Research Article

EFFECT OF SUNFLOWER COLLETS MOISTURE ON EXTRACTION YIELD AND OIL QUALITY†

Running Title: Effect of collets moisture on sunflower oil extraction

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Keywords: sunflower oil, moisture content, solvent extraction, minor compounds

Abbreviations: d.b., dry basis, **SEM**, Scanning electron microscope, **ADF**, Acid Detergent Fiber, **NDF**, Neutral Detergent Fiber, **PLs**, phospholipids, **PE**, L-α-phosphatidylethanolamine, **PC**, L-α-phosphatidylcholine, **PI**, L-α-phosphatidylinositol, **PA**, L-α-phosphatidic acid, **FID**, flame ionization detector, **SD**, standard deviation.

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [**10.1002/ejlt.201800234**].

Received: May 18, 2018 / Revised: October 12, 2018 / Accepted: October 30, 2018

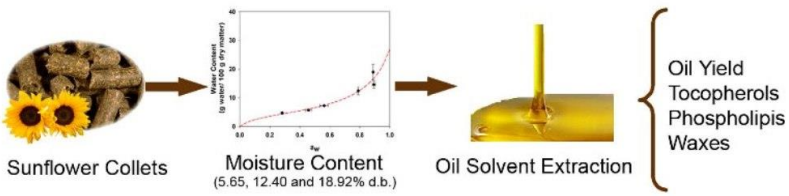
Abstract

The aim of this work was to analyse the effect of moisture content of sunflower expanded material on the quality of the extracted oil by determining its wax, phospholipids and tocopherol content and composition. Sunflower oil extraction was carried out in a batch extractor at 50 ºC using hexane and three moisture levels (5.65, 12.40 and 18.92% dry basis). The increasing moisture content caused an increment in both oil yield and total amount of minor compounds. The total wax content ranged between 808 and 1118 ppm, the wax fraction was composed mainly of esters of between 44 and 58 carbon atoms (>67%), the distribution did not change with the moisture content. In all the experiments, α- and β-tocopherols and β-tocotrienol were detected, being the level of α-tocopherol higher than 93% and easier to extract at 12.40% moisture. Furthermore, the phospholipids content in the oil increased with moisture content but maintaining its profile composition. The obtained results can be explained in terms of water activity and the effect of moisture and hexane extraction on solid structure, studied by scanning electron microscopy. In brief, the moisture content of the collets not only affects the oil yield but also the content of minor compounds, the intermediate moisture rendering the better oil yield and quality.

Practical applications

The data obtained provides updated information on oil yield and quality associated with moisture content of sunflower collets, useful to select the optimal operating conditions for oil extraction and to establish a trade-off between performance and quality.

Graphical abstract



Sunflower collets were conditioned in its moisture content to determine the isotherm sorption and the effects on the oil extraction and quality.

1 Introduction

In principle, oilseed extraction consists of four basic steps: 1) seed cleaning and pretreatment, 2) oil extraction (expelling, solvent extraction or a combination of both), 3) working up the expelled oil and/or the miscella, 4) working up the extraction meal [1]. Considering extraction in the strict sense, three general types of processes are used to crush oilseeds; hard pressing, prepress solvent extraction and direct solvent extraction. Extraction way used depends on the raw material, the amount of residual oil, the amount of protein, the investment cost available and the local environmental laws. High oil content seeds, as sunflower, are processed by using a combination of mechanical pressing and solvent extraction [2].

Presently, the solvent extraction is the most popular method for separation of oil from oilseeds mainly because of its high extraction efficiency

(over 99%) as well as its capability to handle large quantities of material. Even though the process is well known in the oil industry, oil processors aim to respond to new consumer requirements, tending to improve oil quality without losing focus on obtaining the most benefits (or highest yields) in the shortest time possible [3].

The design of an efficient extraction procedure requires a thorough knowledge of all the factors which may influence the extraction process. The pretreatment and processing given to the oil-bearing solid frequently plays a very important role to minimize deterioration and maintain a good quality of both contained oil and meal [1]. The literature reports influence of variables such as particle size, meal to solvent ratio, and contact time on the oil yield and extraction rate [4-6]. Another not less important parameter throughout the entire extraction process is the moisture content of raw material.

