J Am Oil Chem Soc (2011) 88:265–270
DOI 10.1007/s11746-010-1662-2

ORIGINAL PAPER

Protein, Lipid, Aliphatic and Triterpenic Alcohol Content of Caper Seeds "*Capparis spinosa*"

Nizar Tlili · Taissir El Guizani · Nizar Nasri · Abdelhamid Khaldi · Saida Triki

Received: 19 April 2010 / Revised: 19 July 2010 / Accepted: 23 July 2010 / Published online: 13 August 2010

Abstract *Capparis spinosa* has a large natural distribution over the Mediterranean basin. It is used in traditional medicines, and it is one of the most commonly found aromatics in the Mediterranean kitchen. In this paper, the total storage protein and lipids of Tunisian *Capparis spinosa* seeds were investigated, and the quantities were ca. 27% and ca. 33%, respectively. In this study also the composition of the aliphatic and triterpenic alcohols of *C. spinosa* was characterized for the first time. Aliphatic alcohol contents were ca. 45 mg kg^{-1} of total extracted lipids. Three compounds were identified, hexadecanol, octadecanol and tetracosanol, of which octadecanol was the major compound (ca. 28 mg kg^{-1}). Triterpenic alcohol content was 396.82 mg kg^{-1}. Citrostadienol was the major compound (ca. 170 mg kg^{-1}). β-Amyrin, gramisterol, cycloartanol and 2,4 methylcycloartenol were also detected and identified.

Keywords Caper (*Capparis spinosa)* · Seeds · Storage protein · Oil · Aliphatic alcohol · Triterpenic alcohol

Introduction

Plant seeds are important sources of proteins and oils for nutritional, industrial and pharmaceutical applications. Proteins are a necessary component of the diet of humans and other mammals. Accordingly, consumption of proteins, as a general class of macronutrients, is not normally associated with adverse effects. Production of purified vegetable protein is gaining increasing commercial importance due to the consumer preferences for vegetable sources of food and cosmetic ingredients [1]. In addition, the safety of proteins introduced into crops via genetic modification using recombinant DNA techniques for humans or animals may not be known [2]. Moreover, protein-calorie malnutrition (PCM) is a major nutritional syndrome affecting more than 170 million preschool children and nursing mothers in developing Afro-Asian countries [3]. For these reasons, proteins from seeds of non-conventional plants should be explored. These types of plants, which are found in abundance, are not yet being utilized. Such seeds may have good quality proteins that can be used to meet the increasing demand for dietary proteins [4].

Seed oils have attracted much attention because of their biological properties and effects. Recently, research has increased to investigate new plant sources of oil, especially from underexploited seeds [5, 6]. It has been suggested that minor compounds of unsaponifiable matter of most vegetable oils protect against cardiovascular complications and could reduce the risk of heart attacks by 15–45% [7, 8]. There has been recent interest in long-chain aliphatic alcohols as dietary supplements or nutraceuticals in the treatment of various chronic diseases, including diabetes and hypercholesterolemia [9].

To our knowledge, very few studies have been carried out on caper seeds' storage components. As a spontaneous perennial shrub plant, the caper has a large natural distribution in the Mediterranean sea basin. The most important economic species is *Capparis spinosa*. This species has increased in economic importance in the Mediterranean

N. Tlili (✉) · T. El Guizani · N. Nasri · S. Triki
Laboratoire de Biochimie, Département de Biologie, Faculté des Sciences de Tunis, Université Tunis El-Manar, 2092 Tunis, Tunisia
e-mail: Nizar.Tlili@fst.rnu.tn

N. Tlili · A. Khaldi
Unité de Recherche Gestion et Valorisation des Ressources Forestières, INRGREF, BP 10, 2080 Ariana, Tunisia

region over the last years. Before commercialization, the fresh aerial parts are stored in salt, vinegar or brined and used as an appetizer with olives, cheese and nuts or as a complement to meat, salads and pasta. Additionally, fruits with small, soft seeds are preferred for the production of pickles [10–12]. Previous studies have brought attention to the richness of *C. spinosa* seed oil, which contains essential fatty acids, sterols, tocopherols and carotenoids [13–15].

To our knowledge, very few studies have been carried out on *C. spinosa* storage compounds. The objective of this study is to quantify the storage protein and lipids and describe for the first time the aliphatic and triterpenic alcohol contents and compositions of *C. spinosa* seed samples.

Materials and Methods

Chemicals

All solvents used in the experiments were purchased from Fluka (Ridel-de Haën, Switzerland). 1-Eicosanol was purchased from Sigma-Aldrich (Steinheim, Germany). TLC silica plates (silica gel 60G F254, 20 × 20 cm^2, 0.25 mm thickness), potassium hydroxide pellets and anhydrous sodium sulphate were obtained from Merck (Darmstadt, Germany).

