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Enhancing oilseeds cold pressing techniques based on HAZOP analysis for prickly pear seeds, *Opuntia ficus-indica* (L.) Mill.

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Abstract

The main goal of the present study was to determine optimal conditions for the extraction of oil with high added value by cold pressing technique. Namely the feeding conditions of the *Opuntia ficus-indica* (L.) Mill. (OFI) seeds, as well as the definition of the manipulable variables of the Kern-Kraft 20 press were specified. These specifications combine HAZOP (Hazard and Operability) analysis and experience plan to find out correlation between yield and oil quality with the pressure, temperature, nozzle diameter, residence time, pressing speed while considering high hardness level of the seed under study. The seed oil extracted according to the proposed approach was highly unsaturated where linoleic acid is the main fatty acids (60.42 % of total fatty acids), followed by oleic acid (21.65 %), palmitic acid (12.24 %), and stearic acid (3.88 %), respectively. Furthermore, it is worth mentioning that *beta*-sitosterol and *gamma*-tocopherol were the principal components of sterols and tocopherols representing 67.56 % of total sterols and 330 $mg.kg^{-1}$ of total tocopherols. The proposed approach applied to prickly pear seeds had preserved the tocopherol fraction (796.70 $mg.kg^{-1}$ of total tocopherols) about two times more than other cold-pressed seed oils approaches. Based on trial-and-error conditions, no additional operating problem even has been reported with a seed of high level of hardness. Seed should be introduced with a humidity level of 10 % without grinding. Moreover, no heating should be supplied and an optimal pressing speed of 30 and a nozzle diameter of 15 mm.

Introduction

The oilseed industry allows the production of oil as a high value-added product with specified quality that meets the requirements of downstream health industries, namely cosmetics, pharmaceuticals and nutraceuticals. In recent years, a growing demand has been observed which consequently promotes extractive techniques under the concept of bio-refining (Laurent *et al*. 2011). These industries extract vegetable oils through various techniques, the most commonly cost-efficient are based on solvent extraction and cold pressing. Although the latter has a reduced yield compared to the former one, it is more appropriate for health-related purposes since it is solvent free and preserves oil quality in terms of fatty acids and the levels of bioactive elements such as: tocopherol, squalen, and sterol (Koski *et al*. 2002; Bail *et al*. 2008).

However, during the cold pressing method if the

press set points conditions are not well specified and seed properties (such as hardness, oil content, moisture content, granulometry, etc.) are not taken into account, serious problems could emerge in terms of yield. For instance, significant oil loss in the cake, quality deterioration, productivity loss, production cost could be caused. Other problems such as operability risks could occur, such as operating loss, mechanical equipment failure and occupational risks.

In the literature there are some guidelines that indicate the appropriate conditions for oil seed extraction (Schaufler and Schaufler *et al.* 2013). Nevertheless, less research has been done on seed hardness, whose approach has been mainly based on trial and error procedure. In order to achieve adequate cold pressing and produce high-quality oil without operating issues, a cold pressing method is carried out on prickly pear (*Opuntia ficus-indica* (L.) Mill.) seeds, using HAZOP analysis to study the operational and hazardous conditions related to the cold pressing process. Prickly pear seeds are considered a raw-material for cosmetic oil production. They contain valuable oil. However, they present a relatively high level of hardness. Consequently, these seeds are chosen as reference to study seeds leading to low yield as well as operability risks.

The adopted approach combines HAZOP analysis and experience plan, so it is possible to specify the optimal conditions for oilseed extraction based on cold pressing. Indeed, HAZOP as the method of qualitative approach allows to identify and record hazards based on formalized and systematic thinking through a multidisciplinary team examining potential intent deviations (Kletz 1999; Reniers *et al.* 2005). HAZOP significantly reduces the number of experiments to carry out in order to find out the best correlation between yield and quality of the extracted oil and the manipulated variables of the cold pressing, namely pressure, temperature, nozzle diameter, residence time, pressing speed.

To assess the oil quality, the Gas chromatography (GC) and High-performance liquid chromatography (HPLC) were performed to determine the composition of fatty acids, sterols and tocopherols, respectively.

Experimental

Oil extraction by cold pressing method

Seeds of the prickly pear (*Opuntia ficus-indica* (L.) Mill. (OFI) with high hardness are the most used seeds for oil extraction in Morocco. Samples from the Aït Baamrane region in southern Morocco were obtained and their properties were studied (weight, humidity, grain size, and others). Seed oil was extracted by the cold pressing method (Fig. 1) at a laboratory scale. It is a technological mechanical process of extracting oil from the seeds by applying the required pressure according to seed characteristics. The cold pressing was performed using a screw press Kern & Kraft Press (KK-20, Germany) with capacity of 20 $kg.h^{-1}$ of seed, equipped with engine of 2.0 kW, a speed variator, various nozzle diameter as well as a heating system for temperature control.

