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Fatty acid and amino acid profiles in the fruits of *Prunus spinosa L. subsp. dasyphylla* (Schur) Domin from Europe-in-Turkey

Tamer Özcan* and Gülriz Bayçu

Istanbul Universiy, Faculty of Science Department of Biology- Division of Botany, Istanbul, Turkey

Abstract

Fatty acid and amino acid compositions were determined in the fruits of *Prunus spinosa* subsp. dasyphylla with GC and amino acid analyser. Total oil (0.22%) and total protein (1.35%) were quantified in lower levels. Major fatty acids were palmitic (34%), oleic (20.85%) and stearic acids (16.20%), respectively. Linoleic (6.07%), eicosatrioneic (3.17%), α-linolenic (1.95%), gamma-linolenic (1.76%), myristic (1.61%), arachidic (1.17%) and lauric acids (1.10%) exhibited the lower concentrations. The other fatty acids were examined below 1%. Total percentage of the saturated fatty acids were observed higher (56.56%) compare to the unsaturated fatty acids (35.68%). Total concentration of mono and poly-unsaturated fatty acids were 22.73% and 12.95% respectively. Valuable ratio of linoleic: α-linolenic acid (3.103) were detected with respect to dietary reference for fatty acid intake. Amino acid concentrations ranged from 356.7 (aspartic acid) to 47.6 (threonin) (mg/100 g dry wt.). The highest values among essential amino acids were obtained in leucin (122.6), isoleucine (99.2), valine (87.8) and phenylalanin (84.7). Tyrosin (51.3) and lysine (50.6) were quantified at the lowest levels. All essential amino acids were observed in sufficient levels compared to FAO reference values for life stage groups. Investigated traits in the fruits of *Prunus spinosa* subsp. dasyphylla may be useful in explaining the natural product potential and taxonomical variations based on different populations.

Keywords: *Prunus spinosa*, fruit, fatty acid, amino acid, natural product, taxonomy

*Correspondence Author:
Istanbul Univesity, Faculty of Science, Department of Biology - Division of Botany Istanbul - Turkey
E- mail: tameroz@istanbul.edu.tr

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Introduction

Prunus spinosa (Blackthorn or Sloe) is a species of *Prunus* native to Europe, western Asia covering Turkey, Iran, Caucasus and locally in northwest Africa. The species is locally naturalised in New Zealand and eastern North America. It is a deciduous large shrub or small tree growing to 5 m tall, with blackish bark and dense, stiff, spiny branches. The flowers are 1.5 cm diameter, with five slightly creamy-white petals; they are produced shortly before the leaves in early spring, and are hermaphroditic and insect-pollinated. The fruit is a drupe 10–12 mm diameter, black with a pale purple-blue waxy bloom, ripening in autumn; it is thin-fleshed, with a very strongly astringent flavour when fresh (Browicz, 1972; Rushforth,1999). *P. spinosa* is represented by subsp. *dasyphylla* (Schur) Domin in southern and the southeastern part of the distribution range. As a variable species in its morphological characters, it is well adapted to different habitats and reaching the elevations of 2200 m. *P. spinosa* subsp. *dasyphylla* doesn't occur in Mediterranean region or in the drier parts of east and south-east Anatolia. It has certain ecological preferences characterized by a wet climate and grows well in humid places of the western Black Sea, Thrace, Aegean region and central Anatolia (Dönmez and Yıldırımlı, 2000). A limited number of studies on the revision of species complex of genus *Prunus* based on morphological characteristics were published in Turkey (Boissier,1872; Browicz, 1996). An important revision of the genus *Prunus* was prepared by Browicz in the Flora of Turkey (1972). Taxonomical status of *Prunus* was also discussed recently, using chromosome numbers, palynological and anatomical results in addition to detailed morphological observations, and a new key for the identification of the