The effects of moisture have been recognized as an important factor in the efficiency of extraction since the 1940s and 1950s when first studies were initiated to understand the mechanisms of solvent extraction. Most reports were focused on their influence on the extraction performance to obtain the higher oil yield without considering its minor components that affect oil quality [7].

The literature shows data on the direct action of moisture on the handling of storage alone or in combination with heat on the yield and quality of oil of different oilseeds [2, 8, 9]. Whole, intact low moisture oilseeds (about 8-10% moisture) may be stored for extended a time under suitable conditions. The moisture content of oilseeds often has to be reduced to minimize degradation in storage and to improve the effectiveness of downstream processing. For example, soybeans are often received at 13% moisture or higher and need to be dried to 10% moisture to facilitate efficient hull removal [3].

Karlovic and coworkers [10] investigated the effect of temperature and moisture content in the range 8-12% on the kinetics of oil extraction using hexane from corn germ flakes and determined that the moisture content exhibits a substantial effect: an increase in the moisture content causes an exponential decrease of the extraction rate. In most cases, extractable materials containing 9-11% moisture are recommended to obtain an adequate extraction rate. Hexane and water are immiscible, and higher moisture contents interfere with the penetration of hexane. Lower moisture levels reduce the structural strength of the extractable material leading to the production of additional fines [11]. Obtained oil quality also can be affected by moisture content. In fact, Chu and Lin [12] determined that the total tocopherol content of crude soybean oil from cracked beans with 15 and 18% moisture had lower tocopherol contents than those from beans with 12% moisture. The effect of moisture content on the composition of extracted sunflower oil, particularly those minor compounds that affect oil quality, has not been investigated.

The aim of this work was to analyse the effect of moisture content of the collets on the extraction and quality of sunflower oil by determining the content and composition of waxes, phospholipids and tocols. Scanning electron microscope (SEM) was used to determine how the structures of the oilseeds collets change during extraction and to ascertain the best configuration for efficient oil extraction.

2 Materials and methods

2.1 Raw Material and sample conditioning

All experimental determinations were made with sunflower expanded material, known as collets, porous cylinders obtained from pressed sunflower cake by expanding, which was kindly provided by a local factory. The collets were stored in polyethylene container with screw caps in the dark at 4 ºC until used in extraction experiments.

The initial moisture and oil content were determined according to IUPAC method 1.121 and 1.122 [13], respectively. Standard AOCS official methods were used to determine fiber crude and protein content (N x 5.3 factor) [14]. The composition of the fiber fraction in terms of cellulose, hemicellulose and lignin was determined using the Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) methods [15], using an Ankom A220 Fiber Analyser (Ankom, New York, USA). Cellulose was calculated as the difference between ADF and lignin and hemicellulose between NDF and ADF.

The samples were conditioned to the selected moisture levels by drying in a forced air oven at 40-45 ºC or by spraying with pre-calculated amounts of distilled water. Then they were thoroughly mixed and sealed in separate polyethylene containers with screw cups. Finally, the samples were kept in a refrigerator at 4 ºC for at least 48 hours to allow a homogeneous moisture distribution. The moisture content was determined by means of OHAUS (Model MB 45) infrared analytical balance (105 ºC).

2.2 Sorption isotherm determinations

To obtain the sorption isotherm water activities of the samples conditioned at different moisture content were measured at environmental temperature using a thermohygrometer Testo 650 (Testo AG, Germany). Three replicates were carried out to estimate the inherent variability of the determination. Experimental data were fitted to the Guggenheim-Anderson-deBoer (GAB) equation expressed as:

$$X = \frac{X_m.C.k.a_w}{(1-k.a_w).(1-k.a_w+C.k.a_w)}$$

nonlinear regression analysis using the SigmaPlot software [16].

In the above equation, X is the water content on a dry basis (g of water per 100 g of dry matter), a_w is the water activity, and X_m is the water content on a dry basis at saturation of the active adsorption sites (monolayer). *C* and *k* are sorption constants that are related to the energies of interaction of sorbed molecules and the absolute temperature.

For each experiment, the required sample was taken out of the refrigerator and allowed to warm up to room temperature for approximately 2 h.

