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Fatty acid composition and rheological behaviour of prickly pear seed oils

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Abstract

Prickly pear fruits constituted valuable foodstuff for humans and animals in arid and semi-arid regions. Two species of prickly pear from Tunisia, *Opuntia ficus indica* and *Opuntia stricta*, were investigated for fatty acid composition and physicochemical parameters of the seed oil. No significant difference in either physical or chemical parameters was found between the species. The main fatty acids of prickly pear seed oil were C16:0, C18:0, C18:1, C18:2. With an exceptional level of linoleic acid, up to 70%, the content of unsaturated fatty acids was high, at 88.5% and 88.0% for *O. ficus indica* and *O. stricta*, respectively.

Rheological properties were analysed with changes of temperature and shear stress. Variations of viscosity were measured and the viscoelastic parameters were determined during heating and cooling cycles between 20 and 70 °C. Curves of flow were established with up and down cycles of shear stress at different temperatures. These measures highlighted the presence of large aggregates of crystal fatty acids in both *Opuntia* crude oils. Shearing and temperature destroyed this structural state and gave birth to an homogeneous stable suspension.

The structural state of crude oil was confirmed using a contrast phase microscope, and the particle size distribution was obtained by laser granulometry.

Keywords: Prickly pear; Fatty acid; Seed oil; Rheology; Microscopy; *Opuntia*

1. Introduction

About 1500 species of cactus belong to the genus *Opuntia* and are distributed mainly in Africa, Mediterranean countries, southwestern United States, northern Mexico and other areas (Hegwood, 1990). The main studies on the *Opuntia* fruits were the chemical analysis of pulp, skin and seeds (El Kossori, Villaume, El Boustani, Sauvaire, & Mejean, 1998), analysis of volatile constituents of pulp (Di Cesare & Nani, 1992; Flath & Takahashi, 1978), use of pulp in juice production (Espinosa, Borrocal, Jara, Zorilla, & Medina, 1973), production of alcoholic beverage (Bustos, 1981), jam production (Sawaya, Khatchadorian, Safi, & Al-Mohammad, 1983) and the production of cocoa butter equivalents from prickly pear juice fermentation by an unsaturated fatty acid auxotroph (Hassan, Blanc, Pareilleux, & Goma, 1995). An overview of processing technologies concerning the fruits and cladodes of cactus pear has recently been published by Saenz (2000). Other authors have studied the nutritional significance of *Opuntia* sp. (Stintzing, Schieber, & Carle, 2001).

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Crude young 'nopal' have been studied as a source of fibres (Majdoub et al., 2001), proteins and amino acids (Teles, Whiting, Price, & Borges, 1997), and processed in Taifi prickly pear sheets (Ewaidah & Hassan, 1992). The extraction and the characterization of the prickly pear mucilage from sheets was optimized by several authors (McGarvie & Parolis, 1979; Medina-Torres, Brito-De La Fuente, Torrestiana-Sanchez, & Katthain, 2000; Trachtenberg & Mayer, 1981, 1982).

Prickly pear seeds were first characterized by Sawaya, Khalil, & Al-Mohammad (1983), who demonstrated that the seeds of *Opuntia ficus indica* are rich in minerals and sulphur amino acids. A reserve protein from the seeds has been isolated and characterized by Uchoa, Souza, Zarate, Gomez-Filho, & Campos (1998). The prickly pear seed oil composition and its chemical characteristics were investigated by Sawaya & Khan (1982), and then by Salvo, Galati, Lo Curto, & Tripodo (2002).

Coskuner & Tekin (2003) studied the seed composition of prickly pear fruits during the maturation period. Ramadan & Morsel (2003) compared the seed and pulp oil compositions. However, the physical characteristics of prickly pear oil are up to now unknown. The major objective of the present work was to study the physicochemical properties of the seed oil of the *O. ficus indica* and *O. stricta* fruits which are the most abundant species in Tunisia. Rheological behaviour and microscopic structure have been studied and special attention was paid to the effect of temperature, since every food technological operation requires a thermal treatment.