Plant Material

Sampling was performed from seven Tunisian regions in May 2008: Dahmani (D), Mateur (M), Bullarijia (B), Sidi Thabet (ST), Ghar el Melh (GM), Ksar Hadada (KH) and Tatouine (T) (Table 1). From each region, seeds were collected from 8 to 13 plants, mixed and then a representative sample was taken for further analysis.

Protein Contents

Protein contents were determined according to the AOAC official method [16] using a micro-Kjeldhal apparatus. Each sample (100 mg) was digested for about an hour with 100 mg of digestion mixture (8 g K_2SO_4 + 20 g $CaSO_4$ + 2 g selenium) and 6 ml of concentrated H_2SO_4. When digestion was completed, the solution became clear. The solution was then made up to 30.0 ml in a volumetric flask with distilled water. For the nitrogen determination, 10 ml of 2% boric acid solution was first put in a beaker with a few drops of methyl red as indicator. Then 10 ml of the digested mixture, 30 ml of 40% NaOH solution, and 10 ml of distilled water were transferred to the distillation chamber. Ammonia was liberated, and it combined with NaOH to form NH_4OH, which was then put into the boric acid solution to form ammonium borate (pink to yellow color). Distillate (ammonium borate) was then titrated with 0.1 N H_2SO_4. The volume of acid that had been added at the point when the color of the distillate changed from yellow to pink was recorded. Protein was calculated according to the following formula: %protein = %N × 6.25. Analyses were performed in triplicate.

Oil Extraction

The oil content was determined according to ISO (1999) method 659:1998 [17]. About 5 g of the seeds was ground in a mortar until forming a dough and extracted with petroleum ether in a Soxhlet apparatus for 6 h. The solvent was concentrated using a rotary evaporator under reduced pressure at 45 °C. The oil was dried by using a stream of nitrogen and stored at −20 °C until use. Analyses were performed in triplicate.

Saponification of the Lipids

To separate the unsaponifiable fraction, oil from *C. spinosa* seeds was treated with a potassium hydroxide solution to transform the fatty acyl esters into potassium salts that are soluble in water. Total extracted lipids were treated with 50 ml of 2 M KOH-ethanol solution, and the mixture was refluxed, with constant stirring, for 1 h. Then, 50 ml of water was added. The unsaponifiable fraction was extracted

Table 1 Location, protein (%), oil (%) and unsaponifiables matter (%) of harvested samples

Samples	Localité	Latitude	Longitude	Protein	Oil	Unsaponifiables
D	Dahmani	35° 57′ N	8° 48′ E	28.81	32.54	2.2
M	Mateur	37° 02′ N	9° 40′ E	29.93	33.26	2.3
KH	Ksar Hdada	33° 06′ N	10° 19′ E	23.5	34.27	2.5
B	Bellarijia	36° 33′ N	8° 45′ E	28.06	41.19	1.7
T	Tataouine	32° 55′ N	10° 26′ E	25.75	31.50	1.8
ST	Sidi Thabet	36° 55′ N	10° 03′ E	26.5	32.48	2
GM	Ghar El Melh	37° 10′ N	10° 11′ E	30.12	32.09	1.9
Mean ± SD				27.52 ± 2.41	33.90 ± 3.33	2.05 ± 0.28

with 3 × 40 ml of diethyl ether. The organic extract was separated and washed with 3 × 40 ml of distilled water, and then dried over anhydrous sodium sulfate, filtered and concentrated using a rotary evaporator under reduced pressure at 60 °C. Analyses were performed in duplicate.

Separation of the Alcohol Fractions from Unsaponifiables by Thin-Layer Chromatography (TLC)

The unsaponifiable matter (5% in chloroform) was separated on TLC plates (20 × 20 cm^2) coated with KOH–methanol (2 N)-impregnated silica gel (0.25 mm) previously activated by heating at 100 °C for 1 h. The unsaponifiable fraction (250 µl) and internal standard 1-eicosanol (0.2%, w/v) were spotted on the plates. Elution was performed using hexane/diethyl ether 65:35 (v/v) as the mobile phase. The plates were then sprayed with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol, and the alcohol's pink bands appeared under UV light together with the spots of 1-eicosanol used as internal standard. Alcohol bands were scraped off separately and dissolved in warm chloroform (5 ml). The obtained solutions were dried over anhydrous sodium sulfate and filtered through Whatman filter paper. The chloroform was evaporated by nitrogen stream, and alcoholic fractions were dried in an oven at 103 °C.