species has been prepared (Dönmez and Yıldırımlı, 2000). Polymorphism and the wide range of environmental adaptability make complicated the taxonomy of this genus The cpDNA polymorphisms made possible the analysis of genetic relationships among the *Prunus* accessions and most of the recovered relationships are in agreement with current taxonomic hypotheses and artificial crosses (Bouhadida *et al*., 2007). In 24 populations of *Prunus spinosa* sampled across Europe, combinations of all the polymorphisms resulted in 33 chloroplast DNA (cpDNA) haplotypes and two mitochondrial DNA (mtDNA) haplotypes (Mohanty *et al*., 2003). As a wild edible gathered for raw consumption or making juices and jams, the fruits are a good source of nutrients. and rich in antioxidants like vitamins C and E, carotenoids and flavonoids which have both nutritional and medicinal value (Kumarasamy *et al.,* 2004; 2007). *Prunus spinosa* was also used for food in times of scarcity (Luczaj and Szymanski, 2007). Among the recorded species, the fruits of *P. spinosa* subsp. *dasyphylla* known as "güvem" was reported ethnobotanically in the treatment of cardiac diseases as decoction in Europe-of-Turkey (Kültür, 2007). Apart from a limited number of studies (Dağlıoğlu and Atansay, 1998; Çalışır *et al.,* 2005; Marakoğlu *et al.,* 2005), there was not a detailed report on the nutritional contents of the fruits of this taxon having widespread distribution in Anatolia and Thrace. However, some organic acids and valuable vitamin contents compared to dietary reference intakes (FAO/DRIs) for life stage groups were examined in *Prunus spinosa* subsp. *dasyphllum* (Özcan, T. unpublished data). Amino acid and fatty acid composition in the fruits have also great importance from health and nutritional point of view. Essential amino acid compositions and the quality of proteins vary considerably among species (Boren *et al.,* 1995). Different amino acid profiles reflect the specific characteristics of a protein. Variation of protein amino acid profile from high taxonomic categories to cultivars in different plant groups were reported to have taxonomically intelligible patterns (Yeoh *et al.,* 1986; Amer and Sheded, 1998; Pedo *et al*., 1999; Cook *et al.,* 2002). Fatty acid profiles of the seed oils have also great taxonomic values in the plant kingdom (Gibbs, 1974) and has been frequently used as a tool in studies of some plant groups at different taxonomic levels (Vickery, 1971; Yaniv *et al*., 1991). Fatty acid and amino acid compositions were examined in the fruits of *P. spinosa* subsp. *dasyphylla* in order to explain the nutritional values as a wild vegetable genetic resources and populational characteristics providing additional taxonomic and genotypic data.

