FATTY ACID COMPOSITION OF SEEDS OF THREE MALPIGHIA GLABRA L. GENOTYPES (MALPIGHIACEAE)

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Abstract - (Fatty acid composition of seeds of three *Malpighia glabra* L. genotypes (Malpighiaceae)). *Malpighia glabra* (acerola) is a plant native to Antilles and northern South America. Its significant commercial value is due to the high vitamin C content of the fruits. Brazil is the leading producer and the largest consumer market of *M. glabra* in the world. The present study determines total oil yield and fatty acid profile of seeds of three *M. glabra* genotypes. Total oil was extracted using *n*-hexane, and methyl ethers of fatty acids were analyzed by gas chromatography and mass spectroscopy. Total concentration of fixed oil in seeds varied between 63.33 ± 12.24 mg/g and 97.14 ± 31.47 mg/g dry matter. Palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1) and linoleic (C 18:2) were identified. Linoleic acid