2.3 Solvent-Extraction Experiments

The oil was extracted with hexane in a magnetically-stirred batch system immersed in a thermo-regulated water bath at 50 °C during 2 h, conditions used in previous works [17]. The temperature was considered sufficient to ensure oil extraction, furthermore is the temperature used in the industrial process. Sunflower collets samples of approximately 22 ± 1.5 g, were subjected to extraction with a 10:1 ($mL.g^{-1}$) solvent-to-collet ratio, using analytical reagent grade hexane as solvent (90%, b.p. 68–72 °C) [6]. A high solvent-to-collet ratio is necessary to make sure that complete extraction is realized. Both the sample and the solvent were heated up to 50 ºC before mixing them together. The agitation rate was kept constant and at such value to ensure both a well-mixed fluid and a homogeneous particle suspension. At the end of the contact time, the contents of the flask were immediately filtered, and the miscella was concentrated by means of a rotary evaporator. Residual hexane was removed under a nitrogen stream to achieved constant weight. All the extractions were carried out in triplicate.

2.4 Minor Components Analyses

2.4.1 Tocol Analysis

The tocol content was measured using AOCS method Ce 8-89 [14] with a Waters 600E HPLC (Waters Associates, Milford, MA, USA) equipped with a Nucleosil Si-100A column (250 mm length, 4.6 mm i.d., 5 µm particle size, Phenomenex, USA) and a fluorescence detector (Waters 470) with the excitation wavelength set at 290 nm and the emission wavelength set at 330 nm. The mobile phase used was hexane: isopropanol (99.5:0.5 v/v, HPLC solvent, J.T. Baker, Phillipsburg, USA) at a flow rate of 1.0 $mL.min^{-1}$. Tocopherol content was determined by external standard method, areas were converted to concentrations using a standard curve of α-tocopherol (Sigma T#3251, 95%) in hexane (R^2 = 0.9982) in the relevant concentration range for the sample concentrations. Tocopherol content was expressed as $mg.kg^{-1}$ of oil. In addition, rice bran oil was used as standard for the identification of tocotrienols.

2.4.2 Phospholipids Analysis

Quantitative determination of phospholipids (PLs) was carried out by enrichment using a diol solid-phase extraction cartridge (J.T. Backer Inc., Phillipsburg, NJ, USA) and subsequent analysis by HPLC–UV [18]. A Waters 600E HPLC system (Waters Associates, Milford, MA, USA) and a Lichrosorb SI-60 column (250 x 4 mm, 5 µm particle size, Merck, Darmstadt, Germany) were used. Identification of PLs was carried out by comparison with the

retention time of pure standards. The following reference phospholipid standards with purities greater than 98% were supplied by Sigma Chemical Co. (St. Louis, MO): L-α-phosphatidylethanolamine (PE) from soybean, L-α-phosphatidylcholine (PC) from soybean, L-α-phosphatidylinositol (PI) from soybean and L-α-phosphatidic acid (PA) sodium salt from egg yolk lecithin. To obtain calibration curves, standard solutions were prepared by dissolving the phospholipids in HPLC mobile phase to different concentrations. Phospholipids content was expressed as mg. g^{-1} of oil.

2.4.3 Wax Analysis

Column chromatography was performed following the method proposed by Carelli et al. 2012 [19]. Column chromatography was performed in a glass column (i.d.= 15 mm, length= 400 mm) with a double phase of silver nitrate-impregnated silica gel (3 g) placed in the bottom of the column and silica gel 2% hydrated (12 g) placed on the top as a solid stationary phase. Sudan I dye was used to control the completion of the wax elution. The eluted wax fraction was evaporated to dryness, diluted with heptane and analysed by capillary GC. A Perkin Elmer AutoSystem XL gas chromatograph equipped with a FID detector, a temperature programmable on-column injector and a TotalCrom Workstation Version 6.3.1 data processor (Perkin Elmer, MA, USA) were employed. The capillary column was an HP-5 (5% diphenyl and 95% dimethyl-polysiloxane), fused-silica 15 m length x 0.32 mm i.d., 0.25 µm film thickness (Hewlett-Packard, Palo Alto, CA). The operating conditions were: hydrogen at 3 $mL.min^{-1}$ as carrier gas; oven temperature programming: initial temperature of 80 ºC, increasing at 30 $°C.min^{-1}$ to 200 ºC, holding for 1 min, increasing at 3 $°C.min^{-1}$ to 340 ºC holding for 20 min; on column injector programmed from 80 ºC to 320 ºC at 20 $°C.min^{-1}$ and injection volume of 2 µL; FID at 350 °C.