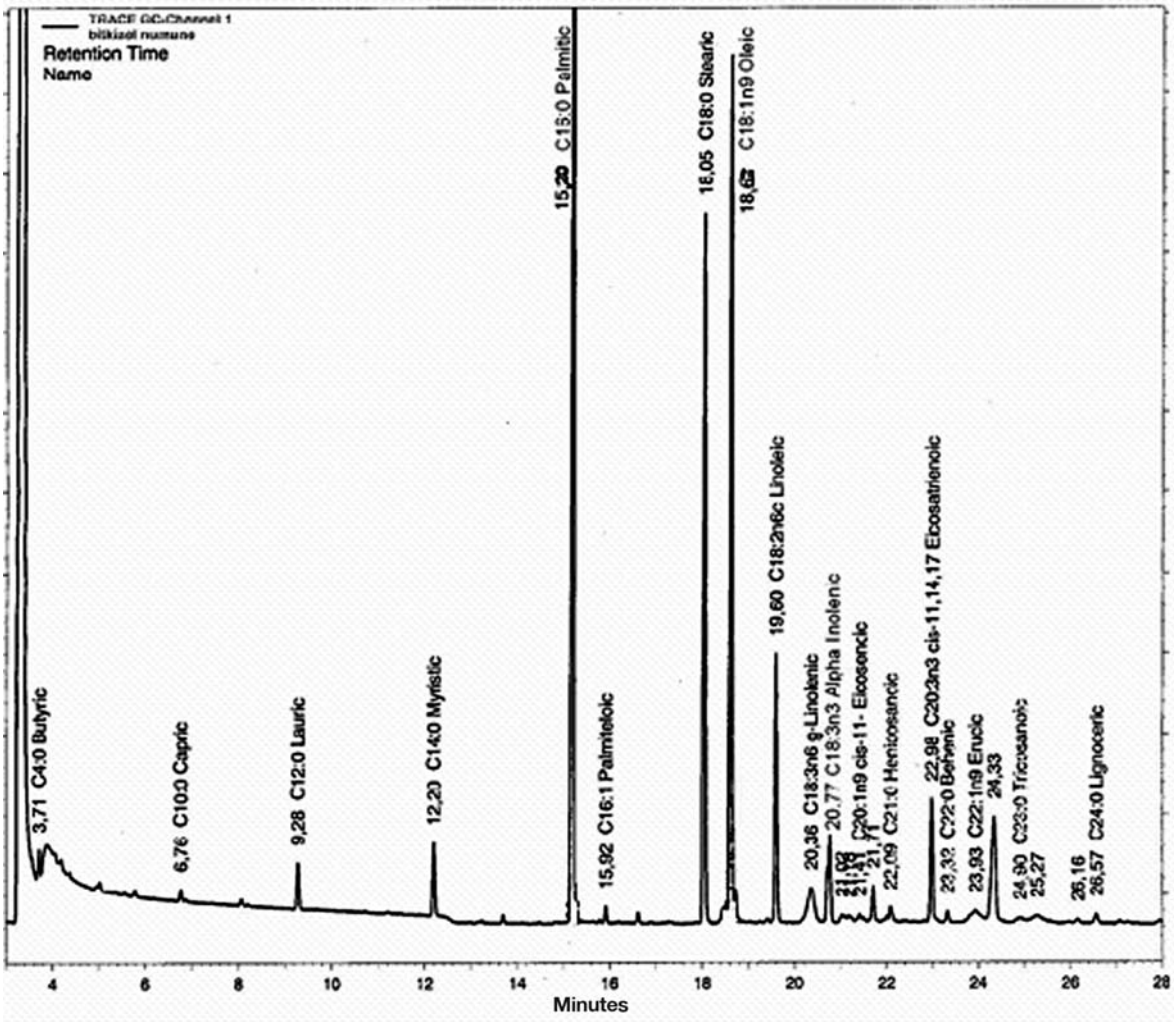
Materials and methods

Fruit specimens of *Prunus spinosa* L. subsp. *dasyphylla* (Schur) Domin were picked at random in the ripen stage from various parts of 20 individual trees of the native population distributed in the central region of Europe-in-Turkey. Collected specimens kept in cool, then they were transported to the laboratory in polypropylene bags and packed in glass vessels in the deep-freezer (-18 ^{o}C) until the analysis carried out. Total protein of the mesocarps removed from the seeds, were determined by Kjeldahl nitrogen method (920.152) according to the Association of Official Analytical Chemists (AOAC, 2000). The percentage of crude protein was estimated by multiplying the total nitrogen content by a factor of 5.30 (AOAC, 2000). The samples were hydrolysed at 110 ± 1°C with 6 N HCl for 24 h and amino acid composition of the samples were determined by Eppendorf LC 3000 amino acid analyser (EzChrom manual). Total oil content was detected with ''Tecator Soxtec System HT''. Powdered material (3 g) from each sample was added to oil in cartridge (W1) with 25–50 mL ether into a weighted extraction pot (W2). Extraction was carried out for 15 min with rinsing for 30–45 min. The extracted seed meals were air dried to remove traces of solvent and oven dried at 100 °C. The pots were cooled in a desiccator and weighed (W3). The following equation was used to calculate percentages of the oil: Oil % = ((W3-W2)/W1) x 100. The oil was transferred into glass sealed amber dark bottles, capped and stored at –18 ^{0}C until analyzed. Fatty acid methyl esters (FAMEs) were prepared from the fruit oil and determined by gas chromatography (GC) according to the method described by (Slover and Lanza, 1979) and (Alasalvar *et al.,* 2003). FAMEs prepared using BF_3 (20%) in methanol were extracted with *n*-hexane and analyzed by GC. Sample (1 co, Bellefonte, PA) coated with poly-(ethylene glycol). The column was connected to a Hewlett-Packard 5890 Series II (Little Falls, Willmington, DE) GC equipped with a flame-ionization detector. The oven temperature was programmed as follows: 180 °C for 2 min, increased to 200 °C at 2 °C/min, held at 200 °C for a further 10 min, and then increased to 215 °C at 2 °C/min and kept there for 10 min. The injector and detector temperatures were 200 and 250 °C, respectively. Helium as the carrier gas was used at a flow rate of 1.5 ml/min. Identification and quantification