2. Materials and methods

2.1. Prickly pear seed

Mature Prickly pear fruits, *O. ficus indica* and *O. stricta*, were collected, respectively, in August and February from the same area (Sfax, Tunisia). The fruits were immediately sorted, washed with running water, air-dried and hand-peeled. A pulper finisher was used to separate the seeds from the pulp. The seeds were washed with distilled water several times, air-dried at ambient temperature and then ground with a Diez crusher.

2.2. Oil extraction

The seed powders oils of *O. ficus indica* and *O. stricta* were extracted with hexane in a Soxhlet extractor for 9 h. The organic phase was then removed using a rotary evaporator under reduced pressure; the oil was flushed with a stream of nitrogen and stored at −20 °C in sealed tubes prior to analyses.

2.3. Physicochemical analyses

The seed weight was appraized, at random, on one hundred seeds. Moisture content was determined by the AOAC method (AOAC, 1984). The oil yield was determined on a seed powder of 5 g. Nitrogen was determined by the Kjeldahl procedure and crude protein was calculated as $N \times 6.25$ (Balogun & Fetuga, 1986). The ash content was determined according to the AOAC method (AOAC, 1990). Refractive index was determined at 20 °C with an Abbe refractometer with temperature adjustment. The density was measured with a densimeter PAAR DMA 60. Saponification number and iodine value were determined using the official method (American Oil Chemists' Society, AOCS, 1993).

2.4. Fatty acid analysis

The fatty acid compositions of both oil samples were analyzed by GC–MS after transesterification. Fatty acid methyl esters were prepared in the presence of 2 N potassium hydroxide in methanol and analyzed on a Hewlett-Packard model 5890 series II gas chromatograph equipped with a flame ionization detector and a polar capillary column: HP Innowax cross-linked PEG, Carbowax 20 M (0.32 mm internal diameter, 30 m length and 0.25 μm film thickness). The operational conditions were: injector temperature 220 °C; detector temperature 275 °C; column temperature 50 °C for 5 min then a gradient of 10 °C/min to 240 °C; carrier gas was nitrogen at a flow of 1.47 ml/min. Three injections were done.

2.5. Rheological measurements

2.5.1. Instrument

The rheological properties were measured using a controlled stress Haake rheometer (Rheostress RS 100). All the rheological studies were conducted using cone-plate geometry: 35 mm diameter and 2° cone. The volume of the sample was 0.4 ml. That equipment is able to control temperature on a plate sensor system to within ±0.1 °C.

For the measures at variable temperature, the variation was at the rate of 5 °C/min.

2.5.2. Flow curves

In order to determine the influence of temperature and the shear rate, measures of viscosity were conducted at constant shear stress (10 Pa) across increasing temperatures from 20 ° to 70 °C and immediately decreasing temperature to 20 °C.

To follow the influence of shear rate on the viscosity, increasing and immediately decreasing cycles of shear stress at constant temperature were performed.

2.5.3. Dynamic tests

The rheological measurements, in oscillatory mode, were performed in the linear viscoelasticity range (1 Hz in frequency and 0.3 Pa in shear stress) with temperature increased from 20 to 70 °C and followed immediately by a decrease of temperature to 20 °C.

2.6. Microscopy

The oils of *O. ficus indica* and *O. stricta* were observed under a contrast phase microscope equipped with a Nikon F301 camera. A scale graduation, introduced on photo, indicated the magnification.

2.7. Particle size distribution

The particle size distribution of crude oil was established by laser light scattering at room temperature, using a Malvern Mastersizer hydro 2000S (Malvern Instruments Ltd, Malvern, UK). All analyses were performed in triplicate.

3. Results and discussion

3.1. General composition (Table 1)

The oil contents for *O. ficus indica* and *O. stricta,* were similar (10.90% and 11.05%, respectively). These values were higher than the crude oil yield in the work of Coskuner & Tekin (2003) (6.91%). The difference observed is probably due to the origin of the fruit. Seeds contain 5.4–3.9% protein and 1.1–1.64% ash, less than values found by Sawaya et al. (1983) on the saudian variety of *O. ficus indica*.

