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Integrated process for extraction and formulation in emulsions of active molecules from fresh passion fruits (*Passiflora edulis Sims*)

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ABSTRACT

Intensified green processes were investigated to extract several classes of active molecules from fresh purple passion fruits, and to prepare emulsions directly by extrusion for the fruit comprehensive utilization. A thermo-mechanical treatment was carried out through a discontinuous process from the edible part and through a continuous process by twin-screw extrusion using the whole fruit. Only mechanical energy was applied to the materials to generate a liquid fraction (filtrate) and a solid residue. Lipid contents of the obtained filtrates ranged from 0.5 to 2.6 g/100 g close to the extractible lipophilic part. Polyphenols extraction was improved when using the whole fruit and with the addition of water in extrusion. *In-situ* emulsions with droplets sizes between 2 and 8 µm were obtained. Emulsions obtained after extrusion showed an enhanced stability. This way represents a green alternative method to solvent extractions applied to the different parts of the fruit, and leads to bio-based emulsions enriched in vegetable oil, natural antioxidants and emulsifiers, such as phospholipids, proteins, polysaccharides, pectins and polyphenols.

1. Introduction

Purple passion fruit (*Passiflora edulis Sims*) is originated from Brazil. It is now widely cultivated in tropical regions, such as India, Australia or South Africa. On Reunion Island in 2017, 32 ha have been dedicated to the passion fruit cultivation, with a production of 500 metric tons according to the French Department of Agriculture and Food statistics. Passion fruit transformation is a growing industry, due to increasing demand for juice in Europe, in Canada and in the United States (Von der Linden, 2007). The juice production generates large amount of agro industrial residues composed of rinds and seeds. Residue represent 76% of the weight of the raw material after fruit processing (Leão et al., 2014). Thus, the expansion of this industry may create environmental issue by causing an increase in the production of wastes (Malacrida and Jorge, 2012).

In the last decade, many studies focused on the valorization of agro industrial wastes. Indeed, the production of high added value molecules from by products is an economically and environmentally attractive solution. Fruit residues like shells and seeds are thus recognized as a valuable source of chemicals for food, cosmetic or pharmaceutical

industries (Da Silva et al., 2014). Previous works showed that passion fruit seeds contain up to 30% of oil rich in unsaturated fatty acids, and especially in linoleic acid (70%) (Delvar et al., 2019). Moreover, the different parts of passion fruit are rich in phenolic compounds (Septembre Malaterre et al., 2016) leading to potential applications in food or cosmetic industries (Oliveira et al., 2016; Morais et al., 2015).

Concerning the passion fruit seed oil extraction, several methods were studied like maceration in solvents of different polarities (Oliveira et al., 2016), supercritical carbon dioxide extraction (SC CO₂) (Zahedi and Azarpour, 2011), ultrasound assisted or Soxhlet extractions (De Oliveira et al., 2013). In these cases, it is usually necessary to separate the seeds from the residual pulp and to dry the fruit materials. Moreover, common extraction organic solvents may have potential environmental and health impacts, and may alter the quality of the extracts. It is also necessary to consider recycling of the solvents or recovery steps. A supercritical fluidic extraction from passion fruit seeds gives a high quality extract (vegetable oil) but requires a high pressure equipment (Zahedi and Azarpour, 2011).

Twin screw technology, as a mechanical treatment adapted to vegetable raw materials, has the advantages to combine compression and

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shearing effects, to perform liquid solid extraction and separation (Evon et al., 2007). The type of screw elements, their length and their disposition determine the screw profile according to the materials. New valorization ways for agro industrial by products have been recently developed with this method. For instance, polyphenols were extracted with water from wood residues (Celhay et al., 2014) and hydroxycinnamic acids were obtained from hemp by products (Candy et al., 2017). Many studies also focused on the extraction of seed oil by extrusion, by expression and by aqueous extraction (Evon et al., 2007; Kartika et al., 2005; Lacaze Dufaure et al., 1999; Magimel et al., 2016). The aim of this work was to implement new extraction processes of molecules of interest from purple passion fruit (whole fruit or co products) and to prepare oil in water emulsion (which is the target product) directly by extrusion for the fruit comprehensive utilization. If extractions are usually performed from the seeds after fractioning the fruit co product, in this study, the objective was to develop a one step process to extract and formulate at the same time several hydrophilic and lipophilic active compounds in oil in water emulsions. To our knowledge, no work mentions the possibility to prepare emulsions from fresh fruit directly by extrusion. Furthermore, most of works have focused in the recovery of the pure seed oil after the extrusion process. So, a stage of centrifugation is usually necessary in order to obtain the pure oil. The new approach proposed here should save processing costs and fruit processing time because the final product, the crude oil in water emulsion, could be used directly in natural formulations which required lipophilic compounds emulsified in water, such as in natural paint, cosmetic, or food formulations.

The strategy of combining the extraction of active molecules (such as vegetable oil, phospholipids, polyphenols and proteins) and their formulation in oil in water emulsion was tested with two methods. The feasibility has been first evaluated in a batch process at laboratory scale, before using the continuous twin screw extrusion at pilot scale. Twin screw extruder was supplied with the whole fruit, including the shell rich in cellulosic fibers, for the fruit comprehensive utilization. In order to prepare enriched oil in water emulsions, the media were characterized in connection with their possible applications in industry (food, cosmetic, paint, etc.).

2. Material and methods

2.1. Plant material

Plant materials consisted of fresh ripe purple passion fruit (*Passiflora edulis* Sims). They were collected from a local industry (Vergers Law Yat) in Reunion Island. The fruits were frozen and stored at -20°C until use

2.1.1. Chemical reagents

Gallic acid, Folin Ciocalteu reagent, DPPH (2,2 diphenyl 1 picrylhydrazyl) and L-fucose were purchased from Sigma Aldrich.

Solvents were analytical grade and were obtained from VWR International.

2.1.2. Raw materials characterization

The dry matter was measured by drying the sample at 103°C for 24 h. The mineral content was determined after calcination at 550°C for 6 h. The following analyses were performed from dried materials. The cellulose, hemicellulose and lignin contents were estimated using the Van Soest and Wine method. The nitrogen content was determined by the Kjeldahl method (Tecator Kjeltex 2200, Denmark) according to the NF V18 100 standard and the protein content was calculated by applying a 6.25 multiplication factor. Lipidic extractives were estimated by Soxhlet extraction in hexane for 4 h. Hydrophilic extractives were obtained after extraction with water for 1 h in a Fibertec apparatus (Tecator M1017, Denmark).

2.1.3. Mechanical pressing of passion fruit seeds

Mechanical pressed seed oil was obtained by pressing dried passion fruit seeds (300 g) in a screw press (Komet, Germany) with a maximum oil flow rate of 1 kg/h, powered by an electric motor (0.75 kW). The press cylinder was heated before extraction to a temperature comprised between 80 and 100°C . The press had a nozzle diameter of 5 mm, a screw length of 18 cm, and a screw speed of 40 rpm.

