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# Characterization of *Xanthoceras sorbifolium* Bunge seeds. Lipids, proteins and saponin content.

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**Abstract**

The increasing demand of renewable source of reduced carbon makes of interest the research on alternative oil crops. *Xanthoceras sorbifolium* B. is a tree that can be cultivated in marginal lands on temperate climates. This tree produces capsular fruits with seeds rich in oil containing unsaturated very long chain fatty acids that has application in cosmetics and biodiesel production. The present work focuses on the integral use of Xanthoceras seeds. Different batches of seeds were extracted and the distribution of fatty acids within the glycerol backbone was investigated by analysing the triacylglycerol species composition of the oil. Furthermore, the nuts extracted meal was characterized in terms of phospholipid content and amino acid composition. Phospholipids accounted for up to 1% of the extracted meal, displaying high levels of galactolipids and phosphatidylinositol species. The extracted meal was rich in protein (35%) with fairly high contents of essential amino acids lysine and isoleucine. Solubility studies showed albumins as the predominant protein classes in this specie. The seeds of Xanthoceras also contain saponins, active compounds present in many traditional Chinese formulations. Triterpenic and steroidal saponins were determined in different parts of the seeds, being predominant those of the triterpenic class, which were accumulated mainly in the seed hull. The possible applications of the different of Xanthoceras-derived products are discussed at the view of the results.

*Keywords:* oil; triacylglycerides; sterols; amino acids; saponins; Xanthoceras sorbifolium

## 1. Introduction

Vegetable oils are the main source of lipids in the human diet, but they are also a natural and renewable source of reduced carbon chains to formulate biofuels, biolubricants or being used as a reactant by the chemical industry (Sharma et al., 2012). Vegetable oils are produced by oleaginous plants, which are those accumulating triacylglycerols (TAGs) in their seeds or fruits. There is a great variety of plants in nature that are a potential source of oil, but only few species are commercially exploited. That means that exist a large amount of germplasm that could give solutions at the problems that are facing the production of vegetable oils at the present (Murphy, 1999). One of the most controversial aspects of the production of vegetable oils for industrial applications is the competition of industrial crops with food production, which causes the raise of the price of food facilities in the international markets (Koh and Ghazoul, 2008). Thus, the production of these oils should come from plants out of the edible oil production circuit, the oil should be easily differentiable from other vegetable oils and production should be possible in marginal lands to avoid competition with most crops. Many crops accomplishing these conditions are under studies in this moment, including *Camelina sativa*, pennycrest, castor or *Jatropha curcas* (Fröhlich and Rice, 2005; Moser et al., 2009; Berman *et al*., 2011, Kumar and Sharma, 2008). In the present work, we studied the tree *Xanthoceras sorbifolium* B. as a possible source of vegetable oil for industry. Xanthoceras also known as yellowhorn, or goldenhorn is a perennial shrub belonging to the Sapindaceae family (Ao et al., 2012). It has been cultivated for long in the north of China for its oil and for traditional medicine. This tree produces fruits consisting in a capsule of 5-6 cm diameter containing three compartments in which seeds of 1 to 1.5 cm diameter can be found. The

number of seeds per compartment can widely vary from 1 to 6. These seeds consisted in a hard black hull and a nutty large embryo rich in oil and protein.

This plant can be grown as a small deciduous tree or large multi-stemmed shrub at full sun or partial shade. It is tolerant of a wide range of soil including high pH, clay, sandy, loam, average, medium or well-drained. Its large radicular system makes it not very demanding in terms of watering, although it resists badly hot climates (Xie et al., 2010). All these facts make Xanthoceras an appropriate crop to be grown in marginal lands in temperate climates.

The most valuable product that can be obtained from Xanthoceras seeds is its oil. This oil accounts for more than the 50% of the weight of the kernel and it is rich in oleic and linoleic acid, being also remarkable the content of unsaturated very long chain fatty acids (VLCFAs), as gondoic, erucic and nervoic acid (Zhang et al., 2010). Although it is edible oil, has been mainly used for non-food applications like cosmetics, traditional medicine or biofuel. In this regard, a large number of works have been published on *X. sorbifolium* as a source of fatty acids for biodiesel use (Zhang, et al., 2016; Yao et al., 2013, Li et al., 2012). The excellent properties of the biodiesel produced from Xanthoceras seed boosted programs of cultivation of this plant in China in the last decade (Chen et al., 2016) and the search of new germplasm of this plant for improved production of biofuels (Yu et al., 2017).

Moreover, other products can be obtained from these seeds. Thus, the extracted cake is very rich in protein and phospholipids, which can be used for animal fed or for the production of lecithins. The seed hulls contain important amounts of bioactive products, being especially remarkable their content in saponins (Li *et al*., 2010). These compounds are steroidal glycosides that have been reported to have a large variety of physiological effects that made them broadly used in traditional medicine (Man et al*.,* 2010; Yu *et al*., 2012). They also act as powerful antioxidants (Zhang and Zhou, 2013) and are an active field of research in

93 phytochemistry due to the variety and complexity of their structures (Chan *et al*., 2008; Fu *et*
94 *al*. 2010).