The following wax standards of almost 99% purity (Sigma Chemical Co., St. Louis, MO) were used for qualitative identification: C32 = lauric acid arachidyl ester ($C_{32}H_{64}O_2$), C36 = stearic acid stearyl ester ($C_{36}H_{72}O_2$), C38 = arachidic acid oleoyl ester ($C_{38}H_{74}O_2$), C40 = arachidic acid arachidyl ester ($C_{40}H_{80}O_2$), C42 = arachidic acid behenyl ester ($C_{42}H_{84}O_2$) and C44 = behenic acid behenyl ester ($C_{44}H_{88}O_2$). The C32 standard was also employed as internal standard. In addition, laboratory purified sunflower waxes from the filter cake of the dewaxing process of sunflower oil was used as standard for the identification of waxes with more than 44 carbon atoms.

2.4.4 Microscopy

Longitudinal sections of collets were sliced with a razor blade, after collets had been plunged into liquid nitrogen to ensure the maintenance of the structure. The slices were adhered to a cover slip, coated with a gold thin film in a sputter coater (Pelco 91000). For the micro-structural analysis, the specimens were observed in a scanning electron microscope (LEO EVO 40XVP, Cambridge, 2004). Digital images were collected at 7.0 kV using 3000x, 6000x and 10000x magnification.

2.5 Statistical analysis

All analysis was carried out in triplicate. The results are presented as mean values + standard deviations (SD). Statistical analysis was carried out by Analysis of Variance using the Infostat software [20]. Fisher's LSD method was used to compare the means of pairs of treatments with a significance level $p \leq 0.05$.

3 Results and Discussion

The sunflower collets were characterized presenting the following mean values: initial moisture content = 7.19 ± 0.03%; oil content = 21.88 ± 0.32%, protein = 33.57 ± 0.30%; NDF = 31.20 ± 0.54%; ADF = 21.65 ± 0.17% and lignin = 4.94 ± 0.13%, all results expressed in percentage dry basis (d.b.).

The moisture levels of sunflower collets samples after the conditioning were: 5.65 ± 0.37, 12.40 ± 1.42 and 18.92 ± 2.72% d.b. ($p = 0.0117$). Samples will be named hereinafter as H_1, H_2 and H_3 according to increasing moisture level, respectively.

With a less than 1% error, it can be stated that the moisture content results in a significant difference in oil yield, differentiating the sample obtained in H_1 of the others (Table 1). So, batch extraction from H_1 using hexane as solvent was less complete than those from H_2 and H_3, confirming that some

water is necessary to obtain a complete oil extraction. The fact that the oil extraction from H_2 and H_3 was not significant different between them and closer to the oil content of the samples obtained by Soxhlet, suggests that the presence of free water is essential to ensure a complete oil extraction. In fact, IUPAC norms establish a minimum of 10% of moisture in the oleaginous material in order to quantify its total oil content by Soxhlet [13].

These results can be explained in terms of the effect of moisture content on the solid structure and the solvent absorption. Figure 1 shows the mean values of the equilibrium moisture content and relative humidity of sunflower collects at environment temperature. As it was expected, the water activity (a_w) of the material to extract increased with their moisture content, from 0.459 to 0.889 ($p = 0.0001$) the sorption behaviour exhibits a sigmoid shape typical of food materials. The isotherm can be divided in three regions: 1) a low aw or monolayer region where water is held by high hydrophilic bonding on polar sites in the solid, mainly carbohydrates and proteins; 2) an intermediate multilayer adsorption region were water is weaker retained by hydrogen bonds inside of small capillaries and in the fiber structure; and 3) a relatively high aw region in which most condensed water is mechanically retained inside void spaces, especially large capillaries, having much properties of free liquid water [21].

Monolayer value predicted for sunflower collets, obtained from the GAB equation, was 4.02 g water/100 g dry matter, being in range of to those reported for meal and whole and ground sunflower seed (3.5-5.2 g water/100 g dry matter) using the same model [21]. Thus, samples H_2 (a_w = 0.790 ± 0.003) and H_3 (a_w = 0.889 ± 0.006) are in the region where most water is free or loosely held in large capillaries while H_1 (a_w = 0.459 ± 0.022) falls in the range intermediate multilayer adsorption.

A comparison of isotherm obtained from sunflower collets and data reported in literature from sunflower seeds, kernels, and hulls is also shown in Figure 1 [22]. The water binding capacity of sunflower collets is in between than that for hulls and kernel, showing that samples with higher oil content and lower hull/kernel ratio had lower equilibrium moisture values. It confirms that the oil does not hinder the access of water to sorption sites and the water binding capacity depends mainly on fiber content and composition [21, 22].