GC-FID Analysis

Alcoholic fractions were treated with a derivatizing reagent obtained from Sigma-Aldrich France Ltd. (pyridine/hexamethyldisilazane/trimethylchlorosilane, 9:3:1, v/v/v). A volume of 0.05 ml of reagent for each milligram of alcohol was added. One microliter of this solution was injected into the gas chromatograph. Samples were analyzed in duplicate by GC in a Hewlett-Packard HP-4890D chromatograph equipped with a HP-5 (5% diphenyl–95% methylpolysiloxane) fused silica capillary column (30 m × 0.32 mm × 0.25-µm film thickness), operated isothermally at 250 °C with an inlet carrier gas (nitrogen) pressure of 100 kPa. The injector with a split ratio of 1:15 was maintained at 230 °C and the flame ionization detector (FID) at 250 °C. Alcohols were identified by using a known samples under the same conditions. Alcohols were expressed as milligrams per kilogram of total extracted lipids by using 1-eicosanol as internal standard.

GC–MS Analysis

The sample, 1 µl, was injected into the GC system, a Hewlett-Packard 5890 series II connected to an HP 5989A mass spectrometer. The GC system was equipped with a 30-m (0.25 mm i.d., 0.25-µm film thickness) HP-5 fused-silica capillary column coated with a stationary phase of 5% cross-linked phenylmethylsilicone. The oven temperature was as follows: raised from 210 to 250 °C at a rate of 6 °C min^{-1}, held at 250 °C for 11 min, then raised from 250 to 310 °C for 12 min. The detector temperature was 350 °C. The split ratio was 1:30. Helium was used as a carrier gas at a pressure of 100 kPa. TMS esters were eluted from the column and passed into the mass spectrometer using electron impact with an ion source of 70 eV.

Statistical Analysis

The experimental data were analyzed using the analysis of variance (ANOVA) and the Statistical Analysis System (XLSTAT 2008). Differences at $p \leq 0.05$ were considered statistically significant by Duncan's new multiple range test.

Results and Discussion

Storage Protein Content

The results in Table 1 show the approximate content of the seed protein of *C. spinosa* from different locations in Tunisia. Protein content ranged between 23.5% (KH) and 30.12% (GM), with an average of 27.52% on a dry weight basis (dw %). These results were slightly higher than the only information about the content of protein from caper seeds reported by Akgul and Özcan [18] for Turkish capers (ca. 22%). This difference might be due to geographic distribution since the protein content of crops varies with the crop cultivars, soil and climatic conditions of an area [19]. As a macronutrient, protein is an essential component of the human diet. These proteins could be used as healthy ingredients in nutraceutical foods and can constitute a new food source for different population sectors. An alternative for improving people's nutritional status is to supplement their diet with plant proteins [3]. In Afro-Asian diets, legumes are the major contributor of protein and calories for economic and cultural reasons [3]. Legumes have been a focal point of interest since they contain 18–25% protein [20], whereas the protein content of cereals is between 10 and 15% [21]. *C. spinosa* seeds have a protein content of 23–30%, which makes them a relatively new and inexpensive source of protein for human use.

Oil Content

The contents of lipids and unsaponifiable fraction in all samples of *C. spinosa* seeds are shown in Table 1. The mean content of total lipids was 33.9 ± 3.33% (dw%), and

varied between 31.5% (T) and 41.19% (B). These values were slightly higher than those reported by Matthaus and Özcan [22] for Turkish caper seeds (27.3–37.6%, with an average 32.2%). This difference was probably due to location distribution since the chemical composition of crops varies with the crop cultivars, soil and climatic conditions of the area [19]. In the case of the unsaponifiable fraction, the mean value was 2.05 ± 0.28% with a minimum of 1.7% in KH and a maximum of 2.5% in B. These results bring attention to the possibility of using the seeds of *C. spinosa* as a new source of oil for food and industrial applications.

Aliphatic and Triterpenic Alcohol Content

Both aliphatic and terpenic alcohols were investigated in caper seeds for the first time. Table 2 shows these compounds isolated from *C. spinosa* seed oil unsaponifiable matter, identified by GC-MS and quantified by using 1-eicosanol as internal standard. Results illustrate the presence of eight compounds: hexadecanol, octadecanol, tetracosanol, *β*-amyrin, gramisterol, cycloartanol, citrostadienol and 2,4 methylcycloartenol. These compounds are widely distributed in the vegetable kingdom. Little information is available on this series of compounds in vegetable oils [23].

The content of total alcohols (Table 3) ranged from 278.14 mg kg^{-1} (M) to 652.65 mg kg^{-1} (D) with an average of 441.61 ± 125.85 mg kg^{-1}. This amount is less than that found in other plants such as acorn, pine and olive oil [23–25].