95 The present work investigates new aspects of Xanthoceras oil and phospholipids. We also
96 examined the extracted cake in terms of protein quality and solubility, evaluating its potential
97 applications. Finally we analysed the total content of sterolic and triterpenic saponins in the
98 seeds kernel and hull. This work does not pretend to study composition of Xanthoceras seeds
99 from different accessions, which have been demonstrated to be subjected to certain variability
100 (Yu et al, 2017), but showing a general approach on typical composition of compounds of
101 interest in commercial Xanthoceras seeds. The potential for integral use of these seeds will be
102 discussed.

103

104 **2. Materials and methods**

105

106 **2.1. Plant Material and oil extraction**

107 *X. sorbifolia* seeds were obtained from F.W. Schumacher Co., Inc. Horticulturists, (East
108 Sandwich, MA, USA). Two independent batches of seeds were used for this research.

109 Xanthoceras seeds were crushed in a hammer mill, followed of the extrusion of the
110 resulting debris in a tubby press. Due to the rheological properties of the crushed seed paste,
111 the physical extraction was not complete and only a part of the oil was obtained. Thus, the
112 extruded material was submitted to extraction with hexane in a Soxhlet arrangement.

113

114 **2.2. Fatty acids determination**

115 For fatty acids determination, lipids were trans-esterified to their fatty acid methyl esters.
116 Methylation reaction was carried out for 1h after the addition of 2 mL of
117 methanol/toluene/sulphuric acid (88/10/2; v/v/v) to 5 mg of oil. Methyl esters were then

extracted with 2 mL heptane and analysed by GC. The chromatographic system consisted of an Agilent 6890 gas chromatograph (Palo Alto, CA) equipped with a Supelco SP-2380 fused silica capillary column (30 m length; 0.25 mm i.d.; 0.20 µm film thickness: Bellefonte, PA). The carrier gas (H2) flow was set at 28 cm/s. Injection and FID temperature was 200 ºC, whereas oven temperature was 170 ºC. Fatty acids were identified by comparing their retention times with those obtained from commercial standards. Their percentage was calculated according to the area obtained from each peak.

**2.3. Triacylglycerol determination**

The TAG species composition of the oil was determined by GC. The chromatographic system was similar to that used for methyl esters but endowed with a Quadrex Aluminium-Clad 400-65HT (30 m length, 0.25 mm i.d., 0.1 µm film thickness: Woodbridge, CT, USA) and a flame ionization detector. The detector and the injector working temperatures were 360 and 370 ºC respectively and H2 was used as the carrier gas at a linear rate of 50 cm/s and split ratio 1:80. The oven temperature was 335 ºC and TAGs were eluted from column applying a head pressure gradient from 100 to 180 kPa. Typically 2 µg of sample were injected in each analysis. TAG species were identified by comparing retention times with those in our data base obtained from previous injections of oils of known TAG composition. New species present in *X. sorbifolium* oil were identified on the basis of interpolation of retention times with the corresponding carbon numbers. Eventually, some species were confirmed by synthesizing the corresponding TAGs and finding their retention times. Response factors reported by Carelli and Cert (1993) were applied for quantification.

**2.4. Phosholipids classes determination**

142 Total lipids were extracted from the meal applying a modification of the protocol reported
143 by Hara and Radin (1978). A weight of 0.1-0.25 g meal was homogenated in a glass tube in 5
144 mL of hexane/2-propanol (3:2, v/v) and heated at 80 ºC for 10 min. Then, phases were
145 separated by addition of 2.5 mL of 6.7% sodium sulphate. The resulting mix was shaken and
146 centrifuged at 1500g for 5 min and the supernatant transferred to a clean tube. A second
147 extraction was applied to the aqueous phase by adding 3.75 mL of hexane: 2-propanol (7:2,
148 v/v) and the resulting organic phase was combined with the former. Then lipids were
149 evaporated under nitrogen and the residue was dissolved in 2 mL of chloroform. Neutral lipids
150 were then separated from polar lipids in a Lichrolut 0.5 g silica gel cartridge (Merck)
151 equilibrated with 2 mL of chloroform as described by Nash and Frankel (1986). The solution of
152 total lipids was loaded onto the column, which was then eluted with 15 mL of chloroform to
153 remove neutral lipids (essentially oil) from the column. Then, the column was washed with 10
154 mL of methanol to elute the polar lipids quantitatively. The polar lipid fraction was evaporated
155 to dryness under nitrogen and dissolved in 1.5 mL of hexane/2-propanol 3:2 and analyzed and
156 quantified by HPLC/ELSD.
157 Polar lipids were analysed in a HPLC Waters 2695 Module (Milford, MA) endowed with a
158 Waters 2420 ELSD. The column used was a Lichrospher 100 Diol 254-4 (5 µm; Merck) applying
159 the method described by Salas *et al*. (2006), based in a linear binary gradient of solvent
160 mixtures containing different proportions of hexane, 2-propanol, acetic acid, water and
161 trimethylamine. The flow was 1 mL/min and the method was regularly calibrated by using
162 commercial high purity standards for each lipid.
163
164 **2.5. Total protein and amino acid determination**

For total protein, the desiccated seed cake was submitted to nitrogen analysis using a LECO CHNS-932 analyser (Leco Corporation, St. Joseph, MI, USA). The protein content was calculated applying a conversion factor of 6.25 to the total N content.

