashew apple (*Anacardium occidentale* L.) extract from by-product of juice processing: A focus on carotenoids. *Food Chemistry*, *138*(1), 25-31.
- Anese, M., Mirolo, G., Fabbro, A., & Lippe, G. (2013). Lycopene bioaccessibility and bioavailability from processed food. *Journal of scientific y Industrial researche*, *72*, 543-547.
- Boon, C. S., McClements, D. J., Weiss, J., & Decker, E. A. (2010). Factors Influencing the Chemical Stability of Carotenoids in Foods. *Critical Reviews in Food Science and Nutrition*, *50*(6), 515-532.
- Bramley, P. M. (2000). Is lycopene beneficial to human health? *Phytochemistry*, *54*, 233-236.
- Carbonell, J., Tarrega, A., Gurrea, M., & Sentandreu, E. (2011). Chilled orange juices stabilized by centrifugation and differential heat treatments applied to low pulp and pulpy fractions. *Innovat Food Sci Emerg Technol*, *12*, 315-319.
- Cissé, M., Vaillant, F., Pallet, D., & Dornier, M. (2011). Selecting ultrafiltration and nanofiltration membranes to concentrate anthocyanins from roselle extract (*Hibiscus sabdariffa* L.). *Food Research International*, *44*(9), 2607-2614.
- Cruz, R., González, J., & Sánchez, P. (2013). Propiedades funcionales y beneficios para la salud del licopeno. *Nutricion Hospitalaria*, *28*(1), 6-15.
- Cuttriss, A., Cazzonelli, C., Wurtzel, E., & Pogson, B. (2011). Carotenoids. In *Advances in Botanical Research.*, vol. 58). Canberra.
- Dhuique-Mayer, C., Tbatou, M., Carail, M., Caris-Veyrat, C., Dornier, M., & Amiot, M. J. (2007). Thermal degradation of antioxidant micronutrient in citrus juice: Kinetic and newly formed compounds. *Journal of Agricultural and Food Chemistry*, *55*, 4209-4216.
- Gomes, F., Costa, P., Campos, M., Tonon, R., Couri, S., & Cabral, L. (2013). Watermelon juice pretreatment with microfiltration process for obtaining lycopene. *International Journal of Food Science & Technology*, *48*(3), 601-608.
- Kuskoski, E., Asuero, A., Troncoso, A., Mancini, A., & Fett, R. (2005). Aplicacion de diversos metodos quimicos para determinar actividad antioxidante en pulpa de frutos. *Cienc. Technol. Aliment.*, *4*(25), 726-732.
- Laorko, A., Li, Z., Tongchitpakdee, S., Chantachum, S., & Youravong, W. (2010). Effect of membrane property and operating conditions on phytochemical properties and permeate flux during clarification of pineapple juice. *Journal of Food Engineering*, *100*(3), 514-521.
- Machado, R. M. D., Haneda, R. N., Trevisan, B. P., & Fontes, S. R. (2012). Effect of enzymatic treatment on the cross-flow microfiltration of açai pulp: Analysis of the fouling and recovery of phytochemicals. *Journal of Food Engineering*, *113*(3), 442-452.
- Nattaporn, W., & Pranee, A. (2011). Effect of pectinase on volatile and functional bioactive compounds in the flesh and placenta of 'Sunlady' cantaloupe. *International Food Research Journal.*, *18*, 819-827.

- Perkins-Veazie, P., & Collins, J. (2006). Carotenoid Changes of Intact Watermelons after Storage. *J. Agric. Food Chem.*, *54*, 5868-5874.
- Perkins, P., Collins, J., Pair, S., & Roberts, W. (2001). Lycopene content differs among red-fleshed watermelon cultivars. *Journal Science Food Agriculture*, *81*, 983-987.
- Pinelo, M., Zeuner, B., & Meyer, A. (2010). Juice clarification by protease and pectinase treatments indicates new roles of pectin and protein in cherry juice turbidity. *Food and Bioproducts Processing*, *88*(2), 259-265.
- Razi, B., Aroujalian, A., & Fathizadeh, M. (2012). Modeling of fouling layer deposition in cross-flow microfiltration during tomato juice clarification. *Food and Bioproducts Processing*, *90*(4), 841-848.
- Sharma, R., Kaur, D., Oberoi, D., & Sogi, D. (2008). Thermal degradation kinetics of pigments and visual color in watermelon Juice. *International Journal of Food Properties*, *11*, 439-449.
- Shi, X. F., Xu, Y., Li, Y. H., Zeng, H. X., & Sun, Y. H. (2011). Optimization of Extraction Process of Lycopene from Watermelon (*Citrullus lanatus*) by Response Surface Methodology. *Applied Mechanics and Materials*, *140*, 385-393.
- Silva, T., Della, R., Penha, E., da Matta, V., & Corrêa, L. (2005). Suco de maracujá orgânico processado por microfiltração. In).
- Tarazona-Díaz, M. P., & Aguayo, E. (2013). Influence of acidification, pasteurization, centrifugation and storage time and temperature on watermelon juice quality. *Journal of the Science of Food and Agriculture*, *93*(15), 3863-3869.
- Tarazona-Díaz, M. P., Alacid, F., Carrasco, M., Martínez, I., & Aguayo, E. (2013). Watermelon Juice: Potential Functional Drink for Sore Muscle Relief in Athletes. *Journal of Agricultural and Food Chemistry*, *61*(31), 7522-7528.
- Tirzitis, G., & Bartosz, G. (2010). Determination of antiradical and antioxidant activity: basic principles and new insights. *Acta biochimica polonica.*, *57*(1), 139-142.
- Tlili, I., Hdider, C., Salvatore, M., Riadh, I., Jebari, H., & Dalessandro, G. (2011). Bioactive compounds and antioxidant activities of different watermelon (*Citrullus lanatus* (Thunb.) Mansfeld) cultivars as affected by fruit sampling area. *Journal of Food Composition and Analysis.*, *24*, 307-314.
- Vaillant, F., & Decloux, M. (2000). *Clarification et Concentration des jus de Fruits Tropicaux Pulpeux Associant Traitements Enzymatiques, Microfiltration Tangentielle et Evaporation Osmotique*. Unpublished These de Doctorat, ENSIA, Paris, France.
- Van Buggenhout, S., Almingier, M., Lemmens, L., Colle, I., Knockaert, G., Moelants, K., Van Loey, A., & Hendrickx, M. (2010). "In vitro" approaches to estimate the effect of food processing on carotenoid bioavailability need thorough understanding of process induced microstructural changes. *Trends in Food Science & Technology.*, *21*(12), 607-618.
- Waliszewski, K., & Blasco, G. (2010). Propiedades nutraceuticas del licopeno. *Salud pública de méxico.*, *52*(3), 254-265.
- Wang, S. (2007). Fruits with high antioxidant activity as functional foods. In J. Shi (Ed.), *Functional food ingredients and nutraceuticals, processing technologies.* Florida.
- Wen'en, Z., Pin, L., & Huihui, G. (2013). Studies on carotenoids in watermelon flesh. *Agricultural Sciences*, *4*(7A), 13-20.
- Wertz, K., Siler, U., & Goralczyk, R. (2004). Lycopene: modes of action to promote prostate health. *Archives of Biochemistry and Biophysics*, *430*(1), 127-134.