2.1.4. Discontinuous process: batch extraction

The batch extraction process consisted of two ways depending on the raw material used. In both ways, the edible part of 15 passion fruits was separated from the shell. For the way 1, all the edible part (juice, pulp, seeds) was used. For the way 2, the juice (223 g) was replaced by the same amount of water (223 g). For both ways, the material was grinded with a blender (Gründig) at maximum speed for 15 s (3 times) with manual homogenization between each grinding and then grinded with a laboratory mixer (Silverson, France) for 2 min. The mixture was filtered on a nylon tissue ($100\ \mu\text{m}$) to obtain Filtrate 1 (way 1) and Filtrate 2 (way 2). The filtrates were emulsified with an Ultraturax apparatus (T25 digital, IKA, Germany) for 2 min at 18000 rpm to prepare emulsions 1A and 2A (ways 1 and 2, respectively). Enriched emulsions 1B and 2B containing 10% of oil were prepared by adding the required quantity of mechanical pressed passion fruit seed oil to the Filtrates 1 and 2 before emulsification.

2.1.5. Twin screw extrusion of whole passion fruit

A co rotating and co penetrating twin screw extruder Clextoral BC21 (Firminy, France) was used to conduct the experiments. The extruder was composed of seven modular barrels of 100mm length. The temperature in the modules 2, 3, 4, 5 and 7 was regulated to 30°C by water circulation (Fig. 1). The module 6 was a filter section (1 mm perforations) to enable the recovery of the liquid fraction. Different screw elements of 25 or 50 mm length were used (Fig. 2). Passion fruits (5 kg) cut in pieces of 2×2 cm were manually fed in the extruder at a

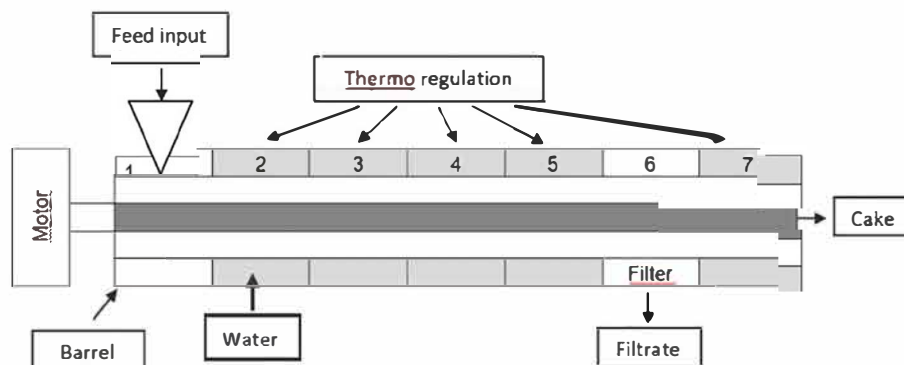


Fig. 1. Schematic modular barrel of the BC21 twin-screw extruder.

T2F : trapezoidal double-thread screw ; C2F : conveying double-thread screw ; BB : bilobe paddle-screw ; CF2C : reversed double-thread screw

The numbers following the type of screw indicate the pitch of T2F, C2F, CF2C and the length of the BB screws.

Profile 1	1		2		3		4		5		6		7				
	T2F 50	T2F 50	C2F 33	C2F 33	C2F 25	BB 90° (6*5)	C2F 33	C2F 25	BB 90° (10*10)	C2F 33	C2F 25	C2F 25	C2F 16	C2F 16	C2F 16	CF2C-25	C2F 33

Profile 2	1		2		3		4		5		6		7				
	T2F 50	T2F 50	C2F 33	C2F 33	C2F 25	BB 90° (6*5)	C2F 33	C2F 25	BB 90° (10*10)	C2F 33	C2F 25	C2F 25	C2F 16	C2F 16	C2F 16	CF2C-16	C2F 33

Fig. 2. Screw configuration for the passion fruit extractions.

flowrate of 6 kg/h (Q_{RM}). Fruit juice acted as a solvent. The screw rotation speed was set to 150 rpm. The raw liquid fraction and the solid fraction (cake) were recovered.

A second extraction was performed by feeding the solid cake of the first extraction (1 kg) in the extruder at a flowrate of 6 kg/h. The screw configuration was modified by changing the pitch of the reversed double thread screw in module 7 (Fig. 2). Water was injected in the module 2 at a flowrate of 4.8 kg/h with a piston pump giving a liquid to solid ratio of 2.8 on a dry matter basis, calculated according to the following formula:

$$ratio_{S/L} = \frac{Q_S \times DM_S}{Q_S \times (100 - DM_S) + Q_W} \times 100$$

where Q_S (kg/h) is the fed extrudate flowrate, DM_S (g/100 g) is the extrudate dry matter and Q_W (kg/h) is the water flowrate.

The particles contained in the raw liquid fractions were filtered on nylon tissue (100 μ m) to obtain Filtrate 3 (first extraction) and Filtrate 4 (second extraction). Enriched emulsions 3A and 3B containing 2.5% and 10% of oil respectively were prepared by adding the required quantity of mechanical pressed passion fruit seed oil to Filtrate 3 before emulsification for 2 min at 18000 rpm.

The solid fraction (extrudate), filtrates and filtration residue (particles) were weighed and their dry matters (g/100 g) were determined (DM_S , DM_F , and DM_P respectively). The solid (Q_S), filtrate (Q_F) and particle (Q_P) flow rates were measured. The yield (g/100 g DM) in solid fraction (Y_S), filtrate (Y_F), and particles (Y_P) were calculated according to the formula:

$$Y_X = \frac{Q_X \times DM_X}{Q_{RM} \times DM_{RM}} \times 100$$

where Q_X (kg/h) is the considered fraction flowrate, DM_X (g/100 g) the considered fraction dry matter, Q_{RM} (kg/h) is the raw materials flow rate, and DM_{RM} (g/100 g) is the raw materials dry matter.

The oil yield Y_O (g/100 g DM), protein yield Y_N (g/100 g DM) and polyphenols yield Y_{PP} (g/100 g DM) were determined according to the formula:

$$Y_x = \frac{Q_F \times DM_F \times R_x}{Q_{RM} \times DM_{RM}}$$

where R_x (g/100 g DM) is the oil, protein or polyphenol content in the filtrate.

2.1.6. Filtrates composition

2.1.6.1. Liquid liquid oil extraction with cyclohexane. A liquid liquid extraction with cyclohexane was performed on the filtrates. 150 g of filtrate and 100 mL of cyclohexane were mixed and were centrifuged at 10^4 g for 10 min (Sigma 6 16K). Aqueous phases and organic phases were recovered. The centrifugation pellets were then suspended in 100 mL of cyclohexane and were centrifuged in the same conditions. The aqueous phase was washed with 50 mL of cyclohexane. The organic phase was washed with 50 mL of distilled water and was dried with anhydrous magnesium sulfate. The solvent was then evaporated to

recover the oil.

2.1.6.2. Total polysaccharides content. Polysaccharides of the filtrates were analyzed by ion exchange chromatography using a CarboPac PA1 column (Dionex, France). The eluent was potassium hydroxide with a 1 mL/min flowrate. A gradient of concentration was used starting at 2 mM for 39 min then increased to 10 mM for 2 min, 100 mM for 8 min and 100 mM for 3 min. The temperature was set to 25 °C. The injection volume was 25 μ L. External standards were used for the polysaccharides identification. Fucose was used as internal standard for the polysaccharides quantification.