The value of viscosity at 20 °C of *O. ficus indica* (53×10^{-3} Pa s) is close to the value reported by Oomah, Ladet, Godfrey, Liang, & Girard (2000) for grape seed oil (49.4×10^{-3} Pa s), whereas the value for *O. stricta* (76×10^{-3} Pa s) is comparable to that of rapeseed oil ($72–82 \times 10^{-3}$ Pa s) (Karlesind & Wolff, 1992).

Table 1
Physicochemical characteristics of the two species of *Opuntia* seed oil

Characteristics[a]	Species	
	O. ficus indica	*O. stricta*
Weight of 100 seeds (g)	1.38 ± 0.08	1.69 ± 0.04
Dry matter (%)	93.00 ± 0.45	95.00 ± 0.30
Oil (%)	10.90 ± 0.10	11.05 ± 0.09
Protein (%)	5.40 ± 0.40	3.90 ± 0.20
Ash (%)	1.10 ± 0.10	1.64 ± 0.15
Viscosity[b] (Pa s)	0.0531 ± 0.0005	0.076 ± 0.001
Refractive index[c]	1.475 ± 0.002	1.469 ± 0.001
Density[c]	0.903 ± 0.002	0.919 ± 0.001
Saponification number	169.0 ± 0.1	174.0 ± 0.3
Iodine value	101.5 ± 1.0	91.6 ± 0.5

[a] Means of three determinations.
[b] At 20 °C and $\tau > 2$ Pa.
[c] At 20 °C.

Table 2
Linear correlation[a] between densities and temperature[b] for the two varieties of *Opuntia* seed oil

Compound	Intercept b	Slope m	R^2
O. ficus indica oil	0.91703	−6.833E−04	0.99
O. stricta oil	0.9336	−6.9121E−04	0.99

[a] Density = $b + m\,T$.
[b] Temperature range: 10–70 °C.

The refractive and iodine indices are comparable with those of rapeseed oil (Karlesind & Wolff, 1992) (respectively, 1.473 vs. 1.475 and 100 vs. 101.5). The saponification value is lower than grape seed oil (188–194) but compared favourably with native rapeseed oil (170–175) (Karlesind & Wolff, 1992).

The density of the seed oil at 20 °C compared favourably with native rapeseed oil and soybean oil (0.910 and 0.921, respectively) (Noureddini, Teoh, & Davis Clements, 1992).

The study of the density of the *Opuntia* seed oil as a function of temperature revealed a linear relationship in accordance with results reported for others oils. It is reported that the densities of fatty acids and triglycerides are linear with temperature, according to the equation $\rho = b + mT$ (Fisher, 1995). The linear coefficients b and m for the *Opuntia* seed oil are shown in Table 2. The square correlation coefficient highlighted a linear correlation between density and temperature.

For triglycerides, the change in density per degree Celsius was in the range of 0.00067–0.00073 (Formo, Jungermann, Norris, & Sontag, 1979). The results on *Opuntia* oils were in accordance with these previous data and with the published density data for coconut, corn and rapeseed oils (Noureddini et al., 1992; Valeri & Meirelles, 1997).

3.2. Fatty acid composition of prickly pear seed oil

The FAME compositions of seed lipids are listed in Table 3. Linoleic acid is the major component (74%), followed by oleic (12.8%) and palmitic acids (7.2%). Both lauric and myristic acids were detected in *O. srticta* oil in low amounts. Prickly pear seed oil was found to be highly unsaturated: 88.5% and 88.0% for *O. ficus indica* and *O. srticta,*, respectively. Besides the linoleic acid (>70%), there is oleic acid (>12%). These results are in agreement with those of Sawaya & Khan (1982) who previously reported the contents of the four most important fatty acids. The lipid pattern of prickly pear is comparable with that of sunflower and grapeseed oils (Tan & Che Man, 2000).