For amino acids analysis, samples (2mg) were hydrolyzed with addition of 6 M HCl (4 mL) at 110 ºC for 24 h in tubes sealed under nitrogen. Amino acids were determined after derivatization with diethyl ethoxymethylenemalonate by high-performance liquid chromatography (HPLC) according to the method of Alaiz et al. (1992).

**2.6. Protein profiling**

In the study of solubility 1 g of the seed meal was lixiviated successively with 10 vol. of water, NaCl 1M, ethanol and NaOH pH 11 at room temperature in a rocker shaker. The supernatants were separated by centrifugation at 1000 x g for 10 min, and the supernatants were lyophilized or evaporated under nitrogen. The resulting residues were analysed for total protein as described in the preceding section.

For molecular profiling 1 g of meal protein was dissolved in a volume of 10 ml of 0.1 M $Na_2BO_3$ pH 8.3, 0.2 M NaCl. Gel filtration chromatography was performed in an FPLC system endowed with a Superose 12 HR 10/30 column (Amersham Pharmacia LKB Biotechnology, Uppsala, Sweden). A volume of 200 µl was injected in the column, with a protein concentration of 1.5 to 2.0 mg/mL. Borate buffer at a flow rate of 0.4 ml $min^{-1}$ was used as mobile phase, and the column output was monitored at 280 nm. Approximate molecular masses were determined using the commercial molecular weight standards blue dextran 2000 (2000 kDa), thyroglobulin (669 kDa), β-amylase (200 kDa), bovine serum albumin (67 kDa) and ribonuclease A (13.7 kDa).

**2.7. Determination of saponins**

Total saponins were determined using the method reported by Hiai et al. (1976). This method is based in the reaction of the C-3 carbon of saponins with vanillin in acid medium to produce chromophores absorbing between 455 and 544 nm. A volume of 0.1mL of ethanolic extract of saponins was mixed with 0.75 mL of methanol plus 0.25 mL 8% vainillin in ethanol. Then a volume of 2.5 mL of 72% $H_2SO_4$ was added and the reaction mix was vortexed. The reaction was run for 10 min at 60ºC and then tubes were transferred to ice. Total saponins were determined by measuring absorbance at 544 nm. The method was calibrated using the commercial saponins diosgenin (Sigma).

Steroidal saponins were determined by reaction with anisaldehyde in ethyl acetate in acid media to produce a chromophore (Baccou *et al*., 1977). A volume of 0.1 mL of ethanolic solution of saponins was mixed with 1 mL of 0.5% anisaldehyde in ethyl acetate plus 1 mL 50% $H_2SO_4$ in ethyl acetate. The reaction was run for 30 min. at room temperature and then steroidal saponins were determined by measuring absorbance at 430 nm. A calibration curve was made with commercial diosgenin (Sigma) for quantification.

## 3. Results and discussion

### 3.1. Oil triacylglycerol species composition.

The *X. sorbifolium* oil obtained accounted for 25% of the total fresh weight of the seeds and 50% of the weight of the seed kernel. It was characterized in terms of fatty acids composition, displaying the expected fatty acids profile according to previous literature (Zhang *et al*., 2010). It displayed low amounts of palmitic acid (5-5.5%) and stearic acid (2-2.5%). The predominant fatty acids were oleic (28-30%) and linoleic (40-45%) and fairly high amounts of the monounsaturated very long chain fatty acids gondoic (6-8%), erucic (8-10%) and nervonic

(2-4%) were found. Amounts of saturated very long chained fatty acids (arachidic, behenic and lignoceric acids) and linolenic acid less than 1% were also detected. One of the aspects remarkable of the fatty acids composition of this oil was the high level of monounsaturated VLCFA, which was in some aspects similar to rapeseed oil composition with lower amounts of the unstable linolenic acid. Thus, these VLCFA could contribute to increase the viscosity of the oil improving its performance as a biolubricant or in cosmetics and keeping higher levels of stability. The TAG species composition of the oil gave account of the distribution of these fatty acids along the glycerol backbone. The variety of fatty acids present in this oil makes the TAG composition very complex. Therefore, up to 28 TAG species were identified and quantified (Table 1). The distribution of fatty acids was similar to that found in other vegetable oils. The predominant species were those containing oleic and linoleic fatty acids dioleoyl-linoleoyl glycerate (OOL) and dilinoleoyl-oleoyl glycerate (LLO). The trioleoyl glycerate (OOO) and trilinoleyl glycerate (LLL) were also present in fairly high proportions. Very long fatty acids mostly appeared in TAGs associated to linoleate or oleate. The content of saturated fatty acids was low and they usually appeared in the form of monosaturated or disaturated TAGs (ie. palmitoyl-dioleoyl glycerate or dipalmitoyl-oleoyl glycerate). No traces of trisaturated TAGs were found, so it was probable that they are excluded from *sn*-2 position of TAGs as it happens in many other vegetable oils. The distribution of fatty acids within the glycerol backbone of oils is dependant of the specificities of the enzymes responsible of its acylation, that are well known acyltransferases located in the endoplasmic reticulum (Stymne & Stobart, 1987). Two diacylglycerol acyltransferase genes have been cloned from Xanthoceras seeds (Guo et al. 2013). These genes were characterized in terms of sequence and expression, and were demonstrated to be functional by heterologous expression in Arabidopsis plants. Nevertheless, no data of specificity of these enzymes was reported to date.