2.1.6.3. Total phenolics content. Total phenolic contents of the filtrates were determined using Folin Ciocalteu method as follows: 20 μ L of filtrates, 10 μ L of Folin Ciocalteu reagent and 170 μ L of sodium carbonate solution (2.36%) were added in a 96 well microplate. After 45 min of incubation at 45 °C, the absorbance was measured at 760 nm with a spectrophotometer (Spectrostar, BMG Labtech). Standard solutions of gallic acid were used to build the calibration curve. Each sample was analyzed in 4 wells. Results were expressed as gram of gallic acid equivalent per 100 g of sample dry basis (GAE/100 g d.b.).

2.1.7. Oil in water emulsions characterization

2.1.7.1. Optical microscopy. The microstructure of filtrates and freshly prepared emulsions 1A, 2A and 3A was observed by optical microscopy with a Nikon Eclipse E600 with a $\times 40$ magnification. The images acquisition was done with a camera Nikon DS Fi2. A drop of sample was placed on a microscope slide and was covered by a cover slip. Images were captured at several location in order to be representative of the sample.

2.1.7.2. Droplet size distribution. Filtrates 1 and 2 and freshly prepared oil in water emulsions 1A, 1B, 2A and 2B of edible part of passion fruit were analyzed for the droplet size distribution using a Mastersizer 2000 laser diffraction particle size analyzer (Malvern). The refractive index of water (1.33) was used for the droplet size distribution calculation. Results are shown as the droplet size distribution profile corresponding to the volume fraction (%) vs. the droplet size (μ m).

2.1.7.3. Rheology. A rheometer MCR 302 (Anton Paar) with a cone plate geometry of 50 mm diameter and 2° angle was used to perform rheological analysis of the samples (Filtrates 1, 2, 3, and emulsions 1A, 1B, 2A, 2B, 3A, 3B). All experiments were carried out at a temperature of 25 °C regulated with a bath thermostat (Huber). The viscosity and shear stress were studied by increasing exponentially the shear strain from 0 to 50 s⁻¹. Then, in order to determine the viscoelastic region of the emulsions, amplitude sweeps were done by increasing shear strain (0.01% 100%) at a frequency of 1 Hz. Storage module G' , loss module G'' and tangent δ were obtained. All experiments were performed in triplicates.

2.1.7.4. Oil in water emulsion stability. The emulsion stability was

Table 1
Chemical composition of the raw materials expressed in g/100 g DM (n = 3).

	Pulp	Seeds	Shells	Whole fruit
Mass repartition (% of fresh fruit)	15.8 ± 0.8	7.7 ± 0.3	50.0 ± 1.1	100
Dry matter (%)	12.7 ± 0.3	78.1 ± 0.4	16.0 ± 0.2	19.9 ± 1.2
Minerals	5.5 ± 0.1	2.1 ± 0.0	20.9 ± 1.5	14.0 ± 1.5
Cellulose	37.0 ± 1.0	52.1 ± 0.4	30.6 ± 0.2	25.6 ± 1.2
Hemicellulose	13.0 ± 1.6	2.4 ± 0.6	11.9 ± 0.1	11.6 ± 0.7
Lignin	7.1 ± 0.1	2.2 ± 0.0	0.0 ± 0.0	1.6 ± 1.4
Protein	7.7 ± 0.1	12.2 ± 0.1	7.5 ± 0.1	8.4 ± 0.0
Pectin	3.3 ± 0.3	-	7.3 ± 1.0	5.2 ± 0.4
Lipophilic extractives	0.7 ± 0.1	23.6 ± 0.9	0.4 ± 0.0	5.8 ± 0.2
Hydrophilic extractives	31.4 ± 0.3	15.7 ± 0.8	44.2 ± 0.2	53.8 ± 0.9
Polyphenol content in hydrophilic extracts (g GAE/100g DM)	3.1 ± 0.1	6.7 ± 0.4	1.5 ± 0.3	1.1 ± 0.3
Polyphenol	1,0	1,1	0,7	0,6

assessed by the determination of the creaming index (CI). Freshly prepared emulsions (16 g) were transferred into transparent glass tubes (22 mm internal diameter, 45 mm height) sealed by plastic caps. The tubes were kept at room temperature (25 °C) for 14 days. The total heights of the emulsions in the tubes (H_T) were noted. The emulsion stability was followed by measuring the height of the transparent serum layer (H_S) formed below the opaque “cream” layer. The creaming index (CI) was then calculated with the following formula:

$$CI (\%) = \frac{H_S}{H_T} \times 100$$

3. Results and discussion

3.1. Raw materials characterization

The chemical compositions of the different parts (pulp, seeds, and shells) of the whole purple passion fruit were determined (Table 1). Cellulose (25.6 g/100 g DM whole fruit) was the main structural component found in all parts of the fruit. The whole fruit presented a high content in minerals (14%) resulting mainly from the shells. As expected, the lipids are mainly concentrated in fruit seeds (23.6 g of lipids per 100 g of dry seeds). The edible part of the fruit, *i.e.*, the pulp and the seeds, are also rich in polyphenols with 3.1 and 6.7 g GAE/100 g DM in their hydrophilic fractions, respectively.

3.2. Discontinuous process: batch extraction

The discontinuous process extraction from edible part of passion fruit was implemented according to two ways to obtain filtrates, *in situ* emulsions and enriched emulsions (Fig. 3).

The results are presented in Table 2. As expected, the starting materials dry matter is lower for the way 2 due to the removal of the juice. Similar quantities of filtrates are obtained for both ways of the batch process (81.1 83.7 g per 100 g of fresh fruit). The dry matter yield showed that the same proportion of initial dry matter is obtained with both ways. The oil is efficiently extracted with a 75% recovery of the lipids of the seeds contained in the edible part. The fruit juice does not influence the oil extraction yield. The protein yield is higher for the way 1, thanks to the juice proteins extraction. Nearly 75% of the proteins of the edible part are recovered in the Filtrate 1. The polyphenols yield is also higher for the way 1, indicating that polyphenols are present in the passion fruit juice. The way 1 allows the recovery of 43% of the extractable polyphenols of the edible part.

3.3. Twin screw extrusion of whole passion fruit

A two step extraction was also performed by twin screw extrusion

on the passion fruit (Fig. 4). In the first step, the extrusion was conducted with the whole fruit (shells, pulp, seeds) using the water contained in the fresh fruit as solvent. The step two was performed with the residual solid cake of the first step. The screw profile was modified and a flow rate of water was added to the extrudate giving a liquid to solid ratio of 2.8 on a dry matter basis. The particles contained in the raw liquid fractions were filtered to obtain Filtrate 3 (first step) and Filtrate 4 (second step).

The operating conditions implemented with passion fruits generated the formation of a fibrous dynamic plug without clogging. The yields of extrudate (Y_E) and particles (Y_P) were used as indicators for the raw materials defibration. The extrudate yield (53%) enables to check the efficiency of the fruit destructuring during the first step (Table 3). The solid/liquid separation was also effective, as reflected by the filtrate flow rate (Q_F) and the extrudate dry matter (DM_E). For the second step of extraction with twin screw extrusion, the decrease of the pitch of the reversed double thread screw in module 7 and the addition of water enabled a further defibrating as shown by the extrudate and particles yields.

From the inlet and filtrates flow rates obtained, extraction yields in lipids, proteins and polyphenols were calculated (Table 4).