Table 3
Fatty acid composition of prickly pear seed oil (g/100 g of total fatty acid)

Fatty acid	Species	
	O. ficus indica	*O. stricta*
Lauric C12:0	–[a]	0.19 ± 0.01
Myristic C14:0	–[a]	0.32 ± 0.01
Palmitic C16:0	9.32 ± 0.19	7.21 ± 0.09
Palmitoleic C16:1	1.42 ± 0.01	0.38 ± 0.02
Stearic C18:0	3.11 ± 0.04	3.83 ± 0.01
Oleic C18:1	16.8 ± 0.47	12.8 ± 0.09
Linoleic C18:2	70.3 ± 0.60	74.8 ± 0.26
U/S[b]	7.11	7.61

[a] Not detected.
[b] Unsaturation ratio = (16:1 + 18:1 + 18:2)/(12:0 + 14:0 + 16:0 + 18:0).

Recently, Coskuner & Tekin (2003) reported a palmitic acid content higher than our results (12% vs. 9.32%) and a content of linoleic acid very much lower (52% vs. 70.29%). The observed difference is possibly due to the degree of maturity of the fruit; indeed, these authors suggested that there was an increase in saturated fatty acid content towards the end of fruit maturation.

3.3. Rheological behaviour

3.3.1. Viscosity variations with the temperature

These measurement were carried out at constant shear stress 10 Pa and two consecutive cycles of increasing and decreasing temperature between 20 and 70 °C. As expected, the viscosity decreases strongly when the temperature increases. The curve of viscosity was higher on the curve segment corresponding to the decreasing temperature of 70–20 °C (Fig. 1). A weak hysteresis was observed. When a second cycle of temperature was applied, the viscosity curve was totally superposed on the curve obtained in the phase 70–20 °C of the first temperature cycle (data not shown). At that time, the structural state was stabilized.

The area of hysteresis observed was due not only to the effect of the temperature but also to the effect of the shearing. That result might be related to the chemical composition of the oil that revealed a significant amount of fatty acids in the solid state at 20 °C. Indeed, palmitic acid and stearic acid have melting points of 62.9 and 69.6 °C, respectively. The initial crude oil was a suspension. During the first cooling, both shearing and temperature destroyed the structural state, especially the aggregates. Because this phenomenon was not immediately reversible, hysteresis became visible when temperature decreased to 20 °C. The smaller size of particles explained the greater strength of resistance to flow in the resulting suspension than in the initial suspension. So the curve of viscosity during cooling was above the curve of viscosity during heating. At that time, the second cycle temperature, up 70 °C and down to 20 °C, showed that there was no influence of shearing. A homogeneous suspension took shape during the cooling phase of the first temperature cycle.

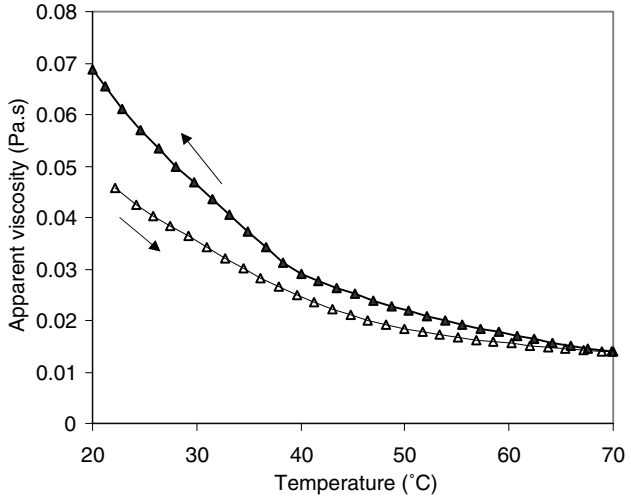

Fig. 1. Effect of temperature increasing (△) and decreasing (▲) on the apparent viscosity at shear stress 10 Pa.