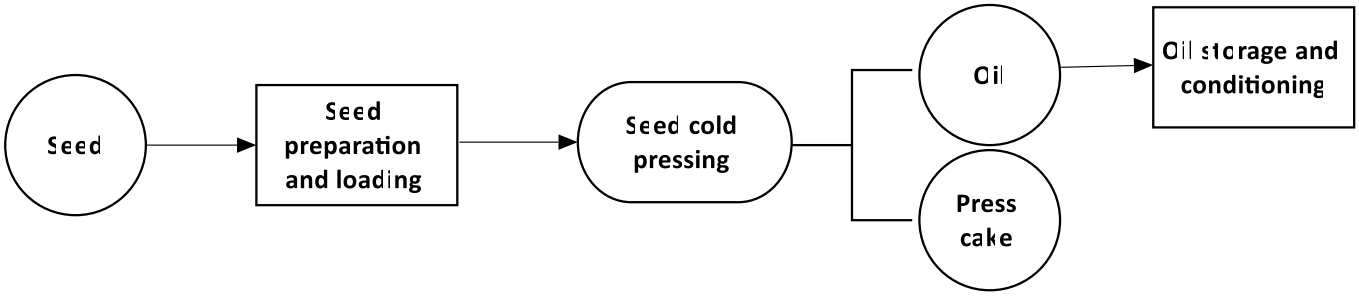

Fig. 1. Scheme of the cold pressing extraction of oil from OFI seeds.

Oil yield

The seed oil content was determined using the Soxhlet method according to ISO 659 (1998). Seeds were grinded using a blender into a fine and homogeneous powder. Twenty grams of powder was subjected to extraction with hexane (100 mL) at 40 - 60 °C in the Soxhlet extractor. After evaporation of

the solvent under reduced pressure, using a rotary evaporator at 40 – 60 °C, the oil content was calculated and expressed as a percentage of seed dry matter according to the following formula (Eq. 1):

$$Y(\%) = 100 \cdot \frac{We}{Wt} \quad (1)$$

W_e : weight of fat extracted (g)
W_t : weight of test sample (seed powder) (g)

After determining seed oil content, the total yield extracted by cold pressing was calculated according to the formula (Eq. 2):

$$Yield = 100 \cdot \frac{Oil\ yield\ extracted\ by\ cold\ pressing}{Seedoil\ content} \quad (2)$$

Fatty acid composition

The fatty acid profile was determined using method ISO 5508 (1990). Before the analysis, fatty acids were converted to fatty acid methyl esters by shaking a solution of 60 mg of oil and 3 mL of hexane with 0.3 mL of 2 N methanolic potassium hydroxide. Fatty acids were analyzed by gas chromatography GC (Agilent G-97-04A) equipped with flame ionization detector (FID) and a columnar capillary filled with a stationary phase (70 % cyanopropyl polysilphenylene, length: 60 m, internal diameter: 0.25 mm, film thickness: 0.250 µm). The carrier gas was hydrogen, and the total gas flow rate was 1.6 mL.min^{-1}. The initial column temperature and the final temperature were 140 °C and 200 °C, respectively. The injector and detector temperature were 280 °C. Fatty acids were identified by comparison of their retention times with standard mixtures.

Sterols composition

The sterol fraction was determined using the method ISO 6799 (1991) by GC (Agilent 19091J-436 HP-5, 5%-phenylmethylsiloxane). After the extraction and isolation of the unsaponifiable, the gas chromatographic analyzes were carried out using a column (length: 60 m, nominal diameter: 250 µm, nominal film thickness 0.25 µm) with a helium carrier gas (flow 1.5 mL.min^{-1}). The column temperature was isothermal at 270 °C, injector and MS detector temperature were 300 °C. The sterols quantification was based on the comparison of the spectra with those in the literature.

Tocopherols composition

Tocopherols were identified based on the international standard ISO 9936 (2006), using HPLC Shimadzu LC-10ad VP 230V CE normal phase Isooctane/Isopropanol (99 : 1, v/v). Solution of 2 g of oil in 25 mL of isooctane (flow rate of 1.2 mL.min^{-1}) was directly used for the HPLC, using octadecylsilane silica (C_{18}) column as stationary phase (length : 250 mm, internal diameter : 4.6 mm, thickness of the film : 5 µm) with the fluorometer detector set at 292 nm, to ensure a high selectivity and to differentiate many forms of isomers antioxidants. Tocopherols were identified by comparing their retention times with those of authentic standards.

