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Cold-pressed cactus pear seed oil: Quality and stability

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SUMMARY: Cold-pressed seed oil from twelve commercially produced cactus pear cultivars was assessed for oil yield, fatty acid composition, physicochemical properties, quality and stability. Large differences in oil content, fatty acid composition and physicochemical properties (IV, PV, RI, tocopherols, ORAC, % FFA, OSI and induction time) were observed. Oil content ranged between 2.51% and 5.96% (Meyers and American Giant). The important fatty acids detected were C16:0, C18:0, C18:1c9 and C18:2c9,12, with C18:2c9,12, the dominating fatty acid, ranging from 58.56–65.73%, followed by C18:1c9, ranging between 13.18–16.07%, C16:0, which ranged between 10.97 - 15.07% and C18:0, which ranged between 2.62–3.18%. Other fatty acids such as C14:0, C16:1c9, C17:0, C17:1c10, C20:0, C18:3c9,12,15 and C20:3c8,11,14 were detected in small amounts. The quality parameters of the oils were strongly influenced by oil content, fatty acid composition and physicochemical properties. Oil content, PV, % FFA, RI, IV, tocopherols, ORAC and *ρ*-anisidine value were negatively correlated with OSI. C18:0; C18:1c9; C18:2c9,12; MUFA; PUFA; *n*-6 and PUFA/SFA were also negatively correlated with OSI. Among all the cultivars, American Giant was identified as the paramount cultivar with good quality traits (oil content and oxidative stability).

***KEYWORDS**: Antioxidants; Induction time; Oxidation; Prickly pear; Yield*

RESUMEN: ***Aceite de semilla de nopal prensado en frío: calidad y estabilidad.*** Se evaluó el rendimiento de aceite, la composición en ácidos grasos, las propiedades fisicoquímicas, la calidad y la estabilidad del aceite de semilla prensadas en frío de doce cultivares de nopal producidos comercialmente. Se observaron grandes diferencias en el contenido de aceite, la composición en ácidos grasos y las propiedades fisicoquímicas (IV, PV, RI, tocoferoles, ORAC, % FFA, OSI y tiempo de inducción). El contenido de aceite varió entre 2,51–5,96% (Meyers y American Giant). Los ácidos grasos mayoritarios fueron C16:0, C18:0, C18:1c9 y C18:2c9,12, siendo el C18:2c9,12 el mayoritario con porcentajes entre 58,56–65,73, seguido de C18:c9 que varía entre 13,18–16,07%, C16:0, 10,97-15.07% y C18:0 entre 2,62-3,18%. Otros ácidos grasos, tales como C14:0, C16:1c9, C17:1c10, C20:0, C18:3c9,12,15 y C20:3c8,11,14 se detectaron en pequeñas cantidades. Los parámetros de calidad de los aceites estuvieron estrechamente influenciados por el contenido total de aceite, la composición de ácidos grasos y las propiedades fisicoquímicas. El contenido de aceite, PV, % FFA, RI, IV, tocoferoles, ORAC y el valor de *ρ*-anisidina se correlacionaron negativamente con OSI. C18:0; C18:1c9; C18: 2c9,12; MUFA; PUFA; n-6 y PUFA / SFA también se correlacionaron negativamente con OSI. Entre todos los cultivares, American Giant fue identificado como el cultivar primordial con rasgos de buena calidad (contenido de aceite y estabilidad oxidativa).

***PALABRAS CLAVE:** Antioxidantes; Higo chumbo; Oxidación; Rendimiento; Tiempo de inducción*

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1. INTRODUCTION

Edible cold-pressed oils are functional products because of their bioactive substances such as polyunsaturated fatty acids, tocopherols, sterols, phenols, carotenoids and chlorophyll. These oils have specific characteristics which provide additional health benefits (Boskou, 2017). The use of non-traditional cold-pressed oils to be introduced into the cosmetic and nutraceutical markets is expected to increase. Recently, research on oils from fig seeds from Morocco (Hssaini *et al*., 2020), lentisk and skeels from Algeria (Brahmi *et al*., 2020) as well as milk thistle oil and their functional properties were reported. Cactus pear seed oil has been viewed as an important vegetable oil because of its related quality composition factors, namely high concentrations of important fatty acids, for example, linoleic acid (C18:2c9,12) (C18:2 n-6); vitamin E (100 mg/100g) and sterols (1 g/100g) (Ennouri *et al.*, 2005). In the past, oil quality was estimated based on oil yield and fatty acid composition alone. Lately, in addition to fatty acid composition, the natural antioxidants, tocopherols and sterols are additional important components which define oil quality (Fernández-Martínez *et al*., 2004). Oil quality has been transformed by the development of oil with enhanced nutritional and functional properties, and these modifications are incorporated when breeding for increased quantities of C18:2c9,12.

Cold-pressed cactus pear seed oil is characterized by a high content of unsaturated fatty acids (Sawaya and Khan, 1982). Past research has demonstrated that vegetable oil with the highest unsaturation level is most likely to undergo autoxidation. Martínez *et al*. (2011) reported that there are elements that can potentially affect the stability and quality of oil in a negative way, for example light, heat, metals, free fatty acids, tocopherols, phospholipids and waxes which contain pro or antioxidant properties. Hydrolytic reactions which are catalyzed by lipases and react with atmospheric oxygen (autoxidation) are a major cause of nutritional and oil quality losses. Naz *et al*. (2004) explained oxidation as an autocatalytic series of reactions that requires low activation energy. These authors also reported that fatty acid composition and the presence of antioxidants in vegetable oil influences oxidation. Lajara *et al.* (1990) and Izquierdo *et al*. (2009) reported that oils containing high contents of oleic acid (C18:1c9) will possibly have greater stability against oxidation and are favored for improved shelf-life. Naz *et al*. (2004) further reported that to minimize the effect of hydrolytic reactions, cold storage and proper packaging must be applied. Martínez *et al*. (2011) stated that the oxidative stability of cold-pressed oils is usually measured by the level of peroxide and *ρ*-anisidine values. The level of unsaturation (polyunsaturated fatty acids) (PUFA), especially the level of α-linoleic acid (C18:2c9,12) and the amounts of antioxidants may perhaps limit the stability of these oils. Tocopherols are reported to be strong antioxidants which can scavenge peroxyl radicals. Choe and Min (2006) indicated that the stability of these oils is commonly set for 6 months or a year. Normand *et al.* (2006) stated that oils with high tocopherol contents have greater stability. Cold-pressed cactus pear seed oil was reported to have higher contents (94.6 mg/100g) of tocopherols than olive oil (22 mg/100g), soybean oil (49 mg/100g) and sunflower oil (49 mg/100g), and close to those of argan oil (85 mg/100g) (Zine *et al*., 2013).

Cactus pear seed oil is a promising source of polyunsaturated fatty acids (PUFA), with significant levels of carotenoids and γ-tocopherols which can be used as antioxidants to preserve lipid components and serve as functional ingredients (Loizzo *et al*., 2019). Proper utilization of this seed by-product can generate up to 1220€ income per ton and is therefore a potentially new economic source (Ciriminna *et al*., 2017). According to Regalado-Rentería *et al. (*2018) prickly pear seed oils are rich in functional metabolites and linoleic fatty acids. Furthermore, this oil could be used in foods as a nutritional supplement, as well as an ingredient in cosmetics and pharmaceuticals. A major advantage is that the residual oilcake can be directly used in animal feed or other secondary products.

Recent research on the oil quality of seed oil from different colored cactus pear fruit seed oil found that cultivar played a major role in the composition of the oils (Regalado-Rentería *et al.,* 2018). Furthermore, oil content and the composition of oils from different origins were reported to indicate differences between cultivars from Sicily and those from Algeria and Morocco; while similarities occurred between cultivars from Sicily and Tunisia, indicating an origin/environment effect (Ghazi *et al.*, 2013; Ciriminna *et al*., 2017; Regalado-Rentería *et al.,*

2018; Loizzo *et al*., 2019). In addition, the effect of different species, e.g. *Opuntia dillenii,* on oil quality was reported. New studies on *O. ficus-indica* seed oils from countries such as Greece (Karabagias *et al*., 2020) and Saudi Arabia (Koshak *et al*., 2020) have been undertaken very recently.

In a previous study done by de Wit *et al*. (2017), the quality aspects of chemically extracted (Soxhlet extraction) seed oils from a few selected cultivars were quantitatively analyzed. The current study focused on the oil yield, composition, quality and stability of cold-pressed seed oil of commercially produced *Opuntia ficus-indica* cultivars. *O. robusta* seed was included as a reference and for comparison purposes. The aim of this study was to determine the quality and oxidative stability of cold-pressed cactus pear seed oil from 12 chosen cultivars. The quality attributes assessed included oil content, fatty acid composition, refractive index, tocopherols, oxygen radical antioxidant capacity, peroxide value and ρ-anisidine value; while stability tests included the oxidative stability index (OSI), as well as induction time extrapolated at 25 and 30 °C.