The oil extraction yield of from passion fruits (246.2 mg/100 g of fresh whole fruit) corresponds to 18% of the lipids of the seeds in the fresh whole fruit (1,4 g/100 g). The global yield obtained after the aqueous extraction of the extrudate 1 (268.9 mg/100 g of fresh whole fruit) indicates that part of the released lipophilic molecules is still retained in the solid residue. The results from other studies, obtained for vegetable oil extraction from sunflower seeds (Evon *et al.*, 2009) and from coriander fruits (Sriti *et al.*, 2012), seem to indicate that it should be possible to improve this extraction yield by modifying the screw configuration and the operating conditions. For oil extraction, the batch process achieves higher yields than the continuous process. The use of the high shear mixer therefore promotes the extraction of lipids in comparison to the extruder. These results could be explained in terms of grinding. The seeds crushed with the mixer led to thin particles, which made the release of the vegetable oil easier compared to extrusion. The first step of the continuous process enables the recovery of 28% of the proteins of the whole fresh fruit (1.4 g/100 g). The aqueous extraction of the extrudate 1 during the second step makes it possible to extract a portion of the proteins contained in extrudate 1 and to achieve a global protein yield of 0.7 g/100 g of fruit, *i.e.* 38% of the potential. The higher yields obtained for the batch process seem to indicate that the proteins of the edible part are more accessible than those of the shells, and that their extraction is favored by the use of the mixer and the application of a greater shear of the material. The proteins of the edible part are mainly from the seeds (68%), which are ground more finely in the batch process.

The polyphenol extraction yield of the first step corresponds to 51% of the extractable polyphenol content of the whole fruit (117.8 mg GAE/100 g of fruit). Thanks to the remarkable increase of this yield (+21%) after the aqueous extraction of the extrudate 1, the continuous process makes it possible to recover 64.5% of the extractable polyphenols from the whole fruit. Extraction of polyphenols is thus improved in extrusion compared to the batch process, particularly through the addition of water.

3.4. Emulsions characterization

3.4.1. Chemical composition

The chemical compositions of the filtrates obtained with the high shear mixer (Filtrates 1 and 2) and with the twin screw extrusion (Filtrates 3 and 4) are presented in Table 5.

Filtrate 1 from the continuous process showed the highest dry matter content (17.2 g/100 g). The continuous process gives Filtrate 3 with lower dry matter content and a lower dry matter yield (Tables 2 and 3). The liquid solid separation taking place in the twin screw

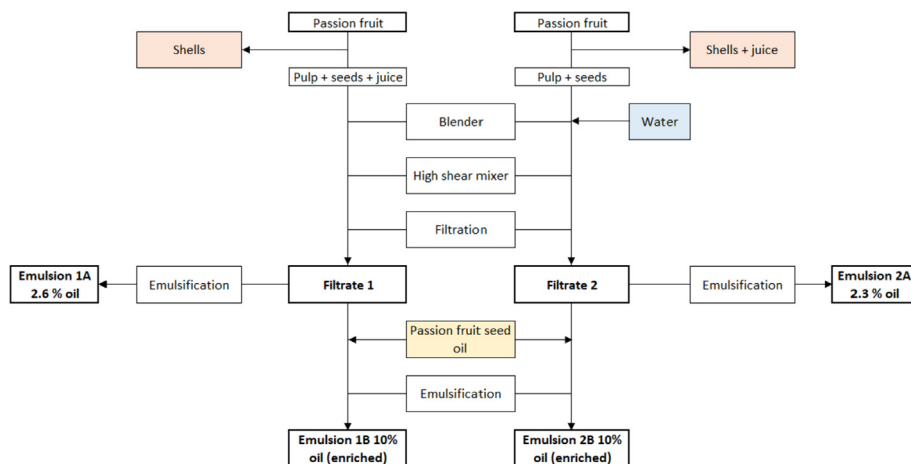


Fig. 3. Discontinuous extraction of edible part of passion fruit.

Table 2
Yields in filtrate and in active molecules for the discontinuous process.

		Way 1	Way 2
Input	Dry matter (g/100 g fresh edible part)	25.4	14.0
	Oil content of the seeds (g/100 g fresh edible part)	2.8	
	Protein content (g/100 g fresh edible part)	2.1	
	TPC of hydrophilic fraction (mg GAE/100 g fresh edible part)	129.5	
Filtrate	Filtrate yield (g/100 g fresh edible part)	81.1 ± 4.0	83.7 ± 4.4
	Dry matter yield (g DM/100 g DM)	54.4 ± 0.1	54.6 ± 0.9
	Oil yield (g/100 g fresh edible part)	2.1 ± 0.2	1.9 ± 0.2
	Protein yield (g/100 g fresh edible part)	1.6 ± 0.1	1.1 ± 0.1
	Polyphenols yield (mg GAE/100 g fresh edible part)	55.2 ± 1.1	33.4 ± 0.8

extruder and the difference in fruit material treatment between the two processes can explain this result.

The filtrates 1 and 2 had higher oil and protein contents than the filtrates 3 and 4, in accordance with the higher yields obtained. The efficient grinding of the seeds performed by the high shear mixer allowed an easier release of these compounds compared to the twin screw extruder. On the other hand, the other compounds extraction (poly saccharides and polyphenols) is enhanced in the continuous process. Filtrates obtained from the extrusion (Filtrates 3 and 4) show higher content in polysaccharides and polyphenols. The Filtrates 4 contents indicate that the aqueous extraction of extrudate 1 allow a further extraction of these molecules. Polyphenol contents in filtrates 3 and 4 were twice as high as those of filtrates 1 and 2, due to the extraction of additional polyphenols contained in the shells. According to the raw materials characterization (Table 1), shells represents in fact half of the

Table 3
Solid and liquid flowrates for the two-step passion fruit twin-screw extrusion (Cletral BC21).

		Step 1	Step 2
Input	Q_{RM} (kg/h)	6.6	6.0
	DM_{RM} (%)	20.0	28.2
	Q_w (kg/h)	0	4.8
	L/S ratio	/	2.8
Extrudate	Q_E (kg/h)	2.6	5.4
	DM_E (%)	27.2	22.3
	Y_E (gDM _E /100 g DM _{RM})	53.5	38.0
	Y_E (g/100 g fresh fruit)	39.1	35.1
Particle	Q_P (kg/h)	0.3	0.7
	DM_P (%)	33.8	27.8
	Y_P (gDM _P /100 g DM _{RM})	7.4	5.8
	Y_P (g/100 g fresh fruit)	4.3	4.3
Filtrates 3 and 4	Q_F (kg/h)	3.3	5.0
	DM_F (%)	13.1	5.2
	Y_F (g DM _F /100 g DM _{RM})	32.4	8.1
	Y_F (g/100 g fresh fruit)	49.2	32.4

Q_{RM} : flowrate of the raw material; DM_{RM} : dry matter of the raw material; Q_w : flowrate of water; Q_E : flowrate of extrudate; DM_E : dry matter of extrudate; Y_E : extrudate yield; Q_P : particles flowrate; DM_P : particles dry matter; Y_P : particles yield; Q_F : filtrate flowrate; DM_F : filtrate dry matter; Y_F : filtrate yield.

extractable polyphenols of the whole fruit.