Previous studies have shown that meal water content affect the sorption of solvent, increasing the amount of adsorbed hexane at equilibrium [21]. Adsorbed water causes some swelling and disruption of the cell structure, thus enhancing adsorption and capillary condensation of the hexane, and improving solvent accessibility to the oil. This effect increases with moisture content, especially above the monolayer value. However, at higher moisture contents free water could fill the pores and interfere with the penetration of hexane, does not affecting significantly the oil yield (Table 1) but reducing the extraction rates. For this reason, intermediate moisture contents are recommended to achieve a better extraction performance.

Table 1 also shows the contents of minor components in the oils extracted. The humidity influences the proportion of no triglyceride components in the crude oil, more polar components are carried over. It can be observed that the oil corresponding to the higher moisture content (H_3) showed a significantly different composition, with a higher content of phospholipids and a lower concentration of tocopherols and waxes.

Tocol-related compounds, tocopherols and tocotrienols, are important lipid oxidation inhibitors in food and biological systems [23]. The tocopherol content and pattern of oils are characteristic and depend on plant genotype, the climatic conditions of growth and harvest, polyunsaturated fatty acid contents in the oil as well as the processing and storage conditions [24]. In all samples only the presence of α- and β-tocopherol and β-tocotrienol was identified, being the level of α -tocopherol higher than 93%. These concentrations were within the range reported by other authors for crude oil obtained from whole sunflower seeds [3].

The total tocopherol contents in the extracted oils were significantly lower in H_3 ($p = 0.0041$) due especially to a lower α-tocopherol content (788 ± 52 $mg.kg^{-1}$ oil), although the relative percentage of the compounds did not show significant differences ($p > 0.13$ in all cases). Previous studies about solvent extraction kinetics from sunflower

collets with 6.73% d.b. moisture showed that tocopherols present a similar behaviour to the oil while phospholipids were more difficult to extract [25]. The fact of a slightly minor content of α-tocopherol in the extracted oil in sample H_3 could be due to a dilution effect caused by a higher amount of phospholipids in this condition (Table 1) as well as a degradation of this tocopherol. In fact, higher moisture content could promote the vitamin E degradation [26]. According to some studies, the loss of tocopherols and tocotrienols is dependent on water activity: when water activity increases, degradation is faster [26].

Total phospholipid content in extracted oil and their profile for the three samples are shown in Table 1. The moisture conditioning prior to extraction leads to a change in the phosphatide content ($p = 0.0231$). The values were between 12.3 and 15.4 $mg.g^{-1}$, increasing with moisture content. Kock [27] determined that the proportion of phosphatides in crude oils depending on the water content of the extracted seed. From an analysis of more than 100 samples he found a proportional relationship, indicating that the portion of phosphatides increases by 0.2% per 1% of seed moisture (between 11 and 14% seed moisture).

Phospholipids are associated with oleosin proteins being essential for the construction of the oil bodies [28]. Moreover, they are the main components of cell membranes being their primary function to maintain the integrity of the cell membrane under different environmental conditions. Thus, an external force is necessary to facilitate their released during oil extraction. One of the aims of the moisture adjustment of the collets is to facilitate cell disruption, solvent penetration and lipid compounds extraction. Table 1 also shows that phospholipids maintain their relative percentage ($p \geq 0.1309$). These experimental results apparently disagree with those in soy flakes that reported an increase of the phosphatidylcholine percentage associated with inactivation of the enzymes by a moisture-heat treatment prior extraction [29]. It should be taken into account that sunflower collects were also pretreated in the plant before extraction experiments.