2. MATERIALS AND METHODS

2.1. Seed material used

Fruit from selected cultivars growing at an experimental orchard outside Bloemfontein (29.0852 °S, 26.1596 °E) was collected during the 2014, 2015 and 2016 seasons. Twelve cultivars, namely Algerian, American Giant, Ficus-Indice, Gymno Carpo, Meyers, Morado, Nudosa, Ofer, Tormentosa, Van As and Zastron were chosen. These *O. ficus-indica* cultivars are commercially produced for fresh fruit consumption. The *O. robusta* cultivar, Robusta, represents a different species and was included because it is commonly found and cultivated as animal fodder in South Africa. The fruit collection was done when the fruit reached 50% color break stage and ±75 kg of fruit per cultivar were collected to produce 2-3 kg of seeds. The seeds were extracted with an industrial liquidizer (Ingram Engineering, Zastron, South Africa) which separates the seeds from the pulp and peels. The seeds were washed with water and collected in a rotating sieved drum. The seeds were then dried under direct sunlight for 2-3 days on shade-netting, placed ± 1 m from the ground to allow for sufficient ventilation. The seeds were manually turned regularly. The drying process aimed to dry out any pulp tissue left on the seed surfaces for easy removal and cleaning before pressing. In a previous study done by de Wit *et al.* (2016), the moisture content in seeds varied from ~3% to ~7.2% due to variations in location, season and especially rainfall. The dried seeds were then vacuum-packed and stored in a freezer at -18 ºC until further analysis.

2.2. Seed oil extraction (cold-pressing)

The dried seeds from all cultivars for each season were cold-pressed in a Komet DD85G twin screw plant oil press (Oekotek, IBG Monforts, Germany) at Nattrend/Bauma Investments CC commercial oil pressing laboratory in Montana Park, Pretoria. The oil extracted using the Komet cold-pressing system is subjected to low temperatures and therefore top-quality vegetable oil which can be utilized for human consumption without any further treatment depending on the raw material used (Schramm, 2016; pers. comm.). The oilcake extrudes out of the oil press in the shape of pellets. The oil was then stored in amber screw-cap 50 ml plastic bottles for one week at room temperature before analysis. The oil analysis for each cultivar for each of the three seasons was done in triplicate.

2.3. Oil yield

The oil yield from the seeds of each cultivar from each season was determined according to the formula:

Oil yield (%) = Mass of oil (g) / Mass of cactus pear seed (g) x 100

2.4. Physico-chemical properties

2.4.1. Fatty acid composition (fatty acid methyl esters) (FAME)

Approximately 20 mg of total lipid (from cold-pressed oil) were transferred to a Teflon-lined screw-top test tube with a glass pasteur pipette. Fatty acids were methylated to methyl esters by using 0.5 N sodium hydroxide (NaOH) in methanol and 14% boron trifluoride in methanol (Park and Goins, 1994). The fatty acid methyl esters (FAME) were quantified using a Varian 430 gas chromatograph with flame ionization detector with a fused silica capillary column (Chrompack CPSIL 88, 100 m length, 0.25 mm ID, 0.2 mm film thickness). Column

temperature was 40–230 °C (held 2 minutes; 4 °C/ minute; held 10 minutes). Fatty acid methyl esters in hexane (1ml) were injected into the column using a Varian CP8400 Auto-sampler with a split ratio of 100:1. The injection port as well as the detector were maintained at 250 °C. The carrier gas was hydrogen, at 45 psi. The makeup gas was nitrogen. Galaxy Star Chromatography Software was used to record the chromatograms. The peaks were identified by comparing the relative retention times of FAME peaks from samples with that of standards from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa). Fatty acids were expressed as the relative percentage of each individual fatty acid as a percentage of the total of all fatty acids in the sample. The following fatty acid ratios and combinations were calculated from the fatty acid data: total saturated fatty acids (SFA), total mono-unsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-6, total omega-3 fatty acid content and the PUFA/SFA (P/S) ratio.

2.4.2. Refraction index (RI)

Refractive Index (RI) was determined according to the Association of Official Analytical Chemists' (AOAC), official method 921.08 (AOAC, 2000) with an Abbè programmed advanced refractometer from ATAGO® (Model RX 5000α) at 40 °C.

2.4.3. Iodine value (IV)

Hanus iodine Value (IV) was determined by the AOAC official method 920.158 (AOAC, 2000), which consists of adding a mixture of iodine and bromine in glacial acetic acid and measuring the excess of unused halogen by titration with sodium thiosulfate.

2.4.4. Tocopherols

High performance liquid chromatography (HPLC) was used to determine the tocopherol amount in each sample using Shimadzu CR8A instruments (Champ sur Marne, France) equipped with a C18-Varian column (25 cm x 4 mm; Varian Incorporated, Middleburg, Netherlands). One gram of each oil sample was added to 5 mL of ethanol (EtOH), filtered (45µm) into a HPLC vial at 25 ºC. HPLC was carried out with an injection volume of 20 µL, a flow rate of 1 ml/min and estimated at UV of 296 nm. The mobile stages were: mobile stage A (Acetonitrile: Methanol: Isopropanol: Water (45:45:5:5, v/v/v/v)) and mobile stage B (Acetonitrile: Methanol: Isopropanol (50:45:5, v/v/v)).

2.4.5. Oxygen Radical Antioxidant Capacity (ORAC)

For the Oxygen Radical Antioxidant Capacity (ORAC) analysis, the method of Prior *et al*. (2003) was used, where samples (10 g each) were diluted with 80% methanol in H_2O (1:1 w/v), and then vortexed for 2 minutes at room temperature and centrifuged for 10 minutes at 500 X g. The peroxyl radicals were generated with 2, 2'-Azobis (2-amidinopropane) dihydrochlorine (AAPH) and B-phycoerythrin (B-PE) was used as the detector of radical activity. The final reaction mixture was prepared in 10-mm-wide quartz cuvettes as follows: 1600 µL of 0.04 µM B-PE in 0.0075 M sodium (Na) – potassium (K) phosphate buffer at pH 7.0 and 200 µL of 50 µM Trolox (6-hydroxy-2,5,7,8-tetramethylchroman -2-carboxylic acid). Phosphate buffer was used as a blank (200 µL of methanol, diluted 1:20 (v/v) with 0.075 M Na-K, at pH 7.0 and Trolox (β and γ tocopherol analogue) as the standards during each run. Samples were pre-incubated at 37 ºC for 15 min. AAPH was added. Flourescence was measured and measurements were recorded every 5 minutes for 35 minutes at 570 nm until a steady state (plateau) of fluorescence decay was reached. The results were then calculated and expressed as µmol of Trolox equivalent (TE). The analyses were done in triplicate.

2.5. Determination of oxidative stability

2.5.1. Free Fatty Acids (FFA)

Free fatty acids were determined according to the method of Pearson (1973), which involves the titration of the oil in an alcoholic medium against potassium hydroxide using phenolphthalein as indicator.

2.5.2. Peroxide value (PV)

Peroxide value was determined by the official method of the AOAC 965.33 (AOAC, 2000). Peroxide value determines hydrogen peroxide, a primary oxidation product, and involves peroxides liberating iodine from potassium iodide by calculating the amount of sodium thiosulfate consumed.

2.5.3. ρ-Anisidine value (ρ-AV)

The *ρ*-AV was determined using the technique of Hamilton and Hamilton (1992). It determines the amounts of aldehydes and ketones during secondary oxidation by reaction with *ρ*-Anisidine (4-methoxyaniline) using a spectrophotometer at 350 nm.

2.5.4. Oxidative Stability index (OSI)

The oxidative stability index (OSI) was determined using the AOAC OSI official method Cd 12b-92 (AOAC, 2000) with a Rancimat 743 apparatus from Metrohm. Three samples were tested per cultivar.

2.5.5. Extrapolated induction time (Shelf-life prediction at 25 and 30 °C)

The oxidative stability index (OSI) was determined using the AOAC OSI official method Cd 12b-92 (AOAC, 2000) with a Rancimat 743 apparatus from Metrohm. Three samples were tested per cultivar. Oxidative stability index (OSI) values were determined at 100, 110, 120 and 130 °C for each sample in duplicate. The extrapolation function of the Rancimat 743 apparatus was used to predict induction times at 25 and 30 °C (Rancimat Manual, 2009) according to the formula:

Predicted induction time = $A \times e^{(BXT)}$

2.6. Statistical analysis

Seed were selected from each of the 2014, 2015 and 2016 harvest. Oil yield was determined for each cultivar for each season. Each measurement was made in triplicate on oil from each cultivar per year. Data were captured for the three years and were reported as average ± standard deviation using NCSS Statistical Software package, version 11.0.20). The Pearson correlation analysis was done to determine the link between the physico-chemical properties of the oil (NCSS Statistical Software package, version 11.0.20).