3.5. Emulsions characterization

Filtrates 1 and 2 were used to prepare emulsion 1A and 2A containing 2.6% and 2.3% of oil, respectively. Additional mechanical pressed passion fruit seed oil was added to the filtrates 1, 2 and 3 to

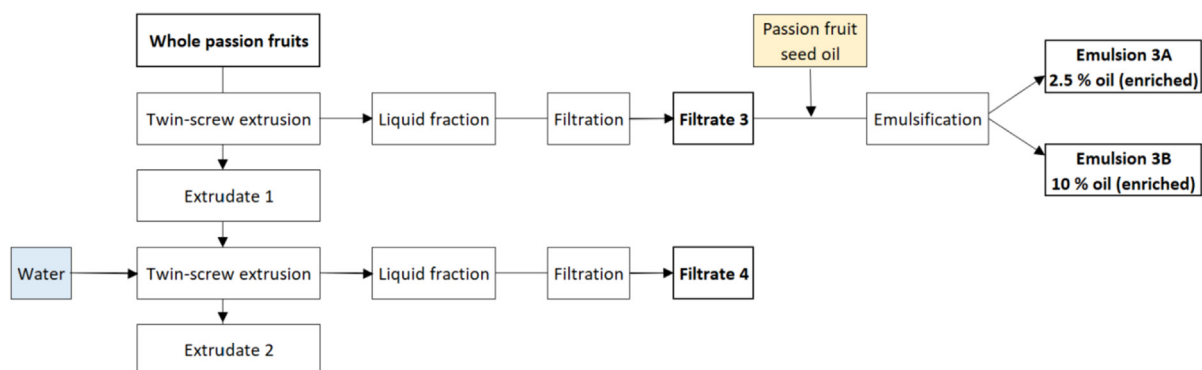


Fig. 4. Continuous extraction by twin-screw extrusion from whole passion fruits.

Table 4
Yields in active molecules in the Filtrates 3 (Step 1) and Filtrate 4 (Step 2).

		Step 1	Step 2	Global process
Oil	Y_H (g/100 g MS _{fruit})	1.2	0.1	1.4
	Y_H (mg/100 g fruit)	246.2	22.7	268.9
Proteins	Y_N (g/100 g MS _{fruit})	2.4	0.9	3.2
	Y_N (g/100 g fruit)	0.5	0.2	0.7
Polyphenols	Y_{PP} (mg GAE/100 g MS _{fruit})	299.8	79.2	379.0
	Y_{PP} (mg GAE/100 g fruit)	59.7	16.3	76.0

Y_O : oil yield, Y_N : protein yield, Y_{PP} : Polyphenols yield.

prepare enriched oil in water emulsions: emulsion 3A with 2.5 g oil/100 g filtrate and emulsions 1B, 2B and 3B with 10 g oil/100 g filtrate. In the filtrates, the dispersion was carried out by the mixer or in the twin screw extruder, whereas a further dispersion with Ultraturax™ was applied to the filtrates and led to emulsions.

Micrographs of filtrates (Fig. 5) showed the lipidic phase dispersion in the aqueous matrix, whatever the process used to put in contact the active molecules (mixer for filtrates 1 and 2 or extrusion for filtrates 3). Thus, the filtrates (F1, F2) and the emulsions (1A, 2A) from discontinuous process presented droplets diameters of less than 10 µm. The continuous process generated a filtrate F3 and an emulsion 3A having a fine dispersion of lipids droplets of sizes 1–5 µm. For all enriched emulsions (B), the addition of vegetable oil resulted in an abundant population of larger droplets, with diameter between 8 and 15 µm.

Droplet size distributions of the samples obtained with the batch process with both ways are shown in Fig. 6A and 6B. No coalescence process was observed as the droplet sizes remained constant throughout the experiments. Filtrates and emulsions B had very similar size distributions exhibiting three main groups of droplets. The most abundant size was around 8 µm. The other probable sizes were about 1.5 µm and 80 µm. For emulsions A, the population of droplets with a size between 1.5 and 6 µm was more important compared to emulsions B and filtrates. The analyses confirm that the emulsification tends to generate a higher number of small droplets.

3.5.1. Rheology of emulsion

The flow behavior of the filtrates and the emulsions were analyzed. The apparent viscosity was plotted against shear rate in Fig. 7. The cold pressed passion fruit oil was used as a standard behaving like a Newtonian fluid. The filtrates and the emulsions showed a rheofluidifying behavior, as their viscosities decreased with the increase of the shear rate.

Samples obtained with the way 1 of the batch process (Filtrate 1, Emulsions 1A and 1B) had the highest viscosities, followed by the extrusion samples (Filtrate 3, Emulsions 3A and 3B) whereas samples of the way 2 had the lowest ($\Delta = 13$ mPa s). Samples 1 (filtrate and emulsions) had higher dry matter ($DM_{F1} = 17.2$ g/100 g) compared to samples 2 ($DM_{F2} = 8.7$ g/100 g), which can partly explain the viscosity

Table 5

Chemical composition of the filtrates obtained from the edible part of the passion fruit with a high shear mixer at laboratory scale (filtrates 1 and 2) and from the whole fruit by twin-screw extrusion (filtrates 3 and 4).

		Filtrate 1	Filtrate 2	Filtrate 3	Filtrate 4
Dry matter	(g/100g filtrate)	17.2 ± 0.6	8.7 ± 0.5	13.1 ± 0.2	5.2 ± 0.0
Oil content	(g/100g filtrate)	2.6 ± 0.1	2.3 ± 0.3	0.5 ± 0.1	0.1 ± 0.0
	(g/100g DM _F)	15.6 ± 1.1	25.5 ± 1.9	3.8 ± 0.1	1.4 ± 0.0
Proteins	(g/100g filtrate)	2.0 ± 0.0	1.3 ± 0.0	1.0 ± 0.0	0.6 ± 0.0
	(g/100g DM _F)	11.4 ± 0.0	14.1 ± 0.0	7.3 ± 0.0	10.7 ± 0.0
Polysaccharides	(g GAE/L filtrate)	48.0 ± 2.4	37.3 ± 1.0	68.7 ± 2.5	23.6 ± 0.6
	(g/100g DM _F)	29.2 ± 1.4	40.5 ± 0.6	52.4 ± 1.9	45.9 ± 1.2
Polyphenols	(g GAE/L filtrate)	0.7 ± 0.0	0.4 ± 0.1	1.2 ± 0.1	0.5 ± 0.0
	(g/100g DM _F)	0.4 ± 0.0	0.4 ± 0.1	0.9 ± 0.0	1.0 ± 0.1

GAE: gallic acid equivalent; DM_F: Filtrate dry matter.

difference. Furthermore, samples 1 and samples 3 obtained from the continuous process (Filtrate 3 and Emulsions 3A and 3B) were richer in polysaccharides coming from the passion fruit juice and thus contributing to increase the viscosity of aqueous media (Ozturk and McClements, 2016). Unlike samples 2, samples 1 and 3 contain natural emulsifiers extracted from the juice or the shells, such as proteins, phospholipids and pectins, thereby accounting for their higher viscosities (Ozturk and McClements, 2016; Schmidt et al., 2015).

Moreover, we notice that emulsions showed higher viscosities compared to their corresponding filtrates, showing the effect of the emulsification step on the viscosity ($\Delta = 3$ –13 mPa s). As expected, the oil enrichment performed from filtrates led to emulsions B with higher viscosities than emulsions A.