The wax fraction ranged from 34 to 58 carbon atoms (C34-C58), rendering total wax contents in the obtained oils between 808 and 1118 $mg.Kg^{-1}$ (Table 1). The lowest wax content corresponded to H_3 sample ($p = 0.0024$). The waxes with less than 40 carbon atoms are considered the oil soluble fraction, waxes with 40 and 42 carbon atoms are the partially soluble fraction, and waxes with more than 44 carbon atoms constitute the crystallized fraction. Within partially soluble waxes, C42 as well as crystallisable wax esters (C44 to C58) are fundamentally drawn from the seed hulls during the oil extraction process [30-32]. As can be seen in the Table 1, the main components were esters belonged to the crystallized fraction, C42-C58. The increase in moisture content affect significantly this fraction ($p = 0.0077$). The content and composition of the waxes depend on the origin of the oil, seed or fruit variety, pretreatment of the raw material, hull content and the temperature and technology used in the extraction process. Oils obtained by solvent extraction have higher wax contents than those obtained by mechanical extraction [9, 33, 34]. Baümler and coworkers [35] found that soluble and partially soluble waxes presented a similar extraction behaviour to that observed in the oil extraction; but, the kinetic curves of the crystallized fraction showed a first quick of adsorption followed by the diffusion stage. This was attributed to the fact that when the solvent comes into contact with the crystallized waxes, they are dissolved and adsorbed inside the particle with the solvent. After that, the solvent dissolves the oil retained in the cells, forming the miscella, and it diffuses towards the exterior of the particle with the waxes. This phenomenon is also influenced by the collet structure, because the hull content in its interior is not in homogeneous proportions [35]. The high presence of free water causing a more difficult transport of the waxes from inside the particle, along with the dilution effect caused by a higher amount of phospholipids, could explain the less wax content in H_3.

Surface structures of the samples at different moisture level were examined with the scanning electron microscope (Fig. 2), before and after being subjected to solvent extraction, to determine how the collet structures change due to moisture content and oil extraction. All samples had a highly porous and tortuous microstructure. When the samples were moistened, cell walls were disrupted; exposing more lipid bodies (Fig. 2 C-E),

this exposure greatly improved the rate of extraction and oil yield. Lipid bodies in full-fat collets (Fig. 2 A-C-E) were full of oil, and definition of cell material was not clear in the micrograph. When the oil was removed from the collet by solvent extraction (Fig. 2 B-D-F), the cytoplasmic network covering the protein bodies could be recognized. The collet subjected to oil extraction (Fig. 2 B-D-F) showed a non-uniform surface with aggregates that could be waxes located superficially [31] or proteins [36]. Thermal denaturation of protein (modification of the protein native structure by breaking the non-covalent interactions) provokes a radical change in their physical and chemical properties [37]. Rouilly and coworkers [36], studying the thermal denaturation of sunflower globulins in low moisture conditions, observed that following denaturation, some corpuscles are still visible but seem opened or emptied, and aggregates of sizes up to 10-15 µm appear. After the rupture of the interactions between polar residues inside the corpuscles leading to a molten state in which the protein's secondary native structure is maintained, the thermal treatment induces rapidly the separation of existing disulphide bonds and/or activation of free sulfhydryl groups which can later form new intermolecular disulphide bonds [38]. This complex phenomenon can lead the formation of either soluble or insoluble complexes between proteins and phenolic residues [39]. The creation of these new bonds, covalent or not, leads to the formation of aggregates. After the solvent extraction was possible to observe these aggregates in collet surface that belonged to sample H_3 (Fig. 3).

4 Conclusions

The moisture content of sunflower collets not only affects the oil yield, but also the content of minor compounds. According to results low moisture content reduces the oil yield while a high humidity gives oil with a significantly different composition, showing a higher content of phospholipids and a lower concentration of tocopherols and waxes. A humidity of about 12% renders both a good oil yield and quality with a high tocopherol content but lower phospholipid content reducing the intensity of degumming.

The authors acknowledge the financial support from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas) and Universidad Nacional del Sur, Argentina.

The authors have declared no conflict of interest.

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Tables

Table 1. Percentage of extracted oil and its minor compounds contents in batch extraction with hexane from collets with different moisture content.