3. RESULTS AND DISCUSSION

3.1. Oil yield

Seed oil content ranged from 2.51 to 5.96% (Meyers and American Giant), respectively (Figure 1). Gymno Carpo and Morado were amongst those which attained the lowest oil contents (2.72 and 2.78%). In a previous limited study, the chemical extraction of seed oils from only seven selected cultivars by de Wit *et al*. (2017), the oil content ranged from 5.65–8.09%. A subsequent elaborated study on seed oil from 42 cultivars by de Wit *et al*. (2018) reported oil contents ranging from 4.09–8.76%. The lower yield values reported in the current study were expected since the oil was recovered by pressing and not by chemical extraction as in the above-

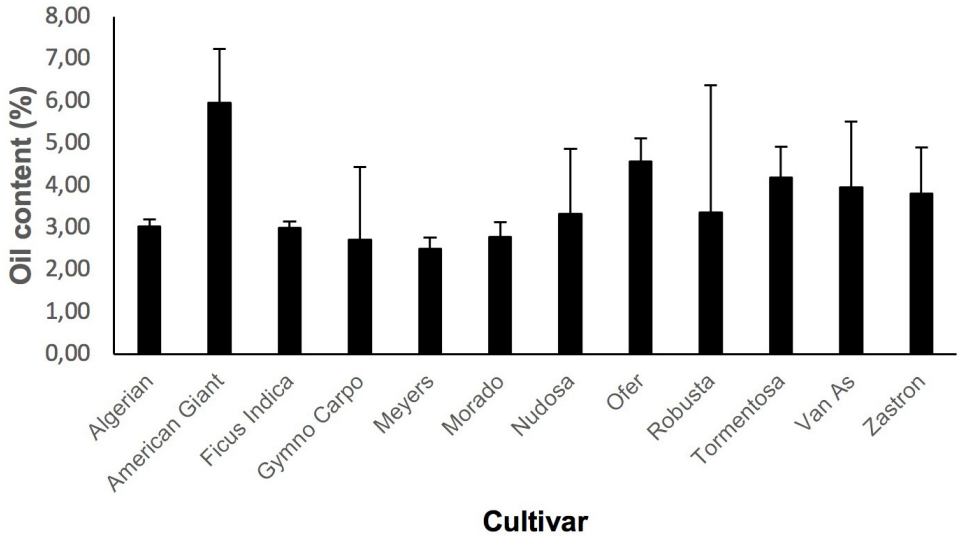


Figure 1. Oil yield (%) of cold-pressed oil for all 12 cultivars. Oil yield was expressed as % Oil yield = Mass of oil (g) / Mass of cactus pear seed (g) x 100. Values in the Figure are mean ±SD of triplicate analyses.

mentioned studies. American Giant also obtained the highest oil content in the study of 42 cultivars by de Wit *et al*. (2018). The values obtained in the current study agree with those reported for the same commercial South African cactus pear cultivars from the Limpopo Province by Labuschagné and Hugo (2010) (2.24 to 5.69%). That study did not include American Giant.

It was also found by Ortega-Ortega *et al*. (2017), Ramírez-Moreno *et al*. (2017), Regalado-Rentería *et al.* (2018) and Loizzo *et al*. (2019) that extraction method had an effect on the oil yield of cactus pear seeds. These authors and others, e.g. Ciriminna *et al*. (2017) also reported the effect of variety and species on the oil yield of cactus pear seeds. High yields of 9.3– 9.5 g oil per 100 g seed were reported for Sicilian cactus pears (*O. ficus-indica*) extracted with hexane by the Soxhlet method, while Ultra-Sound Assisted extraction yielded 5.4–5.6 g/100 g. The highest yields were found in the Turkish and Moroccan varieties (≈ 14 g/100 g seed) (Chougui *et al*., 2013). Lower values than those were reported for Algerian (7.3–9.3 g/100 g seed) and Tunisian fruit seed oils (Chougui *et al*., 2013). The lowest values were found for the Greek wild *O. ficus-indica* variety (yellow to green fruit) of 5.4%, which was also obtained by cold-pressing (Karabagias *et al.*, 2020). Slightly higher yields were reported for *O. microdasys* (Engelm) (9.2%) and *O. macrorhiza* (11.3%) from Tunisia (Chahdoura *et al.*, 2015). Maceration-Percolation (MP) extraction was used by Regalado-Rentería *et al*. (2018) to yield oil contents of up to 15.5% from *O. robusta* species (14.54 - 15.54%). Oil yields for other prickly pear varieties included *O. albicarpa* (8.72%), *O. magacantha* (6.16 – 7.63%), *O. matudae* (9.68%), *O. streptacantha* (10.55 – 11.64%) and *O. dillenii* (6.5%) (Ali Asaad *et al*., 2019).

Fruit color was also indicated as a factor influencing oil yield, e.g. Ramírez-Moreno *et al*. (2017) reported higher yields from a green cultivar compared to a red cultivar. American Giant from the current study is also a green cultivar. These results support research that pointed out that cultivar, crop, environment and origin (rainfall, soil type and soil nutrients, light, temperature) and extraction method, including extraction solvents (hexane > ethanol > ethyl acetate) influence oil yields. According to Karabagias *et al*. (2020), the most important factors are variety and extraction process.

3.2. Physico-chemical properties

3.2.1. Fatty acid composition

The fatty acid compositions of cold-pressed oil from a selection of cactus pear cultivars are shown in Table 1. According to the results presented, there are considerable variations in the fatty acid percentage of the selected cultivars. The dominating fatty acids identified (in decreasing order) were linoleic acid (C18:2 n-6) (C18:2c9,12); oleic acid (C18:1 n-9) (C18:1c9); palmitic acid (C16:0) and stearic acid (C18:0); while palmitoleic acid (C16:1 n-9) (C16:1c9), α-linolenic acid (C18:3 n-3) (C18:3c9,12,15), arachidic acid (C20:0) and lignoceric acid (C24:0) were notable in small amounts in all cultivars. C16:0, C18:0, C18:1c9 and C18:2c9, 12 were the only fatty acids detected at levels of more than 1%. This is in accordance with the results of various authors who reported that cactus pear seed oil contained primarily unsaturated fatty acids namely linoleic and oleic acid, and with a lower but significant content of palmitic and stearic fatty acids (Chougui *et al*., 2013; Ciriminna *et al*., 2017; Ortega-Ortega *et al*., 2017; Ramírez-Moreno *et al.*, 2017, Regalado-Rentería *et al*., 2018; Loizzo *et al.,* 2019). A completely different profile was found for the Greek *O. ficus-indica* which included butyric acid (C4:0), palmitic acid, stearic acid and oleic acid as the main fatty acids (Karabagias *et al*., 2020).

The C18:2c9,12 content varied from 58.56% (Nudosa) to 65.18% (Robusta) (Table 1). A slightly larger range for C18:2c9,12 between 57.75–67.32% was reported by de Wit *et al*. (2017) and de Wit *et al*. (2018) (56.86–65.21%). Labuschagné and Hugo (2010) also reported comparable results for fatty acid profiles with unsaturated fatty acids (C18:2c9,12) ranging between 57.75–67.32%. Linoleic acid (omega-6) is an essential fatty acid and a precursor of arachidonic acid (AA) biosynthesis which is the substrate for eicosanoid synthesis (Ghazi *et al*., 2013). Both present a hypocholesterolemic effect and inhibit colon cancer. Omega-6 PUFA is the source of inflammatory mediators' prostaglandins (PGE) and leukotrienes. Consumption of oleic acid (n-9) and α-linolenic acid (n-3) act as AA antagonists and reduces the production of inflammatory mediators (Koshak *et al*., 2020). Higher levels were found in *O. microdasys* (Engelm) and *O. macrorhiza* as well as *O. dillenii* (> 70%) (Ghazi *et al*., 2013; Chahdoura *et al*., 2015; Ali Alsaad *et al*., 2019); while the current results

Table 1. Fatty acid composition of oil from 12 selected cold-pressed cactus pear cultivars (fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample). Values in the Table are mean ±SD of triplicate analyses.