The value of aliphatic alcohols ranged between 40.74 mg kg^{-1} (GM) and 48.24 mg kg^{-1} (ST), with an average of 45.19 ± 2.27 mg kg^{-1}. The major compound was octadecanol, with an average 28.03 mg kg^{-1}, followed by hexadecanol (11.86 mg kg^{-1}) and tetracosanol (5.3 mg kg^{-1}). Other studies have shown that the major aliphatic alcohol in amaranth oil is docosanol [26], in acorn oil is tetracosanol [23], in pine oil is octacosanol [24] and

Table 2 Identified alcohols in *C. spinosa* seed oil

Peak	Retention time	Relative area (%)	Compounds
1	6.87	0.23	Hexadecan-1-ol
2	8.93	0.50	Octadecan-1-ol
3	14.66	0.10	Tetracosan-1-ol
4	19.47	0.47	*β*-Amyrin
5	19.88	1.23	Gramisterol
6	19.96	1.31	Cycloartanol
7	20.51	2.66	2,4 methylcycloartenol
8	20.90	5.50	Citrostadienol

Table 3 Aliphatic and triterpenic alcohol contents (mg kg^{-1} of dry weight) of *C. spinosa* seed oil

Compounds	Aliphatic				Triterpenic				Total
Samples	1	2	3	Total	4	5	6	7	8	Total	
D	12.25	27.23	5.45	44.93	25.32	66.72	71.24	144.88	299.56	607.72	652.65
M	11.59	29.56	5.23	46.38	22.41	20.09	43.97	73.03	72.26	231.76	278.14
KH	11.92	28.39	5.01	45.32	23.86	43.41	57.60	108.96	165.19	399.02	444.34
B	11.75	28.97	5.12	45.84	24.59	45.21	52.30	100.2	200.2	422.5	468.34
T	11.92	28	5	44.92	24.22	33.33	40	75.69	90.96	264.2	309.12
ST	13.02	29.09	6.13	48.24	25	65	69.13	100.23	206.23	465.59	513.83
GM	10.58	25	5.16	40.74	22.5	44.13	53.23	102.1	162.2	384.16	424.9
Mean ± SD	11.86 ± 0.73	28.03 ± 1.54	5.3 ± 0.39	45.19 ± 2.27	23.98 ± 1.15	45.41 ± 16.48	55.35 ± 11.73	100.72 ± 23.85	170.94 ± 76.25	396.82 ± 125.74	441.61 ± 125.85

in olive oil is hexacosanol [25]. These compounds have been shown to have bioactive properties. Hexadecanol is used extensively in cosmetics and pharmaceuticals as a base for creams, lipsticks, ointments and suppositories because of its mildness and feel on the skin. It is also added to artificial pulmonary surfactant formulations used for surfactant replacement therapy [27]. Tetracosanol is one of the major policosanols. Numerous beneficial effects have been attributed to policosanol, such as enhancing low-density lipoprotein decatabolism and reducing cholesterol levels by inhibiting cholesterol biosynthesis [28, 29]. In addition, it has been shown to decrease endothelial damage, platelet aggregation and the development of foam cells [30, 31].

The content of triterpenic alcohols (Table 3) ranged from 231.76 mg kg^{-1} (M) to 607.72 mg kg^{-1} (D), with an average of 396.82 ± 125.74 mg kg^{-1}. Citrostadienol was the predominant compound, with an average of 170.94 ± 91.41 mg kg^{-1}, followed by 2,4 methylcycloartenol with an average of 100.72 ± 23.85 mg kg^{-1}. β-Amyrin, gramisterol and cycloartanol were also detected with an average of 23.98 ± 1.15, 45.41 ± 16.48 and 55.35 ± 11.73 mg kg^{-1}, respectively. Theses values are less than other values found in other plants such as acorns [23]. These compounds have an important role in the prevention of many diseases. β-Amyrin has been reported to possess anti-inflammatory, antinociceptive, hepatoprotective and anti-allergic properties [32], and the same compound has been found to inhibit the growth of some human cancer cell lines in vitro [33]. Cycloartenol and 2,4-methylenecycloartenol showed anti-inflammatory activity against some compounds inducing inflammation in mice [34].

Conclusions

Results show an important level of *C. spinosa* seed protein (ca. 27%) and high total lipid content (ca. 33%). Moreover, in this study we reported for the first time the aliphatic and triterpenic alcohol in the *C. spinosa* lipid unsaponifiable fraction. Results show that *C. spinosa* seed oil contains a good amount of these compound (ca. 440 mg kg^{-1}). Aliphatic alcohols were present at ca. 45 mg kg^{-1}, and octadecanol is the major compound. Triterpenic alcohols were present at ca. 396 mg kg^{-1}, and citrostadienol was the predominant compound. These compounds have an important role in the prevention of many diseases and can be used in many cosmetic and pharmaceutical products. This study confirms the nutritional and pharmaceutical importance of *Capparis spinosa* seeds.

Acknowledgments We are very grateful to the INRGREF for help with samples collection.

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