240 **3.2. Oil minor components**

241 The main minor components present in oil unsaponificable fraction of oils are sterols and
242 tocopherols. The former are important at the time of setting a fingerprint of the oil, which
243 would help to identify it in oil blends to prevent fraud. Phytosterols are also active compounds
244 in nutrition that act reducing the absorption of cholesterol and contribute, once absorbed, to
245 reduce low density lipoprotein cholesterol in humans (Ostlund, 2002). The sterol content of
246 Xanthoceras oil is shown in Table 2. The total sterol content was 1664 and 1864 ppm in the
247 two batches of seeds analysed, which was lower than those reported for other vegetable oils
248 like sunflower, canola or soybean (Phillips et al., 2002). The predominant sterol species were β-
249 sitosterol and Δ7-stigmasterol, similarly to sunflower, displaying very low contents of
250 campesterol and no brassicasterol. The unsaponificable fraction of Xanthoceras oil accounted
251 for 1% of the total weight of the oil. This oil also contains tocopherols. These compounds are a
252 family of four chemical species that together with tocotrienols form the chemical group known
253 as vitamin E. The intake of this vitamin is necessary to prevent a serial of deficiency diseases.
254 Moreover, they are powerful antioxidants, so their content is important at the time of
255 determining the oxidative stability of oils. The content and composition of tocopherols in
256 Xanthoceras oil is shown in Table 2. The total amount ranged between 390 and 437 ppm,
257 which again is lower than found in other vegetable oils. The predominant specie is gamma-
258 tocopherol, the specie conferring the highest oxidative stability to oils. Tocopherols have been
259 demonstrated to stabilize fatty acid methyl esters, especially the gamma and delta forms
260 (Fröhlich & Schober, 2007). Thus, the tocopherols present in Xanthoceras contribute to
261 increase the stability of the biodiesel produced from this specie. Studies on germplasm of
262 Xanthoceras with increased amounts of these compounds would be of interest for the
263 production of biofuels with improved stability.

264

### 3.3. Polar lipid content of the meal

Other acyl lipids of interest are the phospholipids, which are important components of the oleosomes, in which associated with oleosin proteins the TAGs are stored in seeds. The phospholipids present in the oil during the process of refining are removed in the degumming step and give place to seed lecithins, an important product of the oil extraction and refining process. In the present work, we purified the polar lipid fraction from Xanthoceras extracted meal and characterized it in terms of polar lipids classes. Results obtained are shown in Table 3. The polar lipids amount of the extracted meal was around 1.0-1.2 mg/g, which was an amount similar to that reported in sunflower or soybean (Salas et al., 2006; Nash and Frankel, 1986). However, the lipid class composition found differed from that reported for other seeds like sunflower, Soybean or rapeseed (Salas et al, 2006, van Nieuwenhuyzen and Tomás, 2008). Thus, the predominant specie in the two batches analysed was phosphatidyl inositol (PI), accounting for up to 25% of total polar lipids in batch 1. The content of phosphatidylcholine was unexpectedly low with values between 10 and 16%,at the same level of other phospholipids like phosphatidylethanolamine and phosphatidylglycerol that are usually found in minor proportions in seed lecithins. Nevertheless, the content of photosynthetic glycerolipids like monogalactosyldiacylglycerols, digalactosyldiacylglycerols and sulphoquinovosyl diacylglycerols were unexpectedly high. Thus, these lipids are found at high levels in photosynthetic tissues because they are important structural components of thylakoidal membranes. Xanthoceras produces white seeds like sunflower or castor seeds, which unlike soybeans or rapeseeds are not photosynthetic even at the beginning of their development. Nevertheless, they carry as much as 28% of these polar lipids in batch 2. Finally, it was also remarkable the high content of phosphatidic acid (close to 30%). This compound is not usually found at such high levels in oilseeds and so it is usually produced by degradation of phospholipids by the action of phospholipases of the D type. These phospholipases act on PC