of fatty acid methyl esters were accomplished by comparing the retention times of the peaks with authentic standards. All chemical reagents and standards were obtained from Sigma–Aldrich–Fluka Co. Ltd. Experimental results were presented in the tables and GC chromatogram.

Results

Low level of total protein content was detected in the fruit of *Prunus spinosa* subsp. *dasyphylla* (1.35 g/100g dry wt.). But, considerably high values of 13 amino acids were quantified. Amino acid concentrations ranged from 356.7 (mg/100 g dry wt.) for aspartic acid to 47.6 for threonin. While the lowest values in the examined amino acids belong to threonin, the highest ones were obtained from leucin (122.6) among essential amino acids. Isoleucine (99.2), valine (87.8) and phenylalanin (84.7) have also higher values. Tyrosin (51.3) and lysine (50.6) were quantified at the lowest levels. On the other hand, the highest concentrations of aspartic (356.7) and the lowest levels for serin (57.9) were observed among non-essential amino acids. Glutamic acid exhibited one of the highest values (197.9). The other non-essential amino acids showed mediate levels ranked as prolin (85.8), alanin (78.6) and glycine (70.9). Concentration profile of essential amino acids in the fruits of *Prunus spinosa* subsp. *dasyphllum* were compared to dietary reference intakes (FAO/DRIs) for life stage groups, and all essential amino acids were observed at sufficient levels compared to FAO reference values (Table 1). Very low level of total oil (0.22%) was examined in the



fruits. But, interesting composition of fatty acids were detected (Figure1). Percentages of the fatty acids and total oil content were documented in Table 2. The major fatty acids were palmitic (34%), oleic (20.85%) and stearic acids (16.20%) respectively. Linoleic (6.065 %), eicosatrioneic (3.173 %), alfa-linolenic (1.954%), gamma-linolenic (1.756%), myristic (1.607%), arachidic (1.171%) and lauric acids (1.104%) exhibited the lower levels. The other fatty acids were quantified below 1%. Highest total percentages were observed in the saturated fatty acids (56.562%), compared to the unsaturated fatty acids (35.676%). Total concentration of mono-, and poly-unsaturated fatty acids were 22.728% and 12.948%, respectively. Some ratios of the highest levels of saturated and unsaturated fatty acids were also calculated and the ratio was found as 1.585%. The concentration ratio of total saturated : poly unsaturated (4.368) and total saturated : mono unsaturated fatty acids (2.488) showed relatively higher values. Valuable proportion of linoleic : α-linolenic acid (3.103) were also detected with respect to the dietary reference for fatty acid intake.