Measure	H_1	H_2	H_3
Extracted Oil (% d.b.)	20.83 ± 0.08 [a]	21.35 ± 0.15 [b]	21.23 ± 0.13 [b]
Tocopherols (mg.kg^{-1} oil)	967 ± 12 [b]	1037 ± 48 [b]	843 ± 55 [a]
α- tocopherol (%)	93.19 ± 0.37 [a]	93.80 ± 0.41 [a]	93.44 ± 0.29 [a]
β- tocopherol (%)	1.16 ± 0.03 [a]	1.08 ± 0.42 [a]	1.04 ± 0.02 [a]
β- tocotrienol (%)	5.65 ± 0.39 [a]	5.11 ± 0.07 [a]	5.51 ± 0.28 [a]
Phospholipids (mg.g^{-1} oil)	12.29 ± 0.63 [a]	12.45 ± 1.79 [a]	15.45 ± 1.24 [b]
PE (%)	6.60 ± 0.17 [a]	5.67 ± 0.78 [a]	5.65 ± 0.61 [a]
PA (%)	33.78 ± 1.01 [a]	31.75 ± 3.15 [a]	32.73 ± 0.53 [a]
PI (%)	30.66 ± 0.48 [a]	30.99 ± 1.66 [a]	32.13 ± 4.57 [a]
PC (%)	28.96 ± 1.06 [a]	31.59 ± 3.72 [a]	29.50 ± 3.62 [a]
Total waxes (mg.kg^{-1} oil)	1079 ± 73 [b]	1118 ± 89 [b]	808 ± 3 [a]
Crystallised C42-C58 (%)	67.00 ± 2.22 [a]	68.18 ± 1.49 [a]	70.42 ± 1.7 [a]

Estimated values ± standard error (n=3)
Any two means in the same column followed by the same letter are not significantly different ($p>0.05$) by the Fisher's LSD method.