Cultivar	Fatty acids														
	Myristic	Palmitic	Palmiteloleic	Margaric	Heptadecenoic	Stearic	Oleic	Vaccenic	Linoleic	Arachidic	Eicosenoic	α-Linolenic	Eicosatrienoic (8,11,14)	Eicosatrienoic (11, 14, 17)	Lignoceric
Algerian	0.06 ± 0.01	12.54 ± 0.05	0.55 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	3.04 ± 0.01	13.86 ± 0.02	5.87 ± 0.01	63.29 ± 0.09	0.22 ± 0.01	0.03 ± 0.04	0.23± 0.20	0.12 ± 0.01	0.05 ± 0.06	0.08 ± 0.01
American Giant	0.05 ± 0.01	10.97 ± 0.05	0.38 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	3.74 ± 0.01	16.37 ± 0.02	5.14 ± 0.01	62.49 ± 0.18	0.28± 0.01	0.10 ± 0.01	0.21 ± 0.04	0.14 ± 0.01	0.02 ± 0.01	0.08 ± 0.02
Ficus-Indice	0.06 ± 0.01	12.05 ± 0.08	0.56 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	2.86 ± 0.02	15.74 ± 0.01	6.21 ± 0.02	61.66 ± 0.01	0.22 ± 0.01	0.05 ± 0.04	0.35± 0.01	0.12 ± 0.01	0.02 ± 0.01	0.07 ± 0.02
Gymno Carpo	0.05 ± 0.01	13.24 ± 0.05	0.62 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	2.62 ± 0.01	12.22 ± 0.02	6.16 ± 0.01	64.10 ± 0.03	0.21 ± 0.01	0.08 ± 0.01	0.32 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.07 ± 0.01
Meyers	0.06 ± 0.01	12.31 ± 0.18	0.81 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	3.18 ± 0.01	15.61 ± 0.04	6.53 ± 0.02	60.68 ± 0.28	0.26 ± 0.01	0.12 ± 0.22	0.18 ± 0.01	0.15 ± 0.01	0.02 ± 0.01	0.10 ± 0.01
Morado	0.06 ± 0.01	12.77 ± 0.06	0.59 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	2.81 ± 0.01	13.51 ± 0.01	6.05 ± 0.02	63.28 ± 0.07	0.21 ± 0.01	0.05 ± 0.05	0.36± 0.18	0.11 ± 0.01	0.09 ± 0.07	0.07 ± 0.01
Nudosa	0.07 ± 0.01	15.07 ± 0.07	0.75 ± 0.04	0.02 ± 0.01	0.05 ± 0.01	3.01 ± 0.05	15.78 ± 0.16	5.69 ± 0.07	58.56 ± 0.07	0.26 ± 0.01	0.02 ± 0.03	0.35± 0.01	0.22 ± 0.14	0.11 ± 0.08	0.07 ± 0.01
Ofer	0.06 ± 0.01	12.65 ± 0.21	0.61 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	2.81 ± 0.02	14.98 ± 0.04	6.17 ± 0.02	61.83 ± 0.13	0.22 ± 0.01	0.07 ± 0.01	0.35 ± 0.01	0.12 ± 0.01	0.02 ± 0.01	0.08 ± 0.01
Robusta	0.04 ± 0.01	11.93 ± 0.18	0.41 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	2.68 ± 0.02	13.46 ± 0.06	5.48 ± 0.02	65.18 ± 0.11	0.21 ± 0.01	0.11 ± 0.19	0.22 ± 0.19	0.13 ± 0.01	0.02 ± 0.01	0.07 ± 0.01
Tormentosa	0.06 ± 0.01	12.19 ± 0.04	0.59 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	3.07 ± 0.01	14.99 ± 0.03	6.04 ± 0.09	62.14 ± 0.09	0.24 ± 0.01	0.05 ± 0.05	0.35 ± 0.01	0.13 ± 0.01	0.02 ± 0.01	0.08 ± 0.01
Van As	0.06 ± 0.01	11.80 ± 0.11	0.58 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	2.98 ± 0.01	16.07 ± 0.05	6.30 ± 0.02	61.29 ± 0.10	0.24 ± 0.01	0.17 ± 0.16	0.24 ± 0.20	0.14 ± 0.01	0.02 ± 0.01	0.09 ± 0.01
Zastron	0.06 ± 0.01	11.71 ± 0.07	0.38 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	2.70 ± 0.01	13.18 ± 0.04	5.36 ± 0.01	65.73 ± 0.14	0.20 ± 0.01	0.19 ± 0.15	0.24 ± 0.21	0.11 ± 0.01	0.04 ± 0.05	0.07 ± 0.01

are in agreement with the levels reported for *O. ficus-indica* varieties from Sicily, Saudi Arabia, Turkey and Tunisia (Ghazi *et al*., 2013; Ciriminna *et al*., 2017; Loizzo *et al.,* 2019). Astiasarán and Candela (2000) reported a comparable study on PUFAs (C18:2c9,12) content of various vegetable oils, i.e. soy oil which obtained 48.7%, corn oils (47.7%), sesame oils (44.5%), sunflower oil (49.7%), olive oil ranging from 3.5–21% and cotton oils with 50%. These values are all significantly lower than the values obtained in this current study, implying that cactus pear is the best in terms of oil PUFA yield. PUFAs are able to alleviate symptoms of diseases such as coronary heart disease, stroke and rheumatoid arthritis.

The highest amount of C18:1c9 was detected for American Giant with 16.37% compared with Gymno Carpo, which demonstrated the lowest amount with 12.22%. Robusta contained 13.46% C18:1c9. The highest value for vaccenic acid (C18:1 n-7) (C18:1c7) was observed for Meyers (6.53%) and the lowest for American Giant (5.14%). Robusta (*O. robusta*) obtained 5.48%. Loizzo *et al.* (2019) reported values ranging from 15.8 – 18.1% for C18:1c9 from two *O. ficus-indica* cultivars (red and yellow) extracted by two different methods (Soxhlet and Ultrasound-Assisted). These authors reported values ranging between 4.3% and 5.1% for C18:1c7. The values for C18:1c9 reported by these authors were higher than in the current study, while the C18:1c7 values were somewhat lower than the current study's values. Ciriminna *et al.* (2017) obtained a higher content of vaccenic acid (6.29%) for yellow Sicilian *Opuntia ficus-indica* seed oil, while Chougui *et al.* (2013) reported no vaccenic acid in yellow Algerian fruit. A vaccenic acid content of 4.83% was reported by Tlili *et al.* (2011) for Tunisian cactus pears. Interestingly, seed oil from *O. ficus-indica* from Marocco had no oleic acid (Ghazi *et al*., 2013).

The saturated fatty acid (SFA) palmitic acid (C16:0) ranged between 10.97 and 15.07% and stearic acid (C18:0) ranged between 2.62 and 3.74%. American Giant obtained the lowest content of C16:0 (Table 1) and the highest oil percentage (Figure 1). These C16:0 values are slightly lower than those reported by de Wit *et al*. (2017) which ranged between 12.90 and 17.83% (Monterey and Nudosa, respectively) and the range reported by de Wit *et al*. (2018) namely 12.72–16.05%. The C18:0 content varied between 2.62% (Gymno Carpo) and 3.74% (American Giant) (Table 1), indicating only slight variation in C18:0 levels. Similar values were reported for chemically extracted oil as reported by

Table 2. Fatty acid ratios of oil from 12 selected cold-pressed cactus pear cultivars (fatty acids were expressed as the proportion of each individual fatty acid to the total of all fatty acids present in the sample). Values in the Table are mean ±SD of triplicate analyses.

Cultivar	Saturated fatty acids (SFA)	Monounsaturated fatty acids (MUFA)	Polyunsaturated fatty acids (PUFA)	Omega-6 fatty acids	Omega-3 Fatty acids	PUFA/SFA
Algerian	15.96 ± 0.06	20.34± 0.05	63.70± 0.10	63.42± 0.09	0.28± 0.14	3.99± 0.02
American Giant	15.13 ± 0.04	22.00± 0.01	62.87± 0.05	62.64± 0.18	0.23± 0.14	4.16± 0.01
Ficus-Indice	15.27± 0.05	22.58± 0.06	62.15± 0.01	61.78± 0.01	0.37± 0.01	4.07± 0.01
Gymno Carpo	16.21± 0.06	19.13± 0.03	64.67± 0.03	64.21± 0.03	0.46± 0.01	3.99± 0.02
Meyers	15.94± 0.16	23.10± 0.25	60.87± 0.07	60.83± 0.28	0.20± 0.18	3.81± 0.03
Morado	15.91± 0.07	20.24± 0.04	63.85± 0.04	63.40± 0.07	0.46± 0.07	4.01± 0.02
Nudosa	18.50± 0.05	22.28± 0.24	59.23± 0.24	58.78± 0.20	0.45± 0.07	3.20± 0.02
Ofer	15.83± 0.19	21.84± 0.05	62.32± 0.14	61.96± 0.14	0.37± 0.01	3.94± 0.05
Robusta	14.96± 0.17	19.48± 0.14	65.55± 0.31	65.32± 0.12	0.24± 0.19	4.38± 0.07
Tormentosa	15.66± 0.04	21.69± 0.05	62.65± 0.08	62.27± 0.09	0.37± 0.01	4.00± 0.01
Van As	15.18± 0.10	23.14± 0.1	61.68± 0.10	61.43± 0.11	0.25± 0.20	4.06± 0.02
Zastron	14.76± 0.07	19.12± 0.15	66.13± 0.11	65.84± 0.07	0.28± 0.24	4.48± 0.02

Values in the Table are mean ±SD of triplicate analyses.

de Wit *et al*. (2017) (2.21–3.39%) and de Wit *et al*. (2018) (2.4–4.01%). These results are in agreement to those of Loizza *et al*. (2019) who found that fatty acid content is not affected by extraction method but by variety. Interestingly, Regalado-Rentería *et al*. (2018) found that oil extracted by cold-pressing was richer in UFA and poorer in SFA than oils extracted with solvents. Regarding SFA, the palmitic acid (5.12%) and stearic acid (7.51%) found in *O. dillenii* presented the main saturated fatty acid (> 22%). These are even higher than the SFA found in soy (~3%) (Ali Alsaad *et al*., 2019). Stearic acid has a neutral effect on LDL.

3.2.2. Fatty acid ratios

According to Table 2 SFA ranged from 14.76 (Zastron) to 18.50% (Nudosa). The results for the fatty acid composition presented in Table 1 show that C16:0 and C18:0 were observed to be marginally lower than those reported in the publication of de Wit *et al. (*2017) (16.59–20.65%) and de Wit *et al*. (2018) (16.19–19.12%) yet higher than those obtained by Ennouri *et al*. (2005). These fatty acids will influence SFA ratios, since they are the main contributors to SFA. The stage of development of the fruit may be the cause of variation in fatty acid composition, but in this study the fruits were all picked at the same maturity stage (50% color break), although genotype, climate and weather, as well as environmental effects may be the reason behind this variation. According to de Wit *et al.* (2017), weather conditions, particularly were the main factors contributing to variations in fatty acid composition and oil percentage.