Quality indices (acidity, peroxide value)

In order to check the quality and appropriate conservation of the obtained seed oil, the acidity (A) (expressed as percentage of oleic acid) and the peroxide value (PV) (expressed as milliequivalents of active oxygen per kg of oil) were measured.
Acidity (A) is the amount of free fatty acids expressed as a percentage of oleic acid. The acidity of the prickly pear seed oil was determined according to the method ISO 660 (1996). An amount of 10 g of prickly pear oil was added to 50 mL of neutralized ethanol/diethyl ether mixture (1 : 1, v/v) in the presence of phenolphthalein as indicator, then titrated with potassium hydroxide solution NaOH (0.1 M).
Acidity was calculated as follows (Eq. 3):

$$A\ (\%) = \frac{V \times C \times M}{10 \times m} \quad (3)$$

V: NaOH volume (mL); C: NaOH concentration (mol.L^{-1}); M: Oleic acid molar mass (282 g.mol^{-1})
m: Oil mass in grams (g).

Peroxide Value (PV) is defined as the number of mili-equivalents of oxygen per kilogram of fat. It is determined following the method ISO 3960 (1977).

An amount of 5 g of prickly pear oil was added to 10 mL of chloroform, 15 mL of acetic acid, mixed all with 1 mL of saturated potassium iodide. The mixture was left in darkness, then 75 mL of distilled water was added. The mixture was titrated with sodium thiosulphate (0.01 N), using starch solution as indicator. The Peroxide value was calculated as follows (Eq. 4):

$$PV\left({}^{meqO_2}\!/_{kg}\right) = \frac{(V_x - V_0) \times N}{m} \times 1000 \quad (4)$$

Vx : Sodium thiosulfate volume (mL) for the titration; V0: Sodium thiosulfate volume (mL) for the blank; N: Sodium thiosulfate Normality; m: Oil mass in grams (g).

HAZOP method

During the preliminary experiments, various incidents during the cold press oil extraction of prickly pear seeds were observed due to their high hardness. It caused serious problems in yield (significant oil loss in the cake, quality deterioration, etc.) and operability (operating loss, mechanical equipment failure, occupational risks). For this purpose, the HAZOP method was adopted to determine operational and risk conditions during start-up, steady-state operation, and press shut down. The system was subdivided mainly into three sub-systems as shown in the Table 1.

Table 1. The proposed nodes for HAZOP analysis application.

Sub system	Node
Raw material conditioning & feeding	I: Seed supply conditions and press loading
Production (steady state operation)	II: Seed pressing for oil extraction and cake recovery purposes
Conditioning and storage of the obtained oil	III: Conservation of the extracted oil

In each node the process parameters were identified (Table 3) considering the experiment design of the *in situ* oil extraction, the feedback from oilseed industrial operators and the literature data. (Frączek *et al*. 2005; Lacoste *et al*. 2005; Altuntaş and Yıldız 2007; Soetaredjo *et al*. 2008; Van Hoed *et al*. 2010; Matthäus and Özcan 2011; Nitièma-Yefanova *et al*. 2012; Chougui *et al*. 2013; Schaufler and Schaufler *et al*. 2013; Rabrenović *et al*. 2014; Fuentes-Bargues *et al*. 2016).

The study was performed following the steps of the HAZOP process (Fig. 2). Indeed, the process parameters associated with guidewords (Table 2) were depicted in order to unveil the most significant deviations that could involve risks or dangers in each subsystem. Eighteen significant deviations were generated (Table 3).

Table 2. Standard guidewords and their generic meanings.

Guidewords	Interpretation
No (not, none)	None of the design intent is achieved
More (more of, higher)	Quantitative increase in a parameter
Less (less of, lower)	Quantitative decrease in a parameter
As well as (more than)	An additional activity occurs
Reverse	Logical opposite of the design intention occurs
Other than (other)	Complete substitution - another activity takes place or an unusual activity occurs or uncommon condition exists

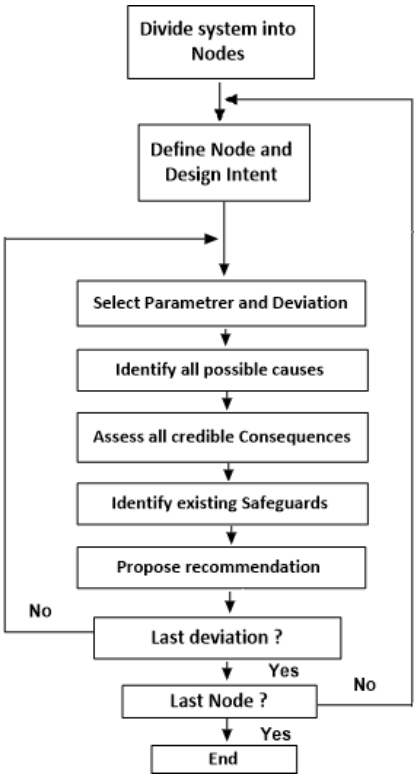

Fig. 2. HAZOP process according to ISO 31.010 (2011).