The viscoelastic properties of the samples were studied by performing small amplitude oscillatory shear measurements. The storage or elastic modulus G' is plotted against the deformation in Fig. 8. Rheological data have been collected in Table 6 to characterize the linear viscoelastic region (G'_{LVR} , G''_{LVR} and $\gamma_{90\%}$) and the crossover points of G' and G'' ($G' = G''$ and $\gamma_{G' = G''}$). The end point of the linear viscoelastic region (LVR) was determined at the point corresponding to the value of G' reduced by 10%.

For all the samples, the storage module G' is 2–3 times greater than the loss modulus G'' in the LVR, thus reflecting the dominant elastic behavior of the filtrates and emulsions in this region. The length of the LVR is however different depending on the type of samples. The comparison of the critical strains (γ_C) (Table 6) shows that the LVR of the emulsions is longer than that of their corresponding filtrate. The emulsions, thanks to their increased viscosity, have a better resistance to deformation than the filtrates and will not flow until a larger deformation (γ_C). The observation of the deformation corresponding to the intersection point of both modules confirms this analysis: the values of $\gamma_{G' = G''}$ are higher for the emulsions than for the filtrates.

Samples 1 showed the longest linear viscoelastic regions, while samples 2 have the shortest. Thus it appears that the factors favoring a higher viscosity (emulsions versus their filtrates, or samples 1 compared to samples 2) confer a better elasticity to the medium. For instance, the higher content of polysaccharides (identified as one of the factors enhancing the viscosity) may also be responsible for the improved stability of samples 1, by inhibiting the droplets movement (Ozturk and McClements, 2016). Moreover, it was highlighted that the proteins can participate in the shear resistance after their adsorption at the droplets surface, which generates electrostatic and steric repulsion between the droplets (Bouyer et al., 2012). So, when keeping the passion fruit juice and its natural emulsifying molecules within the formulation, the pre-formulated samples have better deformation capacities, resulting in a higher flow threshold.

The shorter LVR observed for emulsions B compared to the corresponding emulsions A, could be explained by the larger size droplet population (8–15 µm) in emulsions B (Figs. 5 and 6). Larger droplets promote rearrangement between droplets leading to lower resistance to

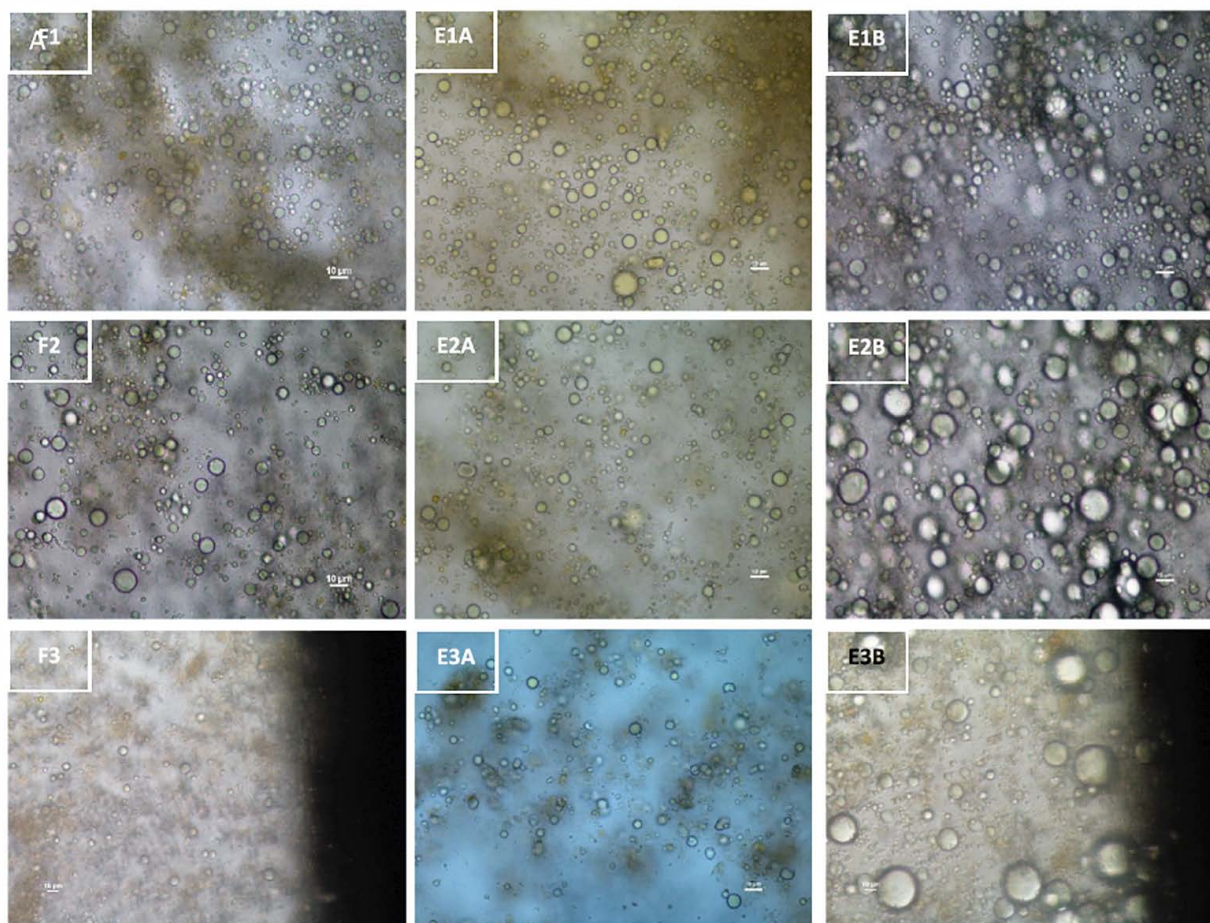


Fig. 5. Optical micrographs of emulsion 1A (5.A), emulsion 2A (5.B) and emulsion 3A (5.C) (scale bar = 10 μm , magnification $\times 40$).

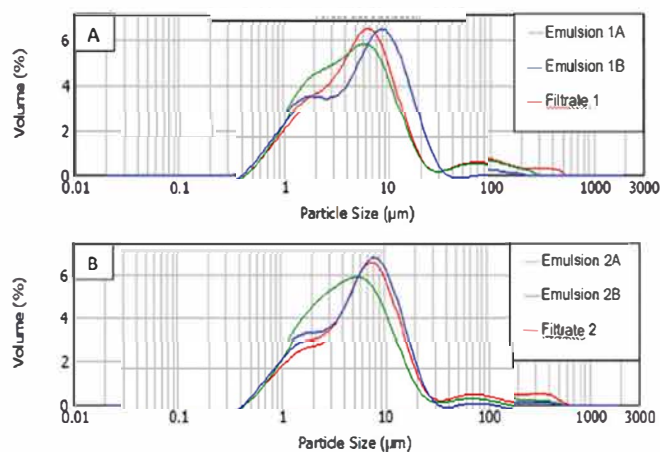


Fig. 6. Droplet size distribution for samples group 1 (6.A) and 2 (6.B).

deformation. The shortening of the DLVE observed for the emulsion 3B compared to the emulsions 3A (Table 6) thus indicate that the 10% enrichment in the extrusion samples also leads to an increase in the diameter of the droplets, in accordance with the microscopic observation of emulsion 3A and 3B (Fig. 5).