3.3.2. Viscosity curves at different temperatures

This experiment aimed to explore variations of the viscosity at constant temperature with increasing and immediately decreasing shear stress. When the shear stress increases, the viscosity increases from 11% to 24% for all temperatures, ≈0.3 Pa. A weak decrease of viscosity followed this rise and a stabilization was then observed from 2 Pa. So the flow of oil became Newtonian when the shear stress was above 2 Pa (Fig. 2). When the shear stress immediately decreased, the behaviour remained Newtonian in the same zone of shearing. This behaviour was reproduced at every temperature but a weakening of phenomenon was observed at 50 °C and a near disappearance at 60 °C. These temperatures were in the same range as the melting points of palmitic (62.9 °C) and stearic (69.6 °C) acids. This flow characterized the physical

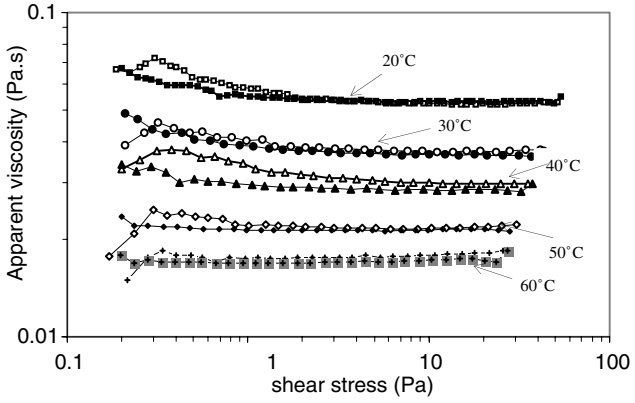

Fig. 2. Effect of shear stress increasing (Hollow symbols) and decreasing (filled symbols) on the apparent viscosity of *O. ficus indica* at various temperatures.

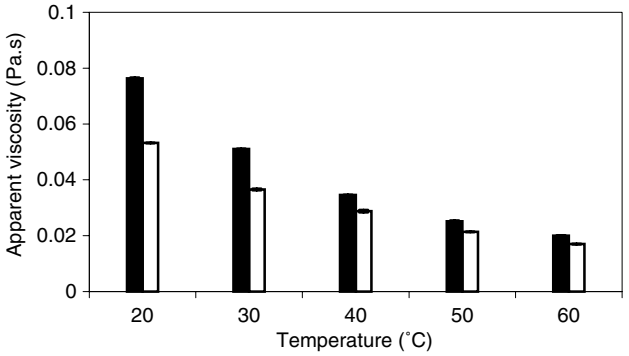

Fig. 3. Viscosity of prickly pear seed oil at different temperatures in the stable linear zone ($\tau > 2$ Pa). ■ = *O. stricta* seed oil, □ = *O. ficus indica* seed oil.

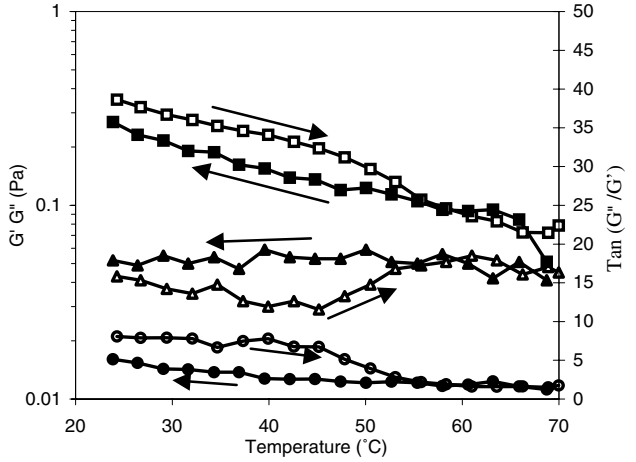

Fig. 4. Changes in the storage modulus G' (△ ▲), in the loss modulus G'' (□ ■) and in tangent loss angle (○ ●) with increasing (△ ○ □) and decreasing temperatures (▲ ● ■) for *O. ficus indica* at 1 Hz in frequency and 0.3 Pa in shear stress.

state of the oil. The crude oil before shearing was a suspension in which dispersed phase was constituted of aggregated particles of fatty acids with a high melting point, as described above. These aggregates resisted the flow and this explained the initial increase of viscosity. They were destroyed quickly by the shearing. Consequently, a small decrease of viscosity and a stable suspension constituted of smallest particles were observed. So the flow became Newtonian when shear stress was above 2 Pa.