Total MUFA ranged between 19.12 and 23.10%, which corresponds to that reported by de Wit *et al*. (2017) (15.78–23.30%) and de Wit *et al*. (2018) (17.4–23.61%). Gymno Carpo and Zastron obtained the lowest MUFA contents (19.13% and 19,12%) (Table 2), while Meyers and Van As showed the highest contents of MUFA (23.14% and 23.10%), respectively. MUFA, such as C18:1c9, ranged between 12.22% (Gymno Carpo) and 16.07% (Van As) (Table 1). Oil containing high concentration of C18:1c9 has been reported to be more stable to oxidation, which is desired for improved shelf-life (Lajara *et al*., 1990).

PUFA was established as the most prominent fatty acid ratio ranging between 59.23 (Nudosa) and 66.13% (Zastron). PUFA from de Wit *et al.* (2017) was at the lower range and ranged from 55.98 – 67.62%. C18:2c9,12 was established as the dominating PUFA with the lowest level of 58.56% for Nudosa and the highest level of 65.18% for Robusta (Table 1), with C20:3c9,12,15 (0.22 to 0.35%) and C20:3c8,11,14 (0.13 to 0.22%) occurring at very low levels.

Table 3. Physicochemical properties of oil from 12 selected cold-pressed cactus pear cultivars analyzed one week after pressing.

Cultivar	Peroxide Value (meq O2/1000 g fat)	% Free Fatty Acids	ρ-Anisidine value (mmol/kg)	Refraction Index at 40 ˚C	Iodine Value	β + γ Tocopherol (mg/100g)	ORAC[1]	OSI[2]	Induction Time extrapolated to 25 ˚C (Years)	Induction Time extrapolated to 30 ˚C (Years)
Algerian	4.23 ± 0.48	3.21 ± 0.04	4.08 ± 0.37	1.4666 ± 0.0001	121.60 ± 4.12	58.27 ± 2.47	5.28 ± 0.43	2.31 ± 0.14	0.52 ± 0.01	0.35 ± 0.01
American Giant	3.02 ± 0.02	1.21 ± 0.03	1.37 ± 0.14	1.4666 ± 0.0001	125.45 ± 2.09	54.19 ± 2.96	4.70 ± 0.25	2.19 ± 0.04	3.44 ± 0.01	2.08 ± 0.01
Ficus-Indice	3.65 ± 0.18	2.19 ± 0.06	2.73 ± 0.21	1.4666 ± 0.0001	123.84 ± 2.09	59.36 ± 7.61	4.54 ± 0.27	3.17 ± 0.09	0.73 ± 0.01	0.49 ± 0.01
Gymno Carpo	5.87 ± 0.02	2.32 ± 0.01	1.00 ± 0.02	1.4666 ± 0.0001	122.32 ± 0.60	55.43 ± 6.05	4.10 ± 0.25	2.54 ± 0.01	2.69 ± 0.01	1.66 ± 0.01
Meyers	4.81 ± 0.12	1.63 ± 0.03	1.42 ± 0.32	1.4664 ± 0.0003	123.07 ± 1.17	59.78± 2.04	3.87± 0.06	1.88± 0.01	4.15± 0.01	2.46± 0.01
Morado	4.19 ± 0.24	1.15 ± 0.01	1.40 ± 0.42	1.4666 ± 0.0001	123.32 ± 0.28	61.31 ± 3.33	4.01 ± 0.18	2.46 ± 0.06	2.14 ± 0.01	1.33 ± 0.01
Nudosa	4.49 ± 1.24	1.74 ± 0.09	1.58 ± 0.12	1.4659 ± 0.0001	123.92 ± 6.36	51.69 ± 4.26	4.14 ± 0.09	2.95 ± 0.10	2.74 ± 0.01	1.69 ± 0.01
Ofer	4.72 ± 0.01	1.19 ± 0.02	1.64 ± 0.36	1.4666 ± 0.0001	122.64 ± 1.18	61.47 ± 1.65	4.30 ± 0.49	2.92 ± 0.02	1.64 ± 0.01	1.04 ± 0.01
Robusta	5.06 ± 0.78	3.41 ± 0.09	1.95 ± 0.54	1.4665 ± 0.0002	122.85 ± 3.85	55.30 ± 9.38	5.07 ± 0.51	2.25 ± 0.01	3.56 ± 0.01	2.16 ± 0.01
Tormentosa	5.80 ± 0.38	3.22 ± 0.03	0.98 ± 0.54	1.4664 ± 0.0001	120.58 ± 2.22	58.45 ± 4.70	4.20 ± 0.41	1.91 ± 0.08	9.40 ± 0.01	5.30 ± 0.01
Van As	3.60 ± 0.41	4.30 ± 0.02	2.94 ± 0.17	1.4664 ± 0.0001	120.59 ± 1.93	59.95 ± 5.74	5.08 ± 0.22	1.78 ± 0.08	2.14 ± 0.01	1.31 ± 0.01
Zastron	5.72 ± 0.58	2.75 ± 0.06	1.94 ± 0.55	1.4668 ± 0.0001	127.43 ± 1.13	56.70 ± 1.37	5.37 ± 0.05	1.74 ± 0.07	4.73 ± 0.01	2.76 ± 0.01

Values in the Table are mean ±SD of triplicate analyses.
1 Oxygen radical antioxidant capacity (µmol Trolox equivalents/g)
2 Oxidative stability Index at 110 ˚C (Hour)

Omega-6 (n-6) fatty acids ranged from 58.78-65.84% (Nudosa and Zastron, respectively). All the cultivars, with the exception of Nudosa, contained n-6 contents at above 60%. Nudosa attained the lowest concentration of n-6 fatty acid ratio with 58.78% (Table 2). Zastron had the highest contents of PUFA and n-6 but obtained the lowest content of MUFA. These contents were found to be slightly higher compared to results from de Wit *et al*. (2017) (55.98 – 67.45%). Pardo *et al*. (2009) concluded that cactus pear seed oils are good sources of omega-6 fatty acids.

The omega-3 (n-3) fatty acid ratio occurred at very low levels (Table 2). The contents ranged from 0.20 (Meyers) - 0.46% (Gymno Carpo), respectively. Omega-3 (n-3) fatty acids, such as C18:3c9,12,15 (C18:3 n-3) occurred at a very low level and ranged from 0.18 - 0.36% (Meyers and Morado in Table 2).

The PUFA/SFA ratio is used to measure the level of unsaturation and is taken as an instrument to measure the oil's tendency to undergo autoxidation. The PUFA/SFA ratio ranged from 3.20 - 4.48% with Zastron occurring in the highest amount and Nudosa at the lowest level (Table 2). American Giant, Ficus-Indice, Morado, Robusta, Tormentosa, van As and Zastron also attained PUFA/SFA contents above 4%. Zastron was also amongst those that had the highest content of C18:2c9,12, while Nudosa occurred at the lowest level (Table 1). *O. dillenii* presented a ratio of 3.22.

The results for the physicochemical properties of the oil from cactus pear cultivars are presented in Table 3.

3.2.3. Refraction index (RI)

The RI values of the oils increase as a result of autoxidation. The RI depends on the chemical composition and temperature of the oil. It also increases with degree of saturation (Brahmi *et al*., 2020) and secondary functions on fatty acid chains. As indicated by the RI values presented in Table 3, little differences were observed among the RI values for all cultivars. The RI values ranged from 1.4659–1.4668 (Nudosa and Zastron). The higher the level of unsaturation, the higher the levels of RI and IV, for example, Zastron obtained the highest RI value (1.4668) and high PUFA content (66.08%) as well as high IV (127.43). Oils with a high unsaturation degree are more likely to undergo autoxidation and tend to oxidize easily when used at high temperatures, for example, PUFA (C18:2c9,12) oxidized ± 50 times faster than C18:1c9 (MUFA). Therefore, an increased RI value could be expected as a result of exposure to heat and light.

American Giant and Gymno Carpo were also amongst the cultivars with high PUFA, RI value and

IV value (Tables 2 and 3). Furthermore, the higher the MUFA content (C18:1c9), the lower the RI and IV-values, for example, Van As attained a higher content of MUFA (23.14%), a low RI value of 1.4664 as well as a low IV value of 120.59 (Tables 2 and 3). These results fall within a similar range (1.464 RI) to those reported by Gharby *et al*. (2011). Previous literature reported a higher RI value of 1.4831 in cactus pear seed oil, 1.473 in rape seed oil, and 1.475 in *Opuntia ficus-indica* (Ennouri *et al*., 2005). Other studies reported slightly lower RI values ranging between 1.4658 and 1.4676 (de Wit *et al*., 2017).

Valuable information can be obtained by determining the density of the oils. Density gives information on the nature of fatty acids, such as chain length, unsaturation degree, and functional groups. Density will also be influenced by the composition and temperature of the oil. Together with RI, it can also indicate the purity of the oil (Brahmi *et al*., 2020). It is therefore recommended to include density analyses in future studies.

3.2.4. Iodine value (IV)

Iodine value is an indication of the total number of double bonds. The iodine value (IV) ranged from 120.58–127.43 (Tormentosa and Zastron). Zastron obtained the highest IV (number of gram iodine absorbed by 100g of oil) of 127.43 followed by American Giant (125.45), Morado (123.32) and Nudosa (123.92). Tormentosa recorded the lowest IV of 120.58 (Table 3). According to Table 2, Zastron and American Giant attained the highest PUFAs (high unsaturation level) as well as high IV and low MUFA content while Nudosa obtained the smallest content of PUFA. The values in this study are higher than those reported by Karleskind and Wolff (1992) ranging from 105.5–107.38 (cactus pear seed oils). De Wit *et al*. (2017) reported slightly lower iodine values ranging between 110.68 (Nudosa) and 126.82 (Robusta).