Table 3. Identification of deviations leading to risks / hazards.

Deviation (parameters/keywords)	More of	Less of	Not	Reverse	More than	Other
Node I						
Seed moisture	′D1	D2	-	-	-	-
Drying / Humidification	-	-	D3	-	-	-
Seed granulometry	D4	D5	-	-	-	-
Seed hardness	D6	-				
Seed cleanness	-	D7	-	-	-	-
Node II						
Press loading	D8	D9			D10	-
Screw speed	D11	-	-	-	-	-
Heating temperature	D12	-	-	-	-	-
Press pressure: nozzle choice	D13	D14	-	-	-	-
Preheating the press	-	-	D15	-	-	-
Node III						
Cleaning the press after use	-	-	D16	-	-	-
Oil storage under cold conditions	-	-	D17	-	-	-
Opacity of the oil storage bottles	-	-	D18	-	-	-

It prevents efficient operation.

Table 4. Summary table of risk causes associated with deviations.

Deviation	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**
Cause	The seeds are stored in a humid place.	The seeds are stored in an air-conditioned and sunny space.	The seed dryer or humidifier is not operative.	The pressed seeds are very thin or have been preliminary grinded.	Grinding large seeds is not done properly to achieve the desired particle size.	Seeds that are intrinsically hard or too dry (under extreme conditions of temperature and pressure).
Consequence	**Oil yield loss:** Seeds become mellow and the oil is strongly related to the water. Moisture content is often the culprit of a bad pressing.	**Oil yield loss:** The seeds get harder and the oil is caught inside the dried cells.	**Oil yield loss:** The pre-drying considerably increased the hardness of seeds. This automatically generates an interrupt, blocking the press head.	**Zero yield:** Because of the fineness of seeds. Seeds came out through pores dedicated to the oil evacuation.	**Productivity loss:** A significant amount of the oil came out the cake.	**Machine breakdown and productivity loss:** The screw cannot grind the seed that remains caught inside the press. Congestion did not let the screw react to advance the material even if the revolutions per minute increased to high enough values.

Table 4. Summary table of risk causes associated with deviations. *Continued*.

Deviation	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**
Cause	Seeds are not washed or contain a significant amount of dust	At start-up, the press is generally loaded. A total loading of press is required to ensure the desired pressure.	During steady-state operation the press is not loaded globally.	Seeds with other elements are introduced (metal parts, plastics, paper, etc.)	The revolutions per minute applied to the motor speed controller are high.	The temperature could increase if the heat is supplied via the press heating ring, or if the pressure inside the press increases, it directly increases the temperature
Consequence	**Quality loss:** Dust containing undesirable elements decreases the oil quality.	**Same consequence of D6**	**Productivity loss:** There is not enough pressure inside the press and a significant amount of oil in the outgoing cake.	**Machine breakdown and quality loss:** Hard objects break the screw and plastic elements can significantly damage the oil quality.	**Machine breakdown and productivity loss:** Short residence time in the press involving high oil content in the cakes it is also possible to have overcrowding between the hopper and the screw because the transfer of material is more important than the pressing speed.	**Quality loss:** The cold pressing seed should be maintained at a temperature below 50 °C

Table 4. Summary table of risk causes associated with deviations. *Continued*.

Deviation	**D14/D13**	**D15**	**D16**	**D17**	**D18**
Cause	The incompatibility between the nozzle diameter and the seed hardness.	Preheating the head press is not carried out for a sufficient period and with the required temperature.	Postpone the machine cleaning.	Oil storage at high temperature.	Oil storage in a transparent bottle.
Consequence	**Productivity loss and operating loss:** If the nozzle is too small, the seeds at the exit of the press are retained and the extraction of the cake will not be executed, we notice that the solid seeds start to come out of the oil outlet pores. However, if the nozzle is too big, there will not be enough pressure, which will probably cause an oil loss in the cake.	**Operating loss and Oil yield loss:** The mechanical gear of the drive parts at the press head does not take place properly and can easily cause mechanical stress that will cause a crack under the applied force. Moreover, this quenching will increase the seed hardness.	**Machine breakdown and operating loss:** cleaning and maintenance must be carried out immediately after pressing because the delay caused a very strong bond between the seeds and the metal of the press head. Forced opening probably will break the press head.	**Quality loss:** Some fatty acids are more fragile than others, especially unsaturated fatty acids which have a high rate of oxidation under certain conditions (heat, oxidation catalyst, etc.) they degrade which produce a change in the properties of the vegetable oil (smell of rancidity, color change). This change is toxic for the organism.	**Quality loss:** Many factors accelerate oxidation: oxygen, light (UV), contact with pro-oxidizing metals (iron or copper), the presence of pigments such as chlorophyll, the presence of enzymes (lipases ...), and the heat that will alter the oil quality and its richness of the vitamin E.