Discussion

Turkey has large diversity in Drupaceae. Nutritive value of its wild fruits has great importance as alternative food and wild genetic resources in plant breeding or genetic engineering. China is the top producer of plum and sloe in 2005 with the output up to 4,635,600 tonnes (FAO, 2007). In the wild plant flora of Turkey, *Prunus spinosa* subsp. *dasyphylla* as a major constituent in some regions have large distributional area and the wide range of environmental adaptability such as calcareous soils, drought and resistance to cold. Wide variability in leaf, petiole and fruit morphology was reported in native wild forms which contain a significant genepool in Anatolia (Browicz, 1972;1996). Therefore, using many genotypes with higher product potential might contribute to its improvement efforts associated with nutritional value. The polymorphism and the wide ecological tolerance make difficult the taxonomy of this genus in the flora of Turkey includir polymorphisms resulted in chloroplast (cpDNA) and mitochondrial DNA (mtDNA) haplotypes account for the genetic relationships among the accessions in *Prunus spinosa* subsp. *dasyphylla* (Mohanty *et al*., 2003; Liu *et al.,* 2007). The fruit of *Prunus spinosa* subsp. *dasyphylla* contains large quantities of biologically active substances such as antioxidant vitamins, carotene, flavonoids, organic acids, medicinal oil, etc., as raw materials used in the pharmaceutical and food industries producing alcohol-free beverages and medicinal oil (Kumarasamy *et al*., 2004). *Prunus padus* and *Prunus spinosa* have the most potent antioxidant extracts (Kumarasamy *et al*., 2007). Investigated species may have a potential as a functional food which provide the body with the required amounts of vitamins, fats, protein, carbohydrates and many other compounds. Considerable amount of total protein and essential amino acids were examined in the fruits. Obtained results in this study for crude protein contents (1.35%) correspond with the result (1.6%) reported from central Anatolia population of wild prune (Çalışır *et al.,* 2005). However, relatively lower amount of protein was reported in plum from Turkey (0.52%) (Cemeroğlu and Acar, 1986). The crude protein content of many edible fruits is usually lower than 5%, and varies considerably. Considering that protein contents and amino acid profiles were determined ultimately by genotypic factors, compositional characteristics of essential amino acids in the fruits of this taxon may worthy investigation in widespread populations growing wide range of ecological conditions in order to determine high yielded genotypes and the optimums for cultivation. The global amino acid composition of a protein, although a cruder variable than sequence, is nevertheless informative and has been correlated with protein structural class (Ojasso and Dore, 1996). Variation of protein amino acid profile from high taxonomic categories to cultivars in different plant groups were reported to have taxonomically intelligible patterns as a diagnostical biochemical trait (Amer and Sheded, 1998; Pedo *et al.,* 1999). The differences in the amino acid concentrations may reflect protein qualities in the delimitation of plant taxa (Özcan, 2006). On the other hand, obtained values of essential amino acids in 100g dry wt. were also sufficient for the requirement of daily intake according to dietary reference intakes for life stage groups (FAO/DRIs). Compared to the plums (ESHA foods database- Food Processor for Windows, Version 7.60, by ESHA Research in Salem, Oregon, USA), *Prunus spinosa* subsp. *dasyphllum* in this study, exhibited considerably higher levels for all investigated essential amino acids. Such rich contents for these amino acids make it irreplaceable as a raw material for the production of pharmaceuticals and diet supplements. Particular focus is given to the lysine requirements of adults, since this indispensable amino acid is most likely to be limiting in the cereal-based diets