Since IV measures the unsaturation of oils, it can also be an indication of the stability of the oil since a high degree of unsaturation suggests a high susceptibility to oxidation. Low IV therefore indicates more resistance to oxidation because of increased saturation and vice versa (de Wit *et al*., 2017).

3.2.5. Tocopherols

Vegetable oils are the most important dietary source of tocopherols. Tocopherols are phenolic compounds that are naturally occurring antioxidants that present biological activity (Ali Alsaad *et al*., 2019). Fernández-Martinez *et al*. (2004) indicated that fatty acids, natural antioxidants, tocopherols and sterols are the key constituents that define oil quality. Normand *et al*. (2006) stated that oils with high tocopherols have greater stability to oxidation. Tocopherols are natural antioxidants in plant foods, particularly those that contain high levels of PUFA as they are able to scavenge lipid peroxyl radicals of unsaturated lipid molecules, inhibiting the propagation of lipid peroxidation.

β and γ tocopherols ranged between 61.47 and 51.69 mg/100g (Table 3). Ofer obtained a higher content of β and γ tocopherols (51.69 mg/100g) followed by Morado (61.31 mg/100g), which means that Ofer and Morado will be more stable to oxidation compared to Nudosa (51.69 mg/100g). The values obtained in the current study are comparable to those reported by Chu *et al*. (2002) with cold-pressed cactus pear seed oil at 94.6 mg/100g, argan oil at 85.0 mg/100g, olive oil at 22.0 mg/100g, soybean at 65.0 mg/100g and sunflower oil at 49.0 mg/100g. Morales *et al*. (2012) found values of 140–220 µg/100 g.

Tocopherol contents are influenced by the extraction method (Loizzo *et al*., 2019). Red cultivars were found to contain higher contents than the yellow cultivars and were mainly in the form of γ-tocopherol. In Moroccan cactus pear seed oil, vitamin E was only present as γ-tocopherol (1.23%) (Ghazi *et al*., 2013). Regalado-Rentería *et al*. (2018) found a tendency for metabolites such as γ-tocopherol to be higher in cold-pressed oils than oils extracted with solvents. These authors reported γ-tocopherol values between 1.71 and 13.86 mg/100 g oil. During the analysis of anti-inflammatory compounds of *O. ficus-indica* seed oils from Saudi-Arabia, β-tocopherol contents of 1.56% were found. This oil was extracted with hexane (Boshak *et al*., 2020).

3.2.6. Oxygen radical antioxidant capacity (ORAC)

Lipid oxidation is a process which is initiated by free radical reactions at the double bonds of unsaturated fatty acids and has been reported to be the key factor in the quality deterioration of edible oils since it modifies the chemical, sensory and nutritional properties of the oils. Márquez-

Ruiz *et al*. (1996) pointed out that autoxidation is another key factor that leads to the development of quality loss in refined oils during storage. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is normally used to predict the stability of cold-pressed oils against oxidation. Most of the literature cited included the use of the DPPH and 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) methods. Cao *et al*. (1996) reported the determination of total antioxidant capacity using the ORAC method, which is currently being used to determine the total antioxidant capacity and measures the plant extract's ability to scavenge peroxyl radicals.

The oxidation of oils happens mainly at the sites of unsaturation and the oxidation rate mainly depends on the number of double bonds and their positions. Table 3 shows that Zastron had the highest antioxidant capacity with an ORAC value of 5.37 μmol/g, followed by Algerian with 5.28 μmol/g. Meyers had the lowest antioxidant capacity (3.87 μmol/g). Kuti (2004) reported higher values which ranged from 1.6–15.8 mMTE/g in yellow-skinned fruits and 1.7–49.2 mMTE/g in the purple-skinned fruits.

It was found by Ramírez-Moreno *et al*. (2017) that extraction solvent had a significant effect on the free radical scavenging capacity of the seed oil and that the green *O. ficus-indica* variety had a higher antioxidant activity regardless of the solvent used. The antioxidant activity of *O. ficus-indica* seed oil from Saudi-Arabia (Brahmi *et al*., 2020) showed a lower DPPH value in cold-pressed oil than in oils extracted by the Soxhlet method. Antioxidant activity was strongly correlated with the amounts of phenolic compounds, including polyphenols and flavonoids. *O. dillenii* seed oil from Iraq demonstrated a strong antioxidant capability due to its ability to reduce oxidation as determined by the DPPH method (Ali Alsaad *et al*., 2019), which reinforced the effect of variety on antioxidant activity.

3.3. Determination of oxidative stability

3.3.1. Free fatty acids (FFA)

The level of free fatty acids (FFA) in oils is measured by the acid value (AV) generated upon the hydrolytic degradation of lipid particles and is therefore an indicator of hydrolytic activity, which consequently adds to the decrease in the time span of usability of the oil. The maximum FFA content limit recommended for cold-pressed oil is 8%, 0.3% for refined oils and 5% for virgin palm oils according to the Codex Alimentarius Commission standard (Codex Alimentarius Commission, 2009). According to the results presented in Table 3, the FFA content ranged between 1.15 and 4.30% (Morado and Van As) and the % FFA of all cultivars was within the recommended limit, implying that cactus pear seed oil is of good quality from a FFA point of view. In a previous study, % FFA ranged between 2.49 and 5.08% in chemically extracted oil (de Wit *et al.*, 2017). The content in cold-pressed oil was thus lower. The production of FFA is a result of lipid hydrolysis which is triggered by chemical or enzymatic actions. Increased free fatty acid values are indicators of increased primary oxidation. Factors such as high temperature and humidity are accountable for an increase in FFA content in oils. An elevated acidity value of 21.2% was reported by Brahmi *et al.* (2020) for Algerian cold-pressed *O. ficus-indica* seed oil. This high value could be ascribed to the enzymatic hydrolysis of seeds during oil pressing, handling or processing, as well as elevated temperatures and the presence of water during the process. It was also mentioned by the authors that free fatty acids could be present since no refining of the oil took place, which could possibly remove acids as impurities.

3.3.2. Peroxide value (PV)

Martín-Polvillo *et al*. (2004) reported that peroxide value (PV) measures the level of hydroperoxide in the oil and is a valuable tool for the indication of a commencement period of oxidation. Furthermore, PV reaches the highest level during the progression of oxidation and following this stage (secondary oxidation), the decomposition rate of hydroperoxides surpasses the rate of their development. Peroxide value is also used to test oxidative rancidity in oils and fats (Karleskind and Wolff, 1992). It was reported that the acceptable PV value for cold pressed oil is < 15 meq $O_2 \cdot kg^{-1}$ (Codex Alimentarius Commission, 1999). According to the results presented in Table 3, all cultivars attained PV values of < 15 meq $O_2 \cdot kg^{-1}$, therefore PV value results are in agreement with the recommended value for cold-pressed oil of good quality.

PV ranged between 3.02 and 5.87. Although Gymno Carpo and Tormentosa were amongst the

cultivars with higher PV values (5.87 and 5.80), oxidation had not yet occurred in these cultivars since their PV values were still within the recommended limit of < 15 meq O_2.kg^{-1}, which indicates that a rancid taste was not yet noticeable (Table 3). Salvo *et al*. (2002) pointed out that a rancid taste in oils begins to be noticeable at PV values of 20–40 meq $O_2 \cdot kg^{-1}$. PUFAs are more susceptible to oxidation than MUFA and SFA. The higher PVs observed for Gymno Carpo and Tormentosa were probably a consequence of their higher unsaturation levels (Tables 1 and 2). American Giant had on average a PV of 3.02 meq $O_2 \cdot kg^{-1}$, which was the lowest compared to that of the other cultivars. Van As also had a relatively low PV value of 3.60 (Table 3). De Wit *et al*. (2017) reported PV values of chemically extracted cactus pear oils which were above the recommended limit and ranged between 9.5 and 23.30 meq $O_2 \cdot kg^{-1}$.

Karleskind and Wolff (1992) reported PV estimations of margarine containing *Opuntia ficus-indica* oil which were far below the levels permitted by the international benchmarks and ranged between 0.38 and 0.39 meq $O_2 \cdot kg^{-1}$. PV measures the extent of rancidity reactions during storage, and therefore indicates quality and stability. A PV of 12 meqO_2/kg was reported for Algerian *O. ficus-indica* cold-pressed seed oils (Koshak *et al*., 2020). PV is therefore affected by genetics, cultivars, growth conditions, soil geography, and harvesting routines as well as handling and storage.