290 and PE and are not active towards galactolipids or PI (Moreno-Pérez et al, 2010). These results
291 suggested that during the process of oil extraction lipases were activated and acted on
292 phospholipids, decreasing the levels of PC and PE and boosting PA. Moreover, lipids non-
293 attacked, such as PI or galactolipids, increased their relative content. This degradative artefact
294 has been reported too during the extraction of sunflower phospholipids at laboratory scale
295 (Salas et al., 2006).
296
297 **3.4. Characterization of protein in the meal of *Xanthoceras sorbifolia***
298 Out of the vegetable oil, oilseeds are also an important source of protein, which are
299 commonly used for animal feeding. The main vegetable source of protein is the extracted
300 soybean meal world commodity that constitutes the base for most compound feed. The
301 extracted meal coming from Xanthoceras kernel was analysed in term of total protein and
302 amino acids composition. The characterization of the meal was started applying elemental
303 analysis (Table 4). This allowed us to estimate the protein content of the extracted meal from
304 the data of total nitrogen, yielding protein contents between 31 and 37%, which was similar to
305 that reported for sunflower, and lower than soybean meal that usually accounts for up to 44%
306 of protein (Sosulski and Sarwar, 1973). The quality of this protein meal was evaluated
307 attending to the amino acids composition, solubility curve and molecular profile.
308 The amino acids composition was determined by total hydrolysis of the protein samples
309 and derivatization of the resulting amino acids for HPLC analysis. Amino acids composition of
310 Xanthoceras meal is shown in Table 5. The predominant amino acids were glutamic plus
311 glutamine, aspartic plus asparagine and arginine, accounting for up to 43% of total amino
312 acids. Nevertheless, the quality of a protein feedstock depends of its content of essential
313 amino acids. In this regard, one of the main quality indexes is the content of lysine.
314 Xanthoceras meal was fairly rich in lysine, containing around 4.3 % of this amino acid, which is

315 in the same range than soybean meal (3-6%). This lysine content importantly improves that
316 found in other oilseed meals such as sunflower, canola or cottonseed (1-2%; Sosulski and
317 Sarwar, 1973). The meal of Xanthoceras was also rich in branched essential amino acids
318 leucine and isoleucine, which accounted for 7.8-7.9% and 4.5-4.7% respectively, which was
319 similar or even higher than that found in soybean meal. The content of sulfur-containing
320 methionine was low, with levels far below the 1%.

321 The protein fraction from Xanthoceras was further characterized in terms of solubility.
322 This factor is important because it gives notice of the protein families present in the meal,
323 which is related with its digestibility and nutritional value. This study was carried out by
324 solubilizing the protein with different buffers, starting with distilled water (pH 6.6) dissolving
325 albumins, NaCl 1M (pH 7.0) dissolving globulins, ethanol dissolving prolamines, and NaOH (pH
326 11.0) dissolving glutelins. Once separated, the buffer was removed and the protein was
327 determined by elemental analysis. The solubility distribution of Xanthoceras proteins is shown
328 in Table 5. The faction of albumins was the most abundant in the two batches of seeds
329 analyzed, accounting for the 40-49% of the total protein. Albumins are globular soluble
330 proteins, which are stored in a high proportion in seeds to feed the embryo. They are usually
331 rich in essential amino acids, although they also can contain anti nutritional factors and cause
332 allergy (Breiteneder and Radauer, 2004). This protein class is found in high proportion in the
333 endosperm of other oil seeds like sunflower. Glubulins and glutenins are also present in fairly
334 high amounts (23-30%). They are also plant storage proteins that are found mainly in legumes
335 and are more difficult to digest. Finally, only traces of prolamins were detected, which was
336 expected due these proteins are usually present in cereals or in the seeds of herbaceous
337 plants. The protein distribution of Xanthoceras meal displayed similarities with that in other
338 cultivated seeds. Thus, in soybean and other leguminous plants, the predominant proteins are
339 globulins (Haard and Chism, 1996) as it is also abundant in sunflower meal (Gonzalez-Perez

340 and Vereijken, 2007), whereas cereals like wheat or rice are rich in gliadins and glutenins
341 (Kinsella, 1982; Ju *et al*., 2001).

342 Moreover, a study of exclusion chromatography on the protein from Xanthoceras meal
343 gives notice of the size of the proteins present in this source. The molecular profile of proteins
344 coming from the different batches is shown in Fig. 1. In both cases, the proteins gave place to
345 six groups of proteins. The proportion and percentage of these groups are represented in
346 Table 6. The largest group corresponded to a fraction of protein having molecular mass higher
347 than 15 kDa, followed by three groups of proteins that displayed weights between 14.5-14.9
348 kDa, 3-2 kDa and under 2 kDa. Unlike in other determinations here there were slight
349 differences between the two batches of seeds processed.

350 All these results showed that Xanthoceras seeds possess a protein fraction rich in lysine
351 and branched essential amino acids, with solubility similar to that in other oilseeds. This
352 protein could be an important by-product of oil production for animal feeding or even food
353 applications. However, more research would be necessary to find important parameters of this
354 protein sus as digestibility, assimilation rates or the present of antinutritional components.