3.5.2. Emulsion stability

The stability of emulsions was assessed by the creaming index, calculated from the serum layer formation. Results are exposed in Fig. 9. After 14 days, the emulsion 3A is the most stable, resulting in a creaming index of less than 20%. This emulsion prepared from the

whole fruit is more stable than the 1A and 2A emulsions (CI: 32%) prepared without the fruit shells, indicating the probable extraction of natural emulsifiers (phospholipids) from the shells that contribute to the emulsion stability. Polyphenols, present in greater amounts in the emulsion 3A than in the emulsions 1A and 2A, can also play a role in the stabilization of the emulsion. Indeed, the work of Di Mattia et al. (2009) and Sabouri et al. (2015) showed that some polyphenols can concentrate on the surface of oil droplets by binding to the emulsifying molecules present at the interface via hydrogen bonds or hydrophobic interactions (Di Mattia et al., 2009; Sabouri et al., 2015). This layer of polyphenols improves the degree of dispersion of the oil and enhances the resistance of the droplets surface to dilation. The addition of catechin and quercetin thus improved the oil in water emulsion stability over a period of 10 days (Sabouri et al., 2015). However, it is difficult to predict these properties, which depend in particular on the nature of the emulsifying molecules and the pH of the solution. Concerning our media, the combination of polyphenols and natural emulsifying molecules extracted during the extrusion seems more favorable to the stabilization of the emulsions than the compounds extracted by the batch process.

Oil enrichment resulted in emulsions B having lower stabilities than the corresponding emulsion A. Thus, emulsion 2B which does not contain emulsifying molecules either from the shells or the juice (water based emulsion) logically has the highest creaming index. The enhanced stability of emulsions A relative to emulsions B is in agreement with the smaller droplet sizes (Fig. 5 and Table 6) and the larger LVR (Fig. 8) obtained for emulsions A.

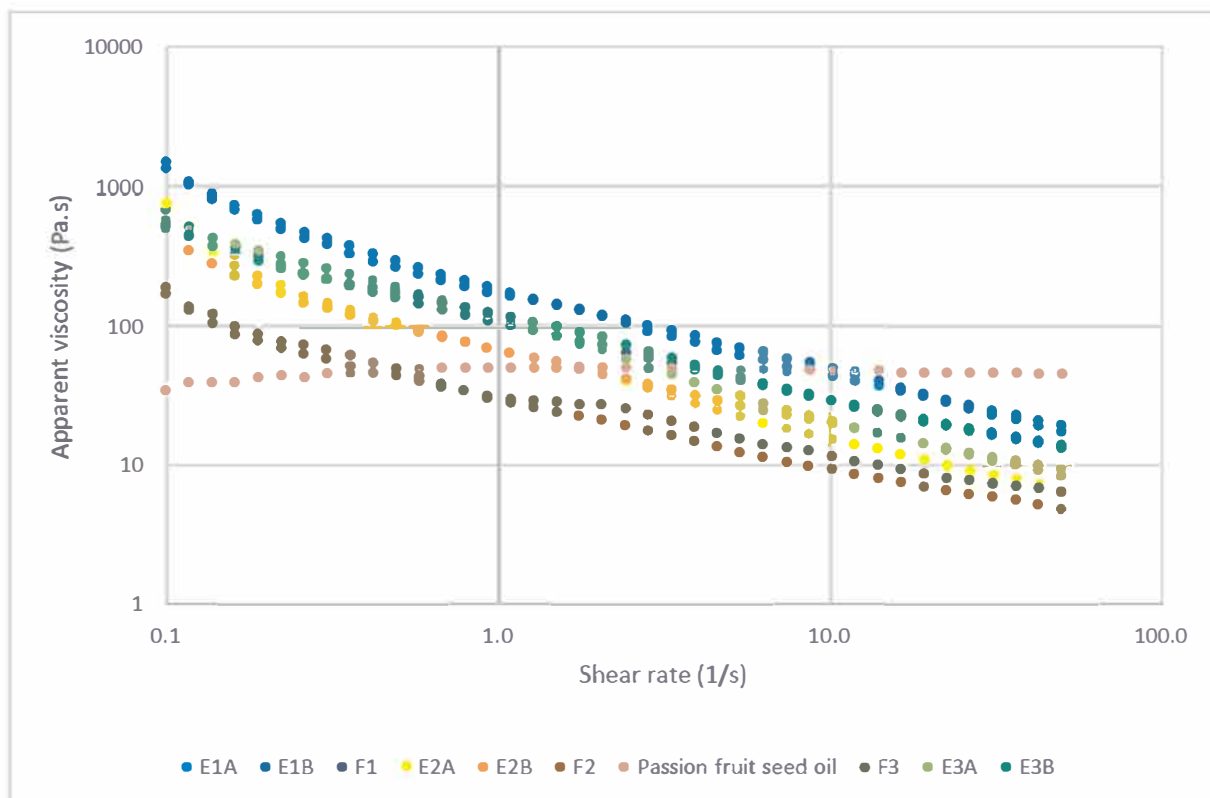


Fig. 7. Flow characterization of filtrates (F), emulsions (E) and control (passion fruit seed oil) - Mean values of triplicate, SD < 5%.

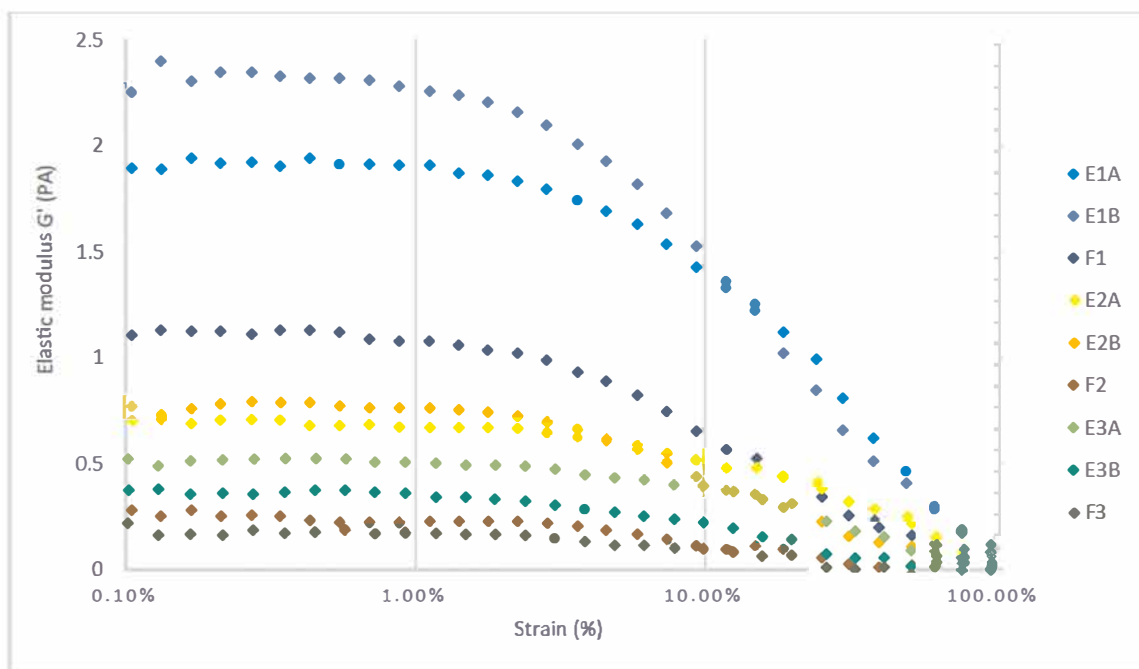


Fig. 8. Evolution of the elastic modulus G' as a function of the strain - Mean values of triplicate, SD < 5%.