These results ratified the hypothesis of the experiments described in the previous paragraph and were in accordance with previous works. Matveenko, Kirsanov, & Remizov (1995) identified two different parts of each flow curve as the high shear rate region and the low shear rate region, separated by a break point. Moreover, Geller & Goodrum (2000) showed that dynamic viscosity of vegetable oils was shear-independent at high shear rates (above 6 s^{-1}). Our measures displayed a Newtonian flow in that shear rate range. The same effect was observed for both prickly pear seed oils. The values of Newtonian viscosity are given in Fig. 3.

3.3.3. Dynamic tests

To eliminate the influence of the shearing and to analyze only the effect of the temperature, rheological measures were conducted in oscillatory mode with up and down cycles of temperature. The reason for oscillatory rheological tests was that the very low shear stress did not destroy the structural state of sample. Curves presented in Fig. 4 showed that the loss modulus G'' was superior to the storage modulus G'. As expected the values for G' and G'' characterized rheological behaviour of a disorganized state at all experimental temperatures.

The effect of the temperature was visible until 55 °C. Rheological dynamic parameters, G' and G'', remained steady between 55 and 70 °C because both palmitic and stearic acids melted; consequently, no more change was observed. At 70 °C, there were no more aggregates in the oil. As a consequence, this medium was totally liquid. During cooling, a recrystallization occurred. The recrystallization led to the formation of fat crystals having similar size and caused a homogeneous organization in the whole volume. The result was that the storage modulus G' was more raised in cooling than in heating due to greater organization of medium than in the initial state. The variations of loss tangent were consecutive to changes of G' and G''.

In previous studies on fat crystal networks, workers observed that a logarithmic linear relationship existed between elastic modulus G' and the solid fat content (Narine & Marangoni, 1999). This result was not observed but in the studied samples of prickly pear seed oil, which were very different from a fat crystal network. The content of solid fats in these samples was significant but very low. So the values of dynamic parameters were very weak and did not permit more observation. This result is confirmed by the measurements from laser light scattering.

3.4. Microscopy

The analysis of rheological behaviour was confirmed by light microscopic observations. The crude oil of *O. stricta* showed large aggregates of fat crystal particles of nearly 60 μm (Fig. 5(a)). The same oil, observed after shearing (Fig. 5(b)), revealed small particles dispersed over all the ranges of vision; the aggregates, which were visible in crude oil, disappeared. The same result occurred with oil treated by heating to 70 °C and then shearing. This structural state of suspension was in agreement with the Newtonian behaviour observed after shearing on the flow curves.

The crude oil of *O. ficus indica* displayed the same microscopic behaviour but the size of aggregates was smaller (30 μm, data not shown).

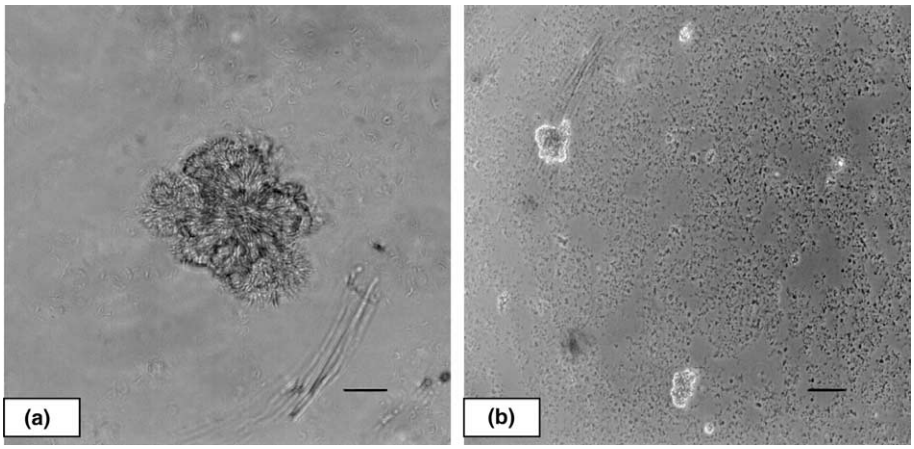

Fig. 5. Contrast phase microscopy of crude (a) and sheared oil (b) of *O. stricta*. Magnification is 400×, scale bar = 15 μm.