355

356

357 **3.5. Content of saponins**

358 As mentioned above one of the most valuable components of Xanthoceras seeds are their
359 saponins. These bioactive compounds consisting in complex glycosides bonded to a lipophilic
360 moiety that can be a sterol or a triterpene, giving place to the two families of saponins. Many
361 plants containing saponins have been used in traditional medicine. Their biological activities
362 are quite diverse and they have been reported as strong antioxidants. Moreover, effect against
363 hypercholesterolemia and anti-inflammatory, anticarcinogenic and anti-coagulant activities
364 have been also reported (Sparg *et al*., 2004).

In the present work the content of saponins in different parts of the Xanthoceras seeds were analysed. Results in Table 7 showed that the crude oil contains low amounts of these compounds, which were more abundant in both extracted meal and seed hull. The total amount of saponins ranged from 3.4 to 7.2 mg/g in these fractions. In both cases, most of the saponins detected were of the triterpenic class, which accounted for 85 to 95 % of total saponins determined. At the view of the results, it was clear that Xanthoceras saponins are mostly accumulated in the seed hull. Thus, depending on the processing of the seed it could be present in the oil or the meal. For a better benefit of this active compounds within the processing of these nuts it would be maybe necessary the separation of the hull and the later extraction of saponins before the step of crushing.

## 4. Conclusions

At the view of results obtained in this study, *Xanthoceras sorbifolia* tree produces nuts rich in oil. The oil composition is similar to that in the oil of oilseed rape, due it contains high levels of oleic, linoleic and unsaturated VLCFA. The distribution of these fatty acids in TAG positions was similar to that in other vegetable seeds. The predominant sterol specie present in this oil was beta sitosterol and delta-7 stigmasterol, being also rich in gamma tocopherol, which enhances its stability and could be of interest to stabilize biodiesel. The extracted meal from Xanthoceras nuts was rich in protein of a good quality, with levels of lysine similar to those in soybean meal, being rich in glutelins and globulins just as the proteins from the meal from other oil crops. The composition and properties of this protein fraction makes it a very promising by-product of Xanthoceras seeds. The meal also contained phospholipids and galactolipids. The saponins produced by this plant remained in the extracted meal and were concentrated in the seed hull. They were not present in the oil. At the view of this result, the cultivation of *X. sorbifolia* in marginal lands in temperate climates could be an attractive

390 alternative for the production of nuts that could be full used for the production of oil and a
391 protein-rich extracted meal with content of lysine higher than other oilseeds. The hull also
392 could be a source of saponins that could be extracted for antioxidant or medical use and finally
393 the extracted hull could be used as a highly energetic biomass for power or heat production.
394
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## Tables

**Table 1**
Triacylglycerol composition of two batches of Xanthoceras sorbifolia oil.

| | %(w/w) | |
|---|---|---|
| | Batch 1 | Batch 2 |
| POP | 0.7 ± 0.0 | 0.3 ± 0.0 |
| PLP | 2.2 ± 0.1 | 0.9 ± 0.0 |
| POS | 1.0 ± 0.0 | 0.3 ± 0.0 |
| POO | 2.4 ± 0.1 | 2.4 ± 0.2 |
| PLS | 1.0 ± 0.1 | 0.6 ± 0.0 |
| POL | 7.8 ± 0.1 | 6.6 ± 0.4 |
| PLL | 9.4 ± 0.6 | 5.0 ± 0.2 |
| SOS | 0.3 ± 0.0 | 0.0 ± 0.0 |
| SOO | 1.4 ± 0.1 | 1.6 ± 0.0 |
| SLS | 0.2 ± 0.0 | 0.2 ± 0.0 |
| OOO | 4.5 ± 0.2 | 6.9 ± 0.5 |
| SOL | 3.7 ± 0.0 | 4.0 ± 0.2 |
| OOL | 7.4 ± 0.6 | 9.2 ± 0.7 |
| SLL | 2.5 ± 0.2 | 2.4 ± 0.2 |
| OLL | 19.1 ± 0.2 | 20.7 ± 0.3 |
| LLL | 8.1 ± 0.8 | 4.3 ± 0.1 |
| OOA | 0.2 ± 0.0 | 0.2 ± 0.0 |
| GOO | 1.5 ± 0.0 | 2.6 ± 0.2 |
| OLA | 3.0 ± 0.1 | 2.8 ± 0.0 |
| GOL | 3.3 ± 0.2 | 4.2 ± 0.0 |
| GLL | 5.6 ± 0.4 | 6.9 ± 0.6 |

| | | |
|---|---|---|
| OOB | 0.9 ± 0.1 | 0.5 ± 0.0 |
| EOO | 1.3 ± 0.0 | 2.5 ± 0.2 |
| OLB | 1.1 ± 0.1 | 1.1 ± 0.1 |
| EOL | 3.0 ± 0.0 | 3.6 ± 0.1 |
| ELL | 5.4 ± 0.3 | 7.2 ± 0.6 |
| NOO | 0.7 ± 0.0 | 0.4 ± 0.0 |
| NOL | 1.0 ± 0.1 | 1.0 ± 0.1 |
| NLL | 1.2 ± 0.0 | 1.4 ± 0.1 |

Data corresponded to the average mean of three determinations repetitions ±SD. TAG species were named with three letters each of one corresponding to a fatty acids. P: palmitic, O : oleic, S: stearic, L: linoleic, A: araquidic, G: gondoic, E:erucic and N: nervoic.