4. Conclusion

Extractions from fresh purple passion fruits were performed using green alternative methods to conventional solvent extractions applied to the different parts of the fruit (whole fruit or co products). The strategy was based on a combined extraction of several types of molecules of interest (vegetable oil, natural antioxidants and emulsifiers) to obtain organized media, oil in water emulsions, which could be used as

a base for natural paints, cosmetics, or food formulations. A thermo mechanical treatment of the fruit materials was tested according to two processes. In one hand, a batch process able to treat 500 g of fruit edible part (pulp, seed and fresh juice) by mixing and shearing effects. On the other hand, the continuous process using a twin screw extruder was fed by 6 kg of fresh whole fruit (shells and the edible part of the fruit) per hour.

The oil extraction is favored by the higher shearing of the seeds

Table 6

Rheological data obtained for the small amplitude oscillatory shear measurements. G' and G'' values corresponds to the linear viscoelastic region (LVR).

	G' (Pa)	G'' (Pa)	$\gamma_{0.05} \sigma$	$G' = G''$ (Pa)	$\gamma_{G' = G''}$ (%)
Emulsion 1A	1.92 ± 0.11	0.68 ± 0.19	4.6 ± 0.3	0.64 ± 0.09	29.0 ± 0.5
Emulsion 1B	2.31 ± 0.06	0.74 ± 0.04	3.7 ± 0.2	0.80 ± 0.05	26.2 ± 0.3
Filtrate 1	1.11 ± 0.05	0.43 ± 0.06	2.9 ± 0.3	0.44 ± 0.04	22.1 ± 0.4
Emulsion 2A	0.71 ± 0.02	0.20 ± 0.03	3.7 ± 0.1	0.26 ± 0.02	45.4 ± 0.8
Emulsion 2B	0.75 ± 0.05	0.27 ± 0.05	3.3 ± 0.4	0.31 ± 0.04	21.1 ± 0.2
Filtrate 2	0.26 ± 0.02	0.11 ± 0.01	0.5 ± 0.1	0.13 ± 0.01	8.9 ± 0.2
Emulsion 3A	0.5216 ± 0.02	0.18 ± 0.02	3.9 ± 0.3	0.38 ± 0.00	15.2 ± 0.6
Emulsion 3B	0.35 ± 0.03	0.21 ± 0.07	3.1 ± 0.1	0.26 ± 0.02	4.8 ± 0.0
Filtrate 3	0.20 ± 0.06	0.11 ± 0.06	1.9 ± 0.2	0.15 ± 0.01	3.4 ± 0.3

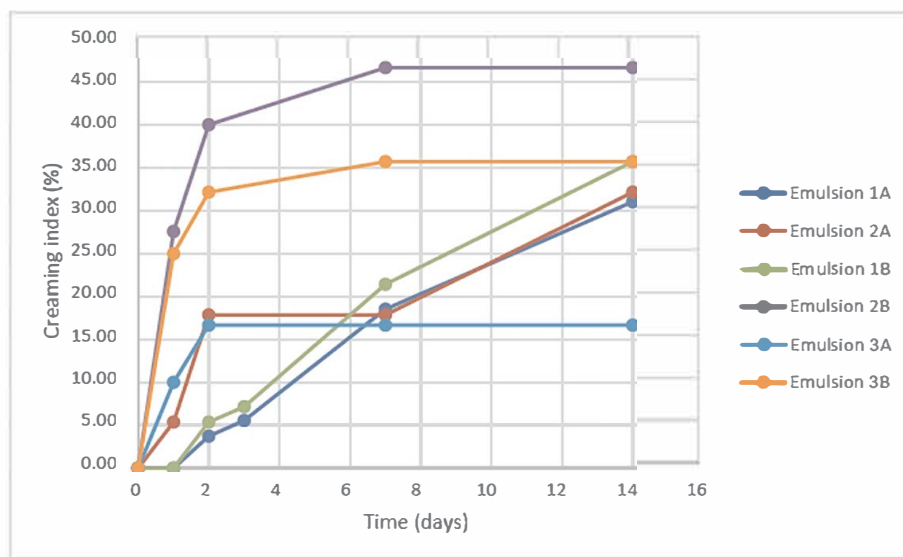


Fig. 9. Influence of the emulsion composition on the creaming index during storage.

occurring during the batch process. The way 1 of the discontinuous process allows the recovery of 75% of the lipids contained in the seeds and to co extract 75% of the proteins and 43% of the edible part extractable polyphenols.

The ratio 'solid to liquid' of the fresh fruit and the described screw profile were found suitable for extrusion, thus allowing an efficient defibrating and pressing of the fruit material. Twin screw extrusion led to filtrates with high contents of polyphenols (1 g GAE/100 g DM filtrates), representing 67.5% of the whole fruit extractable polyphenols. Indeed, the presence of the shells rich in hydrophilic extractives (44.2 g/100 g DM) and the addition of water in the second extraction enhanced the water soluble molecules recovery. Expression and aqueous extraction in the same extruder with an adequate screw profile could still improve the extraction yield as it is suggested by [Evon et al. \(2009\)](#).

The vegetable oil dispersion in the resulting media was highlighted through analyses by optical microscopy and granulometry of filtrates and emulsions (droplets mean diameter around $3 \mu\text{m}$). The pseudo plastic behavior observed for the tested emulsions is of interest for an eventual use of these emulsions for food applications. Indeed, the low viscosity at high shear facilitates the implementation of production processes like pumping and filling, whereas the higher viscosity at low shear improve the taste sensation ([Amid and Mirhosseini, 2013](#)). Emulsions 1A and 1B from the discontinuous process had the highest viscosities, also corresponding to high levels of protein and dry matter. In addition, these emulsions have the highest flow thresholds, which is interesting for paint applications. These features are important to ensure adequate consistency in the final formulation. Emulsions prepared from filtrates after the extrusion of the whole fruit (3A emulsion) proved to be the most stable over time. The coextracted polyphenols,

polysaccharides and other natural emulsifiers probably work in favor of this physical stability. All the filtrates obtained can constitute basic media to prepare oil enriched emulsions. Their polyphenols contents could contribute to the conservation of these natural emulsions.

For twin screw extrusion, a supply of 100 kg would lead to the production of 78.5 kg of pre formulated emulsion. If the same amount of fruit is used for juice production, the co product (seeds and pulp) resulting from this transformation could be treated with the discontinuous process (way 2) to obtain of 41.9 kg of pre formulated emulsion.

The use of thermo mechanical processes to up grade the output gap or the co products of passion fruit would help to minimize energy, solvent costs and extraction steps, thus limiting the environmental impacts. At the same time, the extrudate could be reused for the production of agromaterials by thermo pressing as suggested by the work of [Evon et al. \(2014\)](#).

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