characteristic of populations in large areas of the developing world (Young and Pellett, 1990; Hoshiai,1995). Adequate level of lysine, according to FAO reference value for individual intake, were examined in the fruits of *Prunus spinosa* subsp. *dasyphylla*. A low lysine:arginine ratio in a protein was declared to show health-beneficial effect (Feldman, 2002). High lysine content was reported to be due to the recessive monogenic effect (Karlsson, 1972). Natural hybrids and the range of genotypes in this taxon should be considered for this trait. On the other hand, proline, glycine, arginine and lysine amino acids are accumulated in xerophytic species than mesophytic ones (Amer and Sheded, 1998). Proline (85.8%), glycine (70.9%) and lysine (50.6%) in *Prunus spinosa* subsp. *dasyphllum* as a mesophytic element exhibited relatively lower levels compared to other amino acids generally. For some amino acids, considerable literature exists from human and animal studies; in particular, glutamate, aspartate, and phenylalanine are well represented because of their use as food-flavoring agents (glutamate as monosodium gluatamate (MSG) and aspartate and phenylalanine in aspartame) and lysine for some treatment effects (Garlick, 2004). Aspartic acid and glutamic acid in the fruits of *Prunus spinosa* subsp. *dasyphllum* exhibited the highest quantities as alternative wild resource. Considerably higher level of phenylalanine compared to daily intake was obtained in this study. Concern for the safety of phenylalanine arises from the abnormal brain development known to occur in humans with phenylketonuria. But, in those with a normal ability to metabolize phenylalanine, this amino acid is relatively safe. Regarding taxon may be also evaluated as a favourable source for lysine. Investigated essential amino acids, the building blocks of all proteins in the body and some hormones are termed indispensable amino acids which must be provided in the diet. In this study, sufficient amount of essential amino acids expressing valuable nutritious potential of this product were obtained according to dietary reference intakes and some valuable ratios compared to requirements of life stage groups. Although no single plant would provide humans with adequate levels of all essential amino acids, these product can be prepared with other foods and contribute useful amounts of the amino acids to the diet. Besides distinctive morphological features, fatty acid profiles in the fruits may be valuable parameters in the delineation of *Prunus* at the infrageneric level in the absence of ecological factors, climatic and seasonal variations. Remarkable variation in linolenic acid contents were experienced in the different genotypes, cultivars and mutant lines in the seed samples of *Linum* species (Bickert *et al.,* 1994). Similarly, significant differences were found at section level for palmitic, stearic and oleic acid concentration, in addition to relative concentrations of saturated fatty acids compared to mono, poly and total unsaturated fatty acids in Turkish *Quercus* (Özcan, 2007). Many studies have also reported that phylogenetic relationships are associated with differences in the fatty acid profile of the seed oils (Hohn and Meinschein, 1976; Aitzetmüller, 1999; Velasco and Goffman,1999). Although very low level of fruit oil was determined, fatty acid composition exhibited characteristic pattern in this study. In broad sense, the percentage of major fatty acids, including palmitic, oleic and stearic acids in addition to total saturated and unsaturated fatty acids and their ratios in the fruits of *Prunus spinosa* subsp. *dasyphllum* may also be useful traits at specific, infraspecific and genotypic delimitation based on the populations. Fatty acid patterns were reported to reveal lower intraspecific variability and higher taxonomic resolution (Mayworm and Salatino, 2002). Thus, characteristic fatty acid profiles of each taxa may reflect different pathways involved in oil biosynthesis and accumulation. The relative percentages of fatty acids in this study may be more stable and determinative in order to understand taxonomic and phylogenetic relations of *Prunus*. On the other hand, it was reported that liquid vegetable oils with high concentrations of monounsaturated fatty acids (MUFAs) and polyunsaturated fats (PUFAs) of the n-6 series have a cholesterol-lowering effect and decrease LDL-C, but not HDL-C. Similarly, foods rich in long-chain n-3 PUFAs confer cardioprotective effects beyond their effect on the improvement of the lipoprotein profile. (Feldman, 2002). Suitable ratios between omega-3 and omega-6 fatty acids has great importance for the healthy metabolic activity. Valuable proportions of these fatty acids in the fruit oils of *Prunus spinosa* was obtained compared to the reference intakes of FAO. Relatively lower level in total oil (0.22%) was examined compared to the result from Anatolian population (1.10%) (Çalışır *et al*., 2005). Such differences may result from genotypic or ecological factors in addition to ripening stages of the fruits. The higher level of seed lipid contents in cold climate conditions and decreasing levels of seed oils correlated with dry seasons were reported for different plant groups, but fatty acid quantities exhibit constant profiles generally