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**Table 2**

Insaponificable content, sterols and tocopherols from Xanthoceras sorbifolia oil.

| | Batch 1 | Batch 2 |
|---|---|---|
| Sterol composition (%) | | |
| Brasicasterol | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Campesterol | 0.2 ± 0.0 | 0.3 ± 0.0 |
| Stigmasterol | 3.6 ± 0.5 | 3.7 ± 0.6 |
| Delta 5,23 Estigmastadienol | 3.7 ± 0.6 | 3.3 ± 0.4 |
| Beta-Sitosterol | 47.4 ± 6.0 | 48.5 ± 1.5 |
| Delta 5-Avenasterol | 1.9 ± 0.1 | 1.4 ± 0.0 |
| Delta 7-Estigmastenol | 32.6 ± 2.3 | 32.7 ± 0.1 |
| Delta 7-Avenasterol | 9.6 ± 0.6 | 9.2 ± 1.4 |
| Others | 1.0 ± 0.0 | 0.8 ± 0.0 |
| Total sterol content (mg·kg-1) | 1664 ± 271 | 1853 ± 213 |
| | | |
| Tocoferols (mg/kg) | | |
| alfa | 49.0 ± 3.4 | 36.0 ± 2.6 |
| beta | 4.0 ± 0.5 | 4.0 ± 0.2 |
| gamma | 348.0 ± 47.8 | 313.0 ± 3.1 |
| delta | 36.0 ± 3.8 | 37.0 ± 3.3 |
| Total tocopherol content (mg·kg -1) | 437.0 ± 48.1 | 390.0 ± 5.2 |
| Total insaponificable matter (%) | 1.0 ± 0.1 | 1.2 ± 0.1 |

Data corresponded to the mean of three repetitions ± SD.

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**Table 3**
Polar lipid species composition of Xanthoceras sorbifolia extracted meal.

| | %(w/w) | |
|---|---|---|
| | Batch 1 | Batch 2 |
| MGDG | 13.9 ± 1.5 | 24.4 ± 2.90 |
| PA | 27.9 ± 3.7 | 32.7 ± 1.42 |
| PG | 3.9 ± 0.6 | 6.5 ± 0.82 |
| PE | 6.6 ± 0.4 | 5.7 ± 0.14 |
| PC | 16.2 ± 2.9 | 10.9 ± 1.25 |
| DGDG | 4.6 ± 0.6 | 2.9 ± 0.03 |
| SQVDG | 0.3 ± 0.0 | 0.7 ± 0.12 |
| PI | 26.6 ± 2.5 | 16.3 ± 2.39 |
| Total (mg/g) | 1.1 ± 0.2 | 1.2 ± 0.12 |

Data corresponded to the mean of three repetitions ± SD. MGDG: monogalactosyl diacylglycerol, PA: phosphatidic acid, PG: phosphatidyl glycerol, PE: Phosphatidyl ethanolamine, PC: phosphatidyl choline, DGDG: digalactosyl diacylglycerol, SQVDG: sulphoquinovosyl diacylglycerol, PI: phosphatidylglycerol.

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**Table 4**

Elemental analysis and total protein content of *Xanthoceras sorbifolia* meal

| | % (by weight) | | | | | % |
|---|---|---|---|---|---|---|
| | C | H | N | S | Others | Protein* |
| Batch 1 | 47.5±1.5 | 5.7±0.2 | 5.9±0.2 | 0.4±0.0 | 40.5±1.8 | 36.7±1.2 |
| Batch 2 | 49.1±0.1 | 5.4±0.5 | 5.0±0.5 | 0.3±0.0 | 40.2±0.5 | 31.1±3.1 |

Data corresponded to the mean of three determinations±SD. *Calculated from total nitrogen content.

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**Table 5**
Aminoacid composition of the protein from Xanthoceras sorbifolia meal and Classification in terms of solubility.