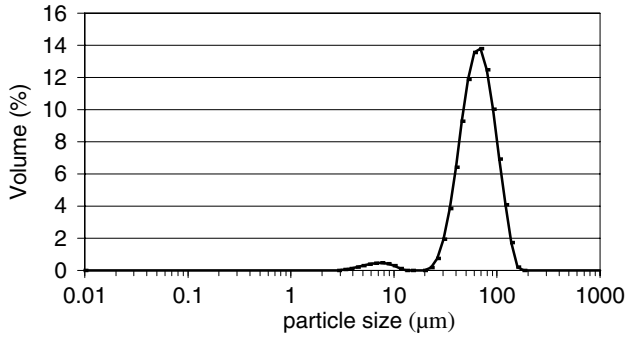

Fig. 6. Particle size distribution of the *O. stricta* seed oil, obtained by laser light scattering.

3.5. Particle size distribution

With the object of analysing the particles in suspension, the crude oil of *O. stricta* was tested with a Malvern Mastersizer. The particle size distribution highlighted a main peak in which the mean diameter of particles, $d_{4,3}$, was 70 μm (Fig. 6). The peak centred at 7 μm only represented 2.87% of total volume, so is of minor importance.

4. Conclusions

The purpose of this research was to determine the physicochemical properties, fatty acid composition and rheological behaviour of seed oils from two species of prickly pear growing in Tunisia: *O. ficus indica* and *O. stricta*.

Seeds represented about 18–20% of peeled fruits. The oil was extracted from ground seeds with hexane (yield nearly 11%). The refractive index and densities were similar for both species. Iodine values and saponification numbers indicated little difference in qualitative composition and quantitative fatty acid content between both oil samples.

Fatty acid analysis by GC–MS revealed (in both *Opuntia* oils) four major fatty acids: palmitic, stearic, oleic and linoleic acid, previously reported in vegetable oils. Palmitoleic acid was present in low quantities. *O. stricta* oil contained also traces of lauric and myristic acids. Both oils were exceptionally rich in linoleic acid, (up to 70%) and their contents of unsaturated fatty acids were high, (about 88%). The fatty acid composition of prickly pear oil was close to those of sunflower and grapeseed oils. These characteristics illustrated the interest of prickly pear as a natural source of edible oil containing essential fatty acids and as an economic utility for Tunisia.

The rheological properties were analysed. Variations of viscosity and viscoelasticity parameters were studied across heating and cooling cycles from 20 to 70 °C. For the investigation on viscosity, a consecutive cycle of temperature was applied. The flow curves were established with up and down cycles of shear stress at different temperatures. Simultaneously, a crude oil sample, a shearing oil sample and an oil sample submitted to a treatment by heat at 70 °C were observed with a contrast phase microscope. Lastly, a crude oil was investigated by laser light scattering to obtain the particle size distribution.

The convergence of rheological measurements and microscopic observations highlighted the structural state of prickly pear seed oil: the crude oil contained large aggregates of unmelted fatty acids. The mean size of aggregates (by microscopy) was 60 and 30 μm for *O. stricta* and *O. ficus indica*, respectively.

The measurment by laser light scattering showed a peak formed by particles with mean diameter of 71.8 μm, corresponding to 14% of the total volume of the *O. stricta* crude oil.

The crude oil of prickly pear seed contains aggregated fatty acid crystals. Shearing and temperature destroyed the aggregates and homogeneous suspension developed: this suspension, with a stable structural state, had Newtonian flow at high shear stress.

The findings shown in this work raise the nutritional value of these under-exploited plants, especially in semi-arid regions of Tunisia, where conventional crops are difficult.

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