3.3.3. ρ - Anisidine value (ρ-AV)

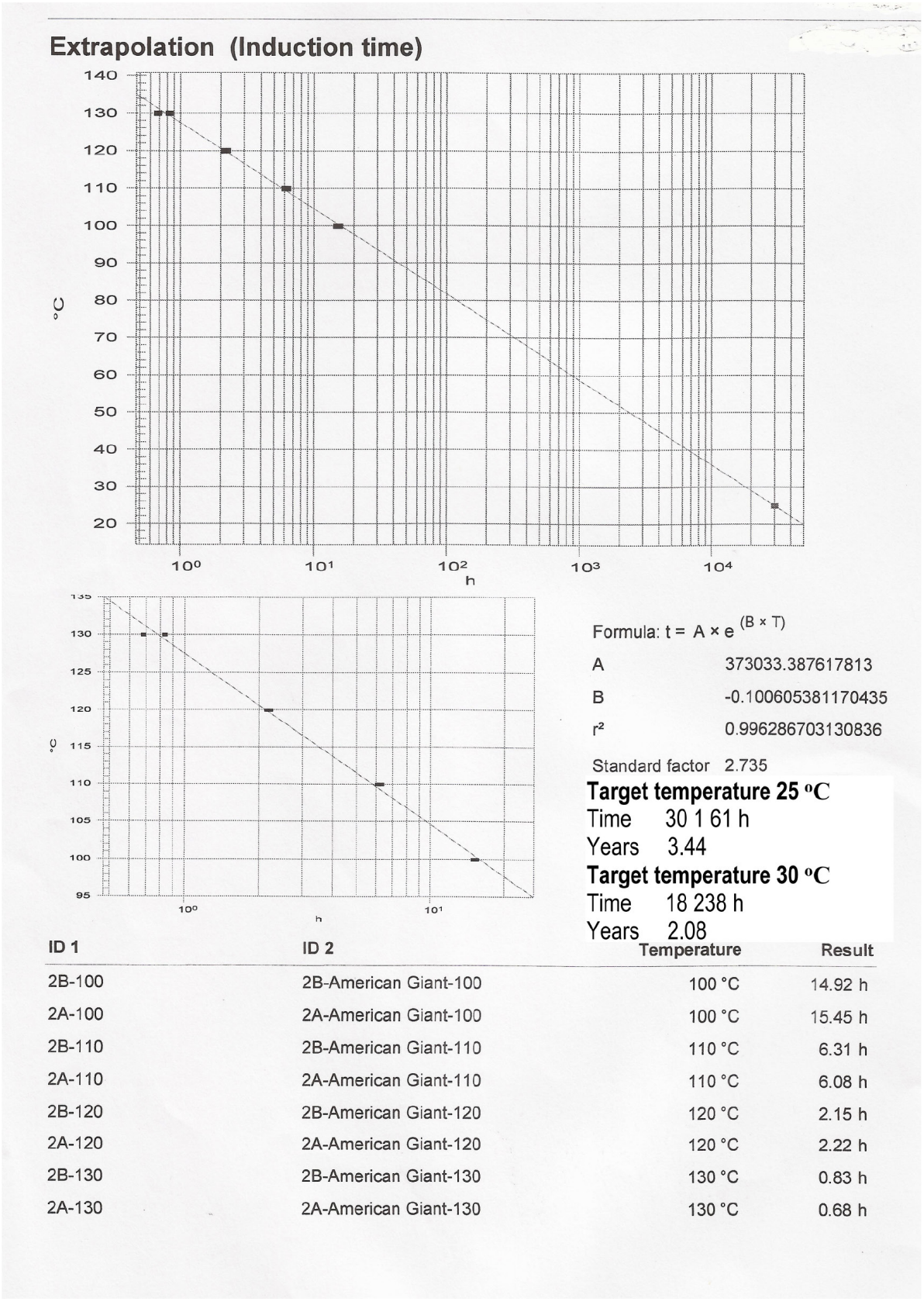
The *ρ*-AV was taken as a tool used to quantify secondary oxidation by surveying the measurement of unsaturated aldehydes as a result of the decomposition of hydroperoxides (Shahidi and Zhong, 2005). The *ρ*-AV is an indicator of oxidative rancidity in oils. The results presented in Table 3 demonstrate that the *ρ*-AV ranged between 0.98–4.08 mmol/kg for Tormentosa and Algerian, respectively. Algerian had the highest *ρ*-AV of 4.08 followed by Van As with *ρ*-AV of 2.94 mmol/kg. Tormentosa acquired the lowest *ρ*-AV content of 0.99 mmol/kg. The results demonstrated that there were only small differences among the *ρ*-AV of American Giant (1.37), Morado (1.40), Ofer (1.64) and Nudosa (1.58). Algerian recorded the highest ρ-Anisidine value and PV value, which is indicative of secondary oxidation products. The high *ρ*-AV may be due to their higher level of unsaturation. The recommended *ρ*-Anisidine value (*ρ*-AV) limit is < 10.0 mmol/kg (Codex Alimentarius Commission, 1999). The *ρ*-AV values obtained were within the acceptable limit (Table 3). Some of these *ρ*-AV values were higher than those reported in the literature which ranged from 0.02 - 3.73 mmol/kg (de Wit *et al*., 2017). Aldehydes made up the highest percentage of cactus pear seed oil volatiles (Karabagias *et al.*, 2020). Aldehydes are responsible for, among others, acrid, burnt-fat, fruit-like, fermented, nutty, deep fat, butter, chicken and fusty odors, flavors and smells. The butyric acid reported by Karabagias *et al*. (2020) is responsible for the buttery flavor of the cactus pear seed oil.

3.3.4. Oxidative stability index

The OSI value gives an indication of the resistance of lipids to oxidation and is also used for quality control of the oils, as well as an indication of the shelf-life of oils. The shorter the reaction time, the more susceptible the oil is to oxidation and therefore higher values will imply more resistance to oxidation. The values ranged between 1.78 and 3.17 h and were lower than the values obtained by de Wit *et al*. (2017), which ranged between 1.79 and 4.15 h. Ficus-Indice had the highest OSI value of 3.17 hours at 110 °C followed by Nudosa (2.92 hours) and Ofer (2.92 hours), implying that these cultivars were more resistant to oxidation reactions. Van As, which attained the highest content of C18:1c9 (Table 1), was determined to be the most favored oil since it is rich in oleic acid and oils rich in C18:1c9 have potential for combining the hypocholesterolemic effect and greater oxidative stability. Oils which are high in MUFA (C18:1c9) such as Nudosa (Table 2) are less susceptible to oxidative degradation and in this manner indicate potential for applications requiring high oxidative stability. Gharby *et al*. (2011) reported OSI of 7 hours at 110 °C for *Opuntia ficus-indica*, 31.23 hours for argan, 7.5 hours for olive oil and 5 hours for soybean and sunflower oil.

3.3.5. Extrapolation of induction time

Induction time extrapolated to 25 and 30 °C was done to predict the shelf-life of the oils based on OSI using Rancimat induction times (Rancimat Manual, 2009). The induction period is an indication of how long the oils will take to reach an end point or be



ID 1	ID 2	Temperature	Result
2B-100	2B-American Giant-100	100 °C	14.92 h
2A-100	2A-American Giant-100	100 °C	15.45 h
2B-110	2B-American Giant-110	110 °C	6.31 h
2A-110	2A-American Giant-110	110 °C	6.08 h
2B-120	2B-American Giant-120	120 °C	2.15 h
2A-120	2A-American Giant-120	120 °C	2.22 h
2B-130	2B-American Giant-130	130 °C	0.83 h
2A-130	2A-American Giant-130	130 °C	0.68 h

FIGURE 2. Predicted shelf-life of American Giant cold-pressed cactus pear seed oil stored at 25 and 30 ºC. The extrapolation function of the Rancimat 743 apparatus was used to predict induction times at 25 and 30 ºC according to the formula: Predicted induction time = A X e (BXT)

oxidized. American Giant is presented as an example in Figure 2. Induction time is also described as the time taken before the rapid increase in oxidation occurs and is used to make relative assessments on oxidative stability (Gharby *et al*., 2011).

The accelerated testing can then be extrapolated to real time conditions. Algerian was found to have a shorter induction time of 5 months, 2 weeks when extrapolated to 25 °C and 3 months, 5 weeks when extrapolated to 30 °C, implying that Algerian will have a shorter shelf-life compared to Tormentosa, with an anticipated time span of usability of 9 years and 4 months when extrapolated to 25 °C and 5 years 3 months when extrapolated to 30 °C (Table 3).

3.4. Pearson correlation analysis

A Pearson correlation analysis was conducted on fatty acid content, fatty acid ratios, physicochemical properties and OSI to establish the relationship among them (Table 4). A significant ($p < 0.001$) negative correlation (–0.6757) was observed between % FFA and OSI (Table 4). This means that cultivars which recorded high contents in % FFA had decreased OSI values or vice versa. This was observed in cultivars such as Algerian which attained a high content in % FFA (3.21%) and low OSI (2.31 h). Nudosa and Ofer possessed the lowest contents in % FFA and obtained the highest OSI values of 2.95 and 2.92 h. De Wit *et al*. (2017) reported higher % FFA results ranging from 2.49% (Tormentosa) with OSI of 3.91h - 5.08h (Algerian) with OSI of 4.15h and the % FFA was higher than the recommended limit, implying that there was increased primary oxidation. El Mannoubi *et al*. (2009) reported much lower % FFA results for cactus pear oil with 0.64%.