Table 5. Corrective measures and nominal values to set up for an adequate cold pressing.

Deviation	**D1/D2**	**D3**	**D4/D5**	**D6**	**D7**	**D8/D9**	**D10**
Measure	Humidity control (measure the seed moisture content) Definition of storage conditions.	The seeds must be dried before pressing.	Sieving to determine the seed size, to choose the right nozzle diameter.	If the seeds are relatively hard, it is necessary to ensure that their moisture is in the standards (10% by weight). It would be preferable to work: without heating the press head, with the larger 15 mm diameter nozzle and with a slow residence time to avoid rapid accumulations at the press crusher barrel. It would be necessary to initially introduce a small amount of seed into the loading tank and leave the machine in normal operation and wait for the appearance of the first cake to come out and then increase the load gradually to reach a full load.	Washing the seeds before use is essential to remove undesirable particles, and it is always followed via air blowing, it makes the pressing much easier.	Provide a loading protocol based on the recommendations and measures presented previously (deviation D6). It is necessary to completely fill the seed press to cause a sufficient pressure and consequently to increase the yield.	Sorting out the raw material is necessary to avoid any undesirable particles during loading.
Nominal Value	Average seed moisture content is an average of the order of 10 %.	Seeds humidity control	The seed size must be compatible with the used nozzle	The water content has a significant effect on the seed hardness. However, using a hardness test could predict grain hardness based on particle size analysis.	Towards zero dust	Fulfilling the press in a steady state.	Pure raw material 100 %

Table 5. Corrective measures and nominal values to set up for an adequate cold pressing. *Continued*.

Deviation	**D11**	**D12**	**D13/D14**	**D15**	**D16**	**D17**	**D18**
Measure	Reducing the screw speed increases the residence time, ensuring more time for pressing, thus increasing the efficiency of the extraction.	It is not necessary to press seeds by heat in order to preserve the maximum of the antioxidant elements ensuring good quality of oil (the heating created during the pressing process alone could reach a high temperature).	Choose the appropriate nozzle according to the seed hardness.	Preheat the press head via the heating ring for a period of 30 min at a temperature of 100 °C, and at the same time rotate the screw at 30 - 50 rpm. The rotation of the screw has a major role in the mechanics, the dilation and the uniformity of the temperature throughout the pressing chamber. It is recommended to activate the screw rotation with heat before the press loading.	Clean the mechanical parts of the press head after use.	Store the oils in a fresh place and avoid excess heat. Preferably a refrigerator in a firm bottle to minimize the oxidation phenomenon.	Using an opaque bottle to avoid sunlight and ultraviolet light that affect the oil quality. It is also important to clean the outer edges and the screw thread of the bottle with a dry tissue as a precaution to avoid the internal humidity of the bottle.
Nominal value	30 rpm	Pressing without temperature or at lower degree. (T < 50 °C)	Nozzle diameter 15 mm (for prickly pear seeds)	Preheat the press headfor 30 min at a temperature around 100 °C and a press speed 30-50 rpm.	Immediately.	Fresh place (T < 20 °C).	Opaque bottle with an inner aluminium wall is covered with a cling film which prevents the direct contact ofthe oil with the metal and avoids oxidation.

Besides, the results of the risks identified by the HAZOP analysis are depicted in Table 4, which are more specifically "operability risks" that affect the press performance, the product quality and the economic operation of the unit. Therefore, HAZOP study forms a useful guide to the maintenance personnel, providing measurements – recommendation and nominal values listed in Table 5, to achieve adequate cold pressing.

Results and Discussion

The optimal conditions of cold pressing method

The cold-pressing operability risks have been identified based on HAZOP analysis. Each process parameters have been studied in order to determine the optimal pressing conditions. The experience plan has been reduced, according to HAZOP results, instead of performing experiments on all the manipulable variables of the press, the seeds were pressed based on the screw speed variable.
The press performance was more effective and without any operating problems when the pressing is based on the corrective measures and nominal values mentioned below (Table 6 and 7).

Table 6. Raw material storage and feeding press conditions.