Figures

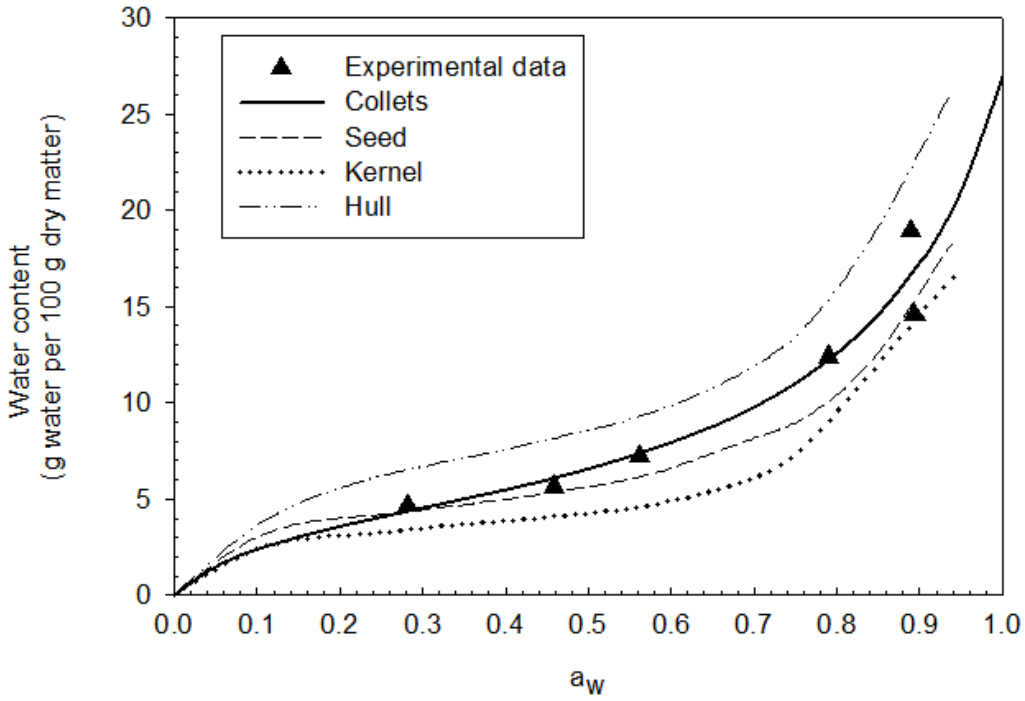

Figure 1

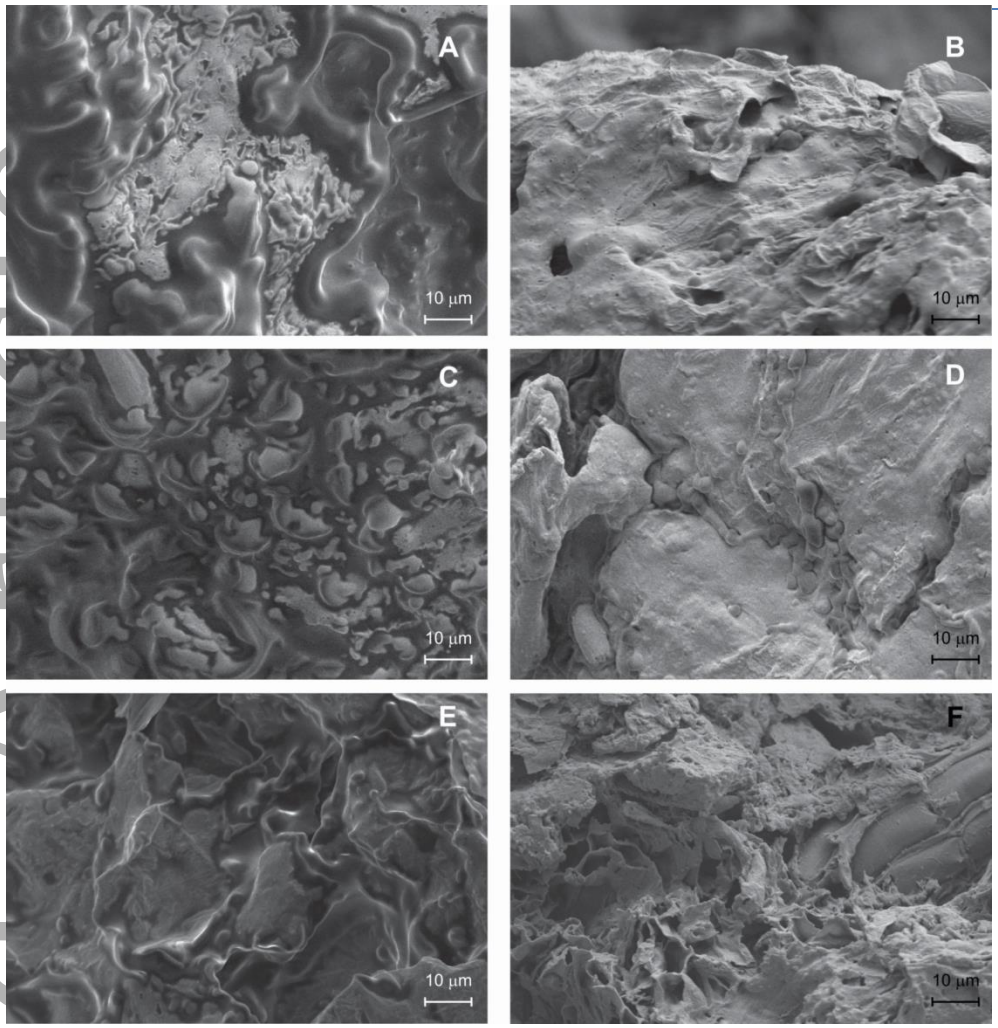

Figure 2

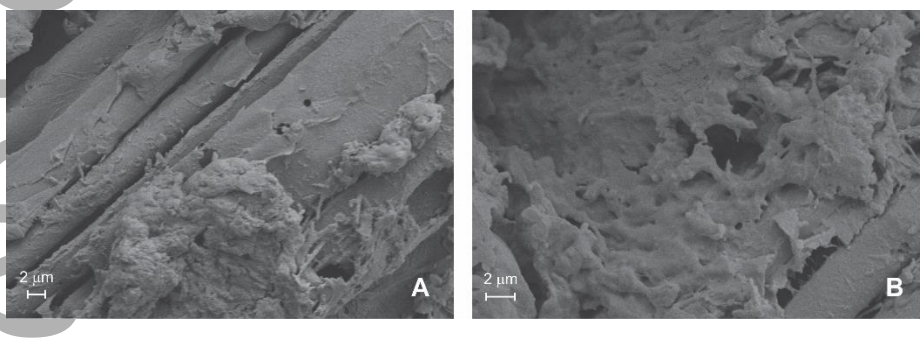

Figure 3

Figure legends

Figure 1: Equilibrium moisture contents of sunflower collets (▲) and predicted values obtained using GAB equations (X_m= 4.2, C= 11.461 and k= 0.8448) at 25 °C. Comparison with sorption isotherms of sunflower seeds, hulls and kernels [22].

Figure 2: SEM photograph of samples H_1, H_2 and H_3 without solvent extraction (A-C-E) and, H_1, H_2 and H_3 subjected to solvent extraction (B-D-F). Magnifications of 3000X.

Figure 3: SEM photograph of sample H_3 submitted to solvent extraction. (A) magnifications of 6000X, (B) magnifications of 10000X.