No significant correlations with OSI were observed for PV, % Oil, *ρ*-Anisidine value, Refraction Index or β + γ Tocopherols. A significant ($p < 0.01$) negative correlation (-0.5762) was observed between OSI and ORAC. An increase in ORAC initiated a decrease in the OSI value. This correlation was especially observed in Zastron and Algerian, whereby high ORAC values were observed for these cultivars along with low OSI values. This correlation shows that these cultivars are less stable to oxidation due to their higher degree of unsaturation. Both C16:0, C16:1c9 and C17:1c10 correlated significantly ($p < 0.001$, $p = 0.001$ and $p < 0.01$) with OSI, as in Nudosa, which obtained the highest content of C16:0 (15.22%) and the highest content of SFA (18.68%) (Tables 1 and 2).

A significant ($p < 0.001$) positive correlation (0.7409) was observed between C16:0 and OSI. This effect was observed for Nudosa, which obtained the highest content of C16:0 with 15.22% (Table 1) as well as the highest OSI value of 2.95 h (Table 3). A significant ($p < 0.001$) positive correlation (0.7230) was observed between OSI and SFA. An increase in SFA initiated an increase in OSI as in Nudosa, which had the highest SFA (18.68%, Table 3) with a high OSI value (2.95 h, Table 3).

C16:1c9 and C17:1c10 had a positive (0.6522 and 0.5690) significant ($p = 0.001$ and $p < 0.01$) correlation with the OSI. This effect was observed for Nudosa which obtained high C16:1c9 (0.75%) and C17:1c10 (0.05%) contents with OSI value of 2.95h. A negative (-0.7146) significant ($p < 0.001$) correlation between PUFA/SFA and OSI value existed. This effect was observed for Zastron, Van As and American Giant, which possessed the highest contents of PUFA/SFA (4.45%, 4.03% and 4.11%, Table 2) and obtained the lowest value of OSI (1.74 h, 1.78 h and 2.19 h, Table 3). With cold-pressing Algerian, American Giant and Ficus-Indice obtained the highest contents of C18:2c9,12 which resulted in a low oxidative stability index and made them less stable to oxidation. However, of these four cultivars, only American Giant had a low % FFA. The reason for the low OSI is the % FFA and the lower unsaturation level (secondary importance). Oil with high unsaturation is most likely to experience autoxidation.

As expected, a significant ($p < 0.001$) positive correlation (0.9996) was observed between OSI and induction time extrapolated to 25 °C and 30 °C. The length of induction time depends on the level of unsaturation of oil. Tormentosa recorded the highest predicted shelf-life at 25 and 30 °C and was also amongst those cultivars that obtained the high MUFA (C18:1c9) content (Tables 2 and 3). Oils with high MUFA contents (C18:1c9) are less susceptible to oxidation degradation and therefore these oils show great potential for applications requiring high oxidative stability.

ρ-Anisidine value had significant ($p < 0.01$) negative correlations (-0.5120 and -0.5297) with

Table 4. Pearson correlation analysis of the of the fatty acid content, fatty acid ratios, physicochemical properties and oxidative stability index, induction time extrapolated to 25 °C and induction time extrapolated to 30 °C of oil from selected cold-pressed cactus pear cultivars.

	OSI		Induction time extrapolated to 25 ºC		Induction time extrapolated to 30 ºC	
	Correlation coefficient (r)	Significance level (P)	Correlation coefficient (r)	Significance level (P)	Correlation coefficient (r)	Significance level (P)
Physicochemical properties:						
OSI (Oxidative stability index)	1.0000	-	-0.4616	0.0154	-0.4539	0.0174
Induction time extrapolated to 25 ºC	-0.4616	0.0154	1.0000	-	0.9996	P<0.001
Induction time extrapolated to 30 ºC	-0.4539	0.0174	0.9996	0.001	1.0000	-
% Oil	-0.1197	0.4869	0.1768	0.3024	0.1785	0.2975
PV (Peroxide Value)	-0.0620	0.7585	0.4585	0.0161	0.4569	0.0166
% FFA (Free Fatty Acids)	-0.6757	0.0001	0.2063	0.3019	0.1915	0.3387
ρ-Anisidine value	-0.1927	0.3355	-0.5120	0.0063	-0.5297	0.0045
RI (Refraction Index)	-0.4080	0.0346	-0.0312	0.8771	-0.0351	0.8619
IV (Iodine Value)	-0.0209	0.9174	-0.0180	0.9289	-0.0086	0.9661
β + γ Tocopherol	-0.1383	0.4915	-0.0747	0.7113	-0.0845	0.6753
ORAC (Oxygen radical antioxidant capacity)	-0.5762	0.0017	-0.1644	0.4125	-0.1749	0.3828
Fatty acid composition (%)						
C14:0 (Myristic acid)	0.2984	0.1306	-0.2782	0.1600	-0.2819	0.1542
C16:0 (Palmitic acid)	0.7409	0.001	-0.3072	0.1191	-0.3024	0.1253
C16:1c9 (Palmitelaidic acid)	0.6522	0.0002	-0.2109	0.2911	-0.2114	0.2897
C17:0 (Margaric acid)	0.1186	0.5556	0.2461	0.2159	0.2517	0.2053
C17:1c10 (Heptadecenoic)	0.5690	0.0020	-0.1896	0.3435	-0.1870	0.3503
C18:0 (Stearic acid)	-0.1058	0.5994	0.0253	0.9001	0.0273	0.8926
C18:1c9 (Oleic acid)	-0.0415	0.8370	0.2614	0.1879	0.2620	0.1867
C18:1c7 (Vaccenic acid)	-0,0554	0.7632	0.1345	0.2356	0.1643	0,3672
C18:2c9,12,16 (Linoleic acid)	-0.2590	0.1920	-0.1425	0.4783	-0.1455	0.4691
C20:0 (Arachidic acid)	0.0453	0.8224	0.0691	0.7321	0.0741	0.7135
C20:1 (Eicosenoic)	-0,0438	0.8141	0.1290	0.3452	0.2645	0.3176
C18:3c9,12,15 (α-Linolenic acid)	-0.0447	0.8249	0.1267	0.5290	0.1269	0.5283
C20:3c8,11,14 (Eicosatrienoic)	0.2705	0.1723	-0.0230	0.9093	-0.0172	0.9320
C20:3c11,14,17 (Eicosatrienoic)	0.3698	0.0576	-0.2043	0.3066	-0.1959	0.3275
C24:0 (Lignoceric acid)	-0.1651	0.4106	-0.0583	0.7727	-0.0671	0.7393
Fatty acid ratios (%)						
SFA (Saturated fatty acids)	0.7230	0.001	-0.3033	0.1241	-0.2977	0.1315
MUFA (Monounsaturated fatty acids)	-0.0150	0.9409	0.2517	0.2053	0.2524	0.2041
PUFA (Polyunsaturated fatty acids)	-0.2512	0.2062	-0.1450	0.4706	-0.1477	0.4622
n-6 (Omega-6)	-0.2562	0.1970	-0.1438	0.4743	-0.1467	0.4653
n-3 (Omega-3)	0.1962	0.3267	-0.0220	0.9131	-0.0165	0.9349
PUFA/SFA ratio	-0.7146	0.001	0.1709	0.3942	0.1658	0.4085

induction time extrapolated to 25 and 30 °C. This effect was observed for Algerian, which attained the lowest induction time extrapolated at both 25 and 30 °C but recorded the highest *ρ*-Anisidine value (Table 4). This cultivar is more likely to experience secondary oxidation (oxidative rancidity) due to its level of unsaturation (PUFA), hence it will have a shorter shelf-life.

4. CONCLUSIONS

The aim of the current study was to determine the oil content, fatty acid composition and physico-chemical quality parameters as well as the stability of cold-pressed seed oil from 12 commercially cultivated cultivars of cactus pear from the Bloemfontein, South Africa area. Large differences in oil content, fatty acid composition and physico-chemical properties (IV, PV, RI, tocopherols, ORAC, % FFA, OSI and induction time) were observed. The quality parameters of the oils were strongly influenced by the oil content, fatty acid composition and physicochemical properties. Quality traits were mainly compared to previous studies on chemically extracted oils from South African cactus pear seed oil.

The main findings pointed out that cold-pressed oils yielded lower oil contents than chemically extracted oils, while green-colored fruit had the highest yields – indicating the effect of cultivar and species. The fatty acids showed similar profiles to those in chemically extracted oils, although the main fatty acid, linoleic acid (C18:2), content was lower. In general, lower PUFA and SFA contents and higher MUFA contents were observed than in the chemically extracted oils. RI, IV and *ρ*-AV was higher than observed in chemically extracted oils, while the PV, FFA and OSI were lower (shorter) – indicating good quality and stable oils. Tocopherol content and antioxidant activity contributed to stability and quality. Extrapolated induction times indicated a shelf-life of up to ~3.5 years when stored at 25 ˚C.

Overall, American Giant, Ofer, Van As, Zastron and Nudosa have proved to be the best performing cultivars in such a way that they contain the highest oil content, antioxidant capacity as well as greater stability to oxidation. Among all the cultivars, American Giant has showed to be the paramount cultivar with good quality traits (oil content and oxidative stability).

Future research should focus on the measurement of total flavonoids, total phenolics and sterols, specifically β-sitosterol, as well as the composition of volatile compounds of seed oils. These would all attribute to regional and varietal identities of the oils. Further perspectives include application of the oils in food products, as well as measuring its ability to function as antioxidants and anti-microbial agents.

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