Seed properties	Nominal values
Seed moisture	Average of the order of 10 %.
Seed hardness	High hardens level
Seed granulometry	The grain size must be compatible with the used nozzle, without grinding the seeds.
Seed cleanness	Towards zero dust

T**able 7**. Optimal conditions of prickly pear seeds cold pressing.

Parameters	Nominal value	Corrective measures
Screw speed	30 rpm	Reducing the speed to 30 rpm increases the residence time thus ensuring more time for the pressing to take place and thus increasing the extraction efficiency.
Temperature	Pressing without heat	Pressing without heat or at lower temperature (T < 50 °C). The heat created during the pressing process is enough. It is not necessary to press seeds by heat in order to preserve the maximum of the antioxidant elements, ensuring good quality of oil.
Press loading	Total filling of the press (100 %)	Load the seeds with small quantities (wrists of the hand) until the appearance of the first cake (10 – 15 s) then completely fill the press. It is necessary to completely fill the seed press to cause a sufficient pressure and consequently to increase the yield.
Nozzle diameter	15 mm	The grain size must be compatible with the used nozzle.
Preheating the press	Preheat the press head for 30 min at a temperature around 100 °C and a press speed 30 – 50 rpm.	The rotation of the screw has a major role in the mechanics, the dilation and the uniformity of the temperature throughout the pressing chamber. It is recommended to activate the screw rotation before the press loading with heat.
Cleaning the press after use press after use	Immediately	Non-cleaning causes a very strong bond between the seeds and the metal of the press head. A forced opening probably will break the press head.
Oil storage under cold conditions	Fresh place (T < 20 °C)	Opaque bottle with an inner aluminium wall is covered with a cling film which prevents the direct contact of the oil with the metal and avoids oxidation.

Oil yield

The cold-press extraction process was carried out 6 times, without heat changing only the pressing speed and considering HAZOP analysis results mentioned previously. The results obtained in Table 8 show that the highest oil yield is 4.85 % on a dry basis when the pressing is at speed of 30 rpm and the lowest oil yield is 3.13 % when the pressing speed is slower at 15 rpm, also the yield decreased to 4.17 % when the

speed increased above 30 rpm. When the screw speed is slower than 30 rpm, it provides more time (long residence time) for the seed pressing, but at the same time the press clogs more, which leads to decrease the seeds flow loading and minimize yield. Indeed, when the screw turns faster, more material is moving through the press and this provides less time for the oil to migrate out and be separated from the meal. So, in both cases, a lower yield is obtained. As a result, the optimum pressing speed is 30 rpm, at this speed, the press oil extraction rate is higher and a lower amount of oil left in the meal.

Table 8. Variation of oil extracted yield with pressing speed.

Test	Screw speed [rpm]	Oil content [%] dry basis
1	15	3.13
2	20	3.78
3	25	4.07
4	30	4.85
5	35	4.57
6	40	4.17

The obtained cold-pressed seed oil yield (4.85 %, on a dry basis) is lower compared to the seed residual oil content (7 %) extracted by hexane. Significant amount of oil remains stored in the cake produced during cold pressing.

According to the data, a comparative study (Mouden *et al*. 2012) that was carried out on *Opuntia ficus indica* (L.) Mill. seeds (origin from Aït Baamrane), through two different extraction methods, one by cold pressing and the other by solvent, the maximum oil obtained was (5.90 %) following the cold pressing method and (7.79 %) following a solvent extraction method. In other studies, as Taoufik *et al*. (2015) and El Hachimi *et al.* (2015) results, the seed oil yield of the same studied variety (OFI originate from Aït Baamrane, Morocco) by the same extraction process (solvent extraction) was (7.6 – 8.74 %), respectively. The difference between the yield could be attributed to the cultivar, geographical origin of fruits, degree of maturity and the storage conditions but also to the extraction protocols and analytic assays (Brahmi *et al.* 2020, El Hachimi *et al.* 2015).

In our case, the cold pressing yield was influenced by seed properties and its storage conditions, press feeding, and operating conditions.

Oil characterisation

The extracted seed oil has some interesting organoleptic properties, namely a greenish yellow color with a strong characteristic odor. According to Ramadan and Mörsel (2003) study, OFI seed oil contains a lower amount of carotenoids (*b*-carotene) compared to pulp oil. Carotenoids may be the source of the light-yellow hues of seed oil, while the pulp oil is characterised by dark orange hues.

Fatty acid composition

The GC results (Table 9) showed that linoleic acid is the dominant fatty acid, with high content that reaches up to (60.42 %) followed by oleic acid (21.65 %), palmitic acid (12.24 %) and stearic acid (3.88 %), respectively. Apparently, the extracted oil is highly unsaturated.