| | % (w/w) | | |
|---|---|---|---|
| | Batch 1 | Batch 2 | Soybean * |
| Aspartic + Asparagine | 9.9 ± 0.1 | 9.8 ± 0.7 | 11.9 |
| Glutamic + Glutamine | 22.1 ± 2.0 | 21.7 ± 1.3 | 18.3 |
| Serine | 6.1 ± 0.7 | 6.0 ± 0.6 | 5.5 |
| Histidine | 2.2 ± 0.1 | 2.2 ± 0.2 | 2.6 |
| Glycine | 5.7 ± 0.0 | 5.6 ± 0.3 | 4.4 |
| Treonine | 3.7 ± 0.4 | 3.9 ± 0.4 | 4.1 |
| Arginine | 10.7 ± 0.9 | 10.7 ± 1.2 | 7.3 |
| Alanine | 4.4 ± 0.0 | 4.4 ± 0.2 | 4.5 |
| Proline | 3.7 ± 0.3 | 3.7 ± 0.2 | 5.5 |
| Tyrosine | 3.2 ± 0.3 | 3.5 ± 0.3 | 3.6 |
| Valine | 6.4 ± 0.3 | 5.8 ± 0.7 | 4.7 |
| Metionine | 0.3 ± 0.0 | 0.5 ± 0.1 | 1.3 |
| Cysteine | 0.4 ± 0.0 | 0.7 ± 0.1 | 1.5 |
| Isoleucine | 4.5 ± 0.4 | 4.7 ± 0.0 | 4.6 |
| Triptophan | 0.5 ± 0.0 | 0.4 ± 0.0 | 1.4 |
| Leucine | 7.8 ± 0.6 | 7.9 ± 0.2 | 7.7 |
| Fenylalanine | 4.2 ± 0.1 | 4.3 ± 0.0 | 4.9 |
| Lysine | 4.2 ± 0.1 | 4.3 ± 0.3 | 6.3 |
| | | | |
| Albumins | 49.6 ± 9.2 | 40.4 ± 1.2 | |
| Globulins | 23.1 ± 1.3 | 28.7 ± 2.7 | |
| Prolamins | 0.5 ± 0.1 | 0.1 ± 0.0 | |

| | | |
|---|---|---|
| Glutenins | 26.8 ± 1.3 | 30.8 ± 7.0 |

Data corresponded to the mean of 3 repetitions±SD.*Data taken from www.soybean.org.

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**Table 6**

Approximate molecular weight and area proportion of different protein groups resulting from molecular profiling from *Xanthoceras sorbifolia* meal protein (Figure 1).

| | Batch 1 | | Batch 2 | |
|---|---|---|---|---|
| Group | Size (KDa) | Area (%) | Size (KDa) | Area (%) |
| 1 | >15 | 6.2±0.6 | >15 | 6.9±0.6 |
| 2 | >15 | 50.0±5.9 | >15 | 43.6±0.3 |
| 3 | 14.9 | 9.7±1.2 | 14.5 | 10.2±0.2 |
| 4 | 2 | 25±0.6 | 3.1 | 30.1±3.0 |
| 5 | <2 | 6.1±0.1 | 1.8 | 7.7±0.5 |
| 6 | <2 | 3.0±0.2 | <2 | 1.5±0.1 |

Data corresponded to the mean of 3 determinations±SD.

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**Table 7**
Saponin content of the parts of *Xanthoceras sorbifolia* seeds.

| | Total saponins | Steroidal saponins | | Triterpenic saponins | |
|---|---|---|---|---|---|
| | mg/g | mg/g | % | mg/g | % |
| Crude oil | 0.64 ± 0.06 | 0.03 ± 0.00 | 4.1 | 0.6 | 95.9 |
| Extracted meal | 3.4 ± 1.17 | 0.49 ± 0.03 | 14.2 | 3.0 | 85.8 |
| Hull | 7.2 ± 0.38 | 0.54 ± 0.00 | 7.5 | 6.7 | 92.5 |

Data corresponded to the mean of 3 determinations±SD

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**Legends to figures**

**Fig.1** Molecular profile obtained by exclusion chromatography of from *Xanthoceras sorbifolia* meal protein. A: Batch 1. B: Batch 2.

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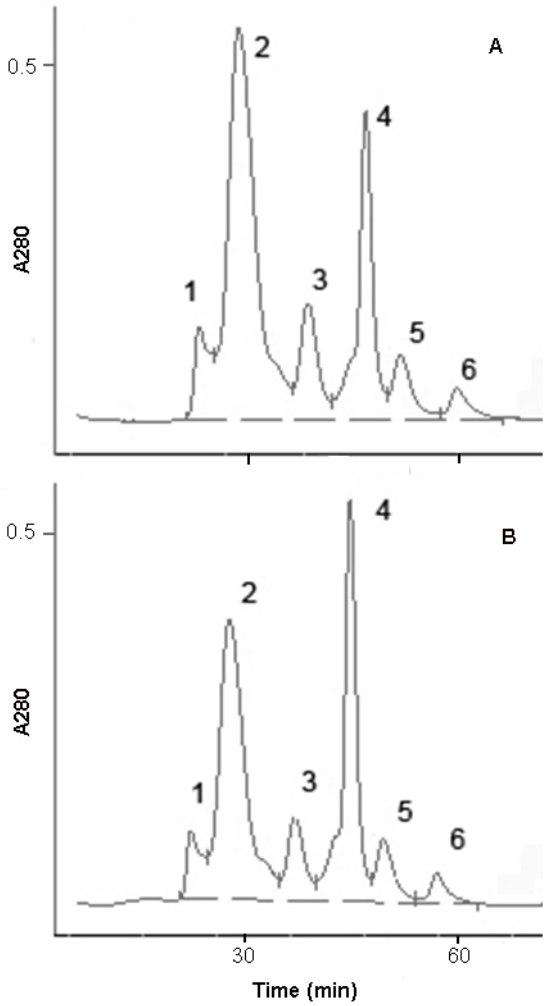
642 Figure 1

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