(Mukherjee *et al*., 1984; Saffarzadeh *et al*., 1999). In plum, total fat was examined at the level of 0.41 gr (in 100 g dry wt.) and higher level of mono-(0.27gr) and poly-(0.09%) unsaturated fatty acids than saturated fatty acids in total (0.03%) were reported. Oleic (0.26gr) and linoleic acid (0.09gr) as omega 6 fatty acid were also major fatty acids (Cemeroğlu and Acar, 1986). Although, relatively higher level of saturated fatty acids in the fruits were examined in this study, considerably higher concentrations in oleic (20.85%) and linoleic acid (essential fatty acid-EFA) (6.06%) were also observed as relatively parallel compared to the results in plum. Similarly, the highest levels in the seed oils of *Prunus spinosa* from Turkey were reported for oleic (57.6%) and linoleic acids (33.5%), but lower level for palmitic acid (6.2%) (Özgül-Yücel, 2005). Parallel results for oleic and linoleic acid in the fruits and the seeds of *Prunus* may account for the common rootstock in phylogenetic relations of this genus. On the other hand, temperate variety oils are less saturated, perhaps due to a natural selection in northern latitudes for oils with a higher energy storage capacity or which remain liquid at a lower temperature (Deferne and Pate, 1996). It was reported that Turkish *Quercus* taxa having xerophytic nature mainly contained higher concentrations of saturated fatty acids (Özcan, 2007). Higher concentrations of saturated fatty acids in *Prunus spinosa* subsp. *dasyphylla* may be related with above arguments considering its global distribution in southern part of the range. Additionally, the fruit of this species was covered with conspicuous epicuticular wax secretions which contain predominantly $C_{12\text{-}36}$ saturated fatty acids in addition to other components such as triterpenoid, primary alchol, aldehydes etc. Epicuticular secretions, an essential structural element of the surface and of fundamental functional and ecological importance for the interaction between plants and their environment were widespread at different densities, ultrastructure and chemistry in different plant groups also provide taxonomical informations (Barthlott *et al.,* 1998). Higher level of palmitic (34%) and stearic acid (16.2%), in addition to myristic (1.6%) and lauric acid (1.1%) in studied species may be included in such secretion, apart from mesocarp tissue. Meanwhile, the sporadic occurrence of unusual fatty acids in seed lipids including gamma linolenic acid have great importance as genetical tools for elucidating the phylogenic relations (Aitzetmüller, 1995;1999). Gamma linolenic, stearidonic and erucic acids were also reported to be more variable unusual fatty acids in genera patterns and the utility of their relative concentrations as indicators of taxonomical relationships of Boraginaceae (Bağcı *et al.,* 2004). Apart from taxonomical importance of general fatty acid patterns, gamma linolenic acid (GLA) as an unusual fatty acids in plants is also valuable product for medicinal and industrial fields (Ugnarius, 1996). Although they were found at lower levels, gamma α-linolenic (18:3n6) and eicosatrienoic acid (20:3n3) as unusual long chain poly unsaturated fatty acids (PUFAs) may be evaluated in taxonomy of *Prunus spinosa* as additional parameters. Typical GC-based fatty acid profile of the fruit oil may also be useful in the characterization of this species according to different populations and genotypes. Relatively lower levels of linolenic acid (1.95%) was determined in the fruit oils of *Prunus spinosa* subsp. *dasyphylla*. Information on the nutritional value for the fruits of this species having widespread distributions in Anatolia will be of use in determining which genotypes should be preferentially utilized and conserved to benefit the overall nutrition of the rural populations especially. Detailed economic analysis of the fruit harvesting and processing in Turkish populations is also needed. Obtained chemometric data from the local population may be evaluated for natural product potential of the wild fruits in addition to taxonomical and eco-populational characteristics of *Prunus spinosa* subsp. *dasyphylla*

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