Table 9. Fatty acid compositions of prickly pear seed oil.

Fatty acids	Concentration [%]
Palmitic acid (C16:0)	12.24
Palmitoleic acid (C16:1 n-7)	0.70
Margaric acid (C17:0)	0.05
Heptadecanoic acid (C17:1)	0.02
Stearic acid (C18:0)	3.88
Oleic acid (C18:1 n-9)	21.65
Linoleic acid (C18:2 n-6)	60.42
α-Linolenic acid (C18:3 n-3)	0.22
Arachidic acid (C20:0)	0.33
Paullinic acid (C20:1 n-7)	0.35
SFA : C16:0+C18:0	16.12
PUFA: C18:	60.42
PUFA/SFA: C18:2/ (C16:0+C18:0)	3.74

SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids; PUFA/SFA = unsaturation ratio.

Our results are in good agreement with Taoufik *et al.* (2015) results, linoleic acid is the dominant fatty acid (61.5 %) followed by oleic acid (21.3 %), palmitic acid (11.9 %) and stearic acid (3.3 %), respectively. There is no difference between the fatty acid concentration of our cold-pressed oil and the fatty acid concentration of the solvent extracted oil from the same OFI, and from the same geographical origin (Aït Baamrane, Morocco).

According to Brahmi *et al*. (2020) results, the Algerian cold-pressed seed oil (OFI) contains

linoleic acid as the main compound represents (55,81%), followed by oleic acid (12.99 %), palmitic acid (10.8 %) and stearic acid (2.97 %), respectively. The cold-pressed seed oil of Brahmi *et al*. (2020) has a lower concentration of fatty acids compared to our results and Taoufik *et al.* (2015) results. Therefore, we conclude the extraction method type does not affect the concentration of fatty acids, the geographical origin of the fruit does.
Indeed, Ramadan and Mörsel (2003) explained the difference between the content of fatty acids which is due to different locations of the cactus cultivation, the content of individual fatty acids differed significantly. Also, it is influenced by geographic and climatic differences (El Finti *et al.* 2013).

Sterols composition

The sterol fraction of OFI seed oil is defined by *beta*-sitosterol as the main component, representing 67.56 % of the total sterols, followed by campesterol (11.87 %) and Δ5-avenasterol (4.62%). Other compounds (stigmasterol, Δ7-avenasterol, cholesterol) were found with a lower percentage (Table 10).

Table 10. Sterol composition of prickly pear seed oil.

Sterols	Concentration [%]
Cholesterol	1.03
β-sitosterol	67.56
Campesterol	11.87
Δ5- avenasterol	4.62
Δ7-avenasterol	2.72
Stigmasterol	2.47

The results are in strong alignment with Taoufik *et al*. (2015), Ramadan and Mörsel (2003). The *beta*-sitosterol represented in their studies 75 % and 72 % of total sterols fraction of OFI seed oil, respectively. The contents of *Δ5*- and *Δ7*-avenasterols were higher in cold-pressed oil in comparison with their contents in both solvent extracted oils (4.8 %, 3.1% – 0.9 %, 0.53 %, respectively).
Phytosterols, including *beta*-sitosterol, campesterol, stigmasterol, and others, are known for having many benefits for human health such as lowering of blood cholesterol. For several decades, it has been appreciated the consumption of plant sterols and stanols that lead to favorable shifts in circulating lipid levels (Jones *et al*. 2000).

Tocopherols composition

The cold-pressed oil contained the *gamma*-tocopherol as the major dominant fat-soluble vitamin representing 97.56 % of total tocopherols. This significant content exceeds the *gamma*-tocopherol content in the OFI solvent extracted oil (Taoufik *et al.* 2015), that was 89.1 % from total tocopherols. Regarding the *alpha*-tocopherol content that represented 1.5 %, it was similar. However, *beta*-tocopherol was not detected in our oil analysis (Table 11).

Table 11. Tocopherols composition of prickly pear seed oil. (nd – not detected)

Tocopherols	[mg.kg^{-1}]	[%]
Alpha	12.65	1.58
Beta	nd	nd
Gama	777.30	97.56
Delta	6.74	0.84
Total tocopherols [mg.kg^{-1}]	796.70	Nd

According to Ramadan and Mörsel (2003), *gamma*-tocopherol was the dominant compound in amount of 330 mg.kg^{-1}, followed by *alpha*-tocopherol (56 mg.kg^{-1}). Our experiment revealed that *gamma*-tocopherol content was significantly higher (777.30 mg.kg^{-1}), followed by *alpha*-tocopherol that represents (12.65 mg.kg^{-1}). Amount of total tocopherols was 796.70 mg.kg^{-1} that was also much more than referred Ramadan and Mörsel (2003) (403 mg.kg^{-1}).
Assessment of prickly pear seed oil yield and quality showed that almost 70 % of high-quality oil has been extracted that contains about double of the total tocopherols in comparison to results of Ramadan and Mörsel (2003). This is due to the methodology followed, which considered the seed properties and its storage conditions, as well as the press feeding and operating conditions. This approach was less devastating and improved the quality of cold-pressed seed oil to 49.4 % compared to results from other studies (Table 12). Pressing without heat enhanced the quality of prickly pears seed oil, preserved its richness in tocopherols sensitive to light (ultraviolet radiation) and heat. In agreement with Bostyn *et al.* (2008), it was clearly explained that tocopherols

degradation is due to the major factor which is the temperature, especially above 100 °C. According to Rueda *et al*. (2016) multivariate factor analysis revealed that tocopherols, particularly the *gamma*-tocopherol, was the strongest variable influencing the discrimination of different oils. Furthermore, *gamma*-tocopherol helps reduce inflammation, guards against certain cancers, and protects against radiation exposure (Gysin *et al*. 2002; Stone *et al*. 2004). The *gamma*-tocopherol, as other tocopherols, also helps moisturize the skin while fighting against wrinkle-forming free radicals (Nagata *et al*. 2010). Prickly pear oil also contains a high level of antioxidants and has antibacterial properties. Some healthful compounds reduce skin inflammation, as well as prevent skin damage and acne (Koubaa *et al*. 2017). OFI seed oil is a suitable and safe carrier for delivering other nutrients that cannot be directly applied to the skin, including vitamin A. OFI seed oil has valuable applications in cosmetic applications (AlZahabi *et al*. 2019). OFI seed oil is highly unsaturated, rich in antioxidants therefore, it could also be added to foods as natural antioxidant additive and fatty acids supplement.

Table 12. Assessment of prickly pear seed oil yield and quality.

	Reference [1]	Reference [2]
Vitamin E concentration [mg.kg^{-1}]	796.7	403
Yield [%]	4.85	9.8
Oil density [kg.m^{3}]		0.9
Optimization [%]		49.4

[1]Cold-pressed prickly pear seed oil extracted from seeds originated from Aït Baamrane, Morocco

[2]Prickly pear seed oil extracted by solvent, originate from Berlin, Germany (Ramadan and Mörsel 2003).

Acidity

Observed acidity of OFI seed oil was 0.8 %. It is a lower value in relation to the standard value approved by Codex Alimentarius (1999) (6 % for cold-pressed oil) and also in comparison with studies Brahmi *et al*. 2020 (2.12 %) and Özcan *et al*. 2011 (1.41 %). These high values might be due to the chemical reactions in seeds during oil harvesting, handling or processing. Oil exposed to inappropriate storage conditions deteriorates its quality in different ways, but most often by hydrolysis or oxidation (Kandji 2001). According to the previous data, OFI seed oil is pure and can be considered virgin oil.

Peroxide value

The PV could be considered an indicator of oils and fats quality and stability (Zahir *et al*. 2017). The PV of OFI seed oil is 9.6 meq O_2.kg^{-1} an acceptable peroxide value at the standard value of 15 meq of peroxides.kg^{-1} of oil for a cold-pressed oil specified by the Codex Alimentarius (1999).

Different cultivars of prickly pear and different extraction methods revealed PV in range from 9.50 to 18.02 meq O_2.kg^{-1} (South African prickly pear, solvent extracted, DeWit *et al*. 2017), 12 meq O_2.kg^{-1} (Algerian prickly pear, cold-pressed, Brahmi *et al*. 2020), and 1.63 meq O_2.kg^{-1} (Turkish prickly pear, solvent extracted, Özcan *et al*. 2011). The PV could be affected by the oil oxidation during the extraction and conservation conditions, decreasing of certain antioxidant substances present in oil such as carotenoids, vitamins A and E, and squalenes (Zahir *et al*. 2017).

Conclusion

The present study established a risk prevention platform to achieve adequate cold pressing performance for the oil industry. The methodology and correct measurements resulting from the proposed approach could be taken into account for the cold pressing of prickly pear seeds, but also other oilseeds with high hardness of seeds.

The seed oil yield was influenced by properties of seeds, seed storage conditions, press feeding, and operating conditions. Presented methodology had no effect on the fatty acid and sterol contents, but affected oil quality in terms of tocopherols content. Oil had high quality, was rich in essential fatty acids and sterols, very high contents of tocopherols, chemically free from organic solvents, with lower value of acidity and peroxide value. This indicated its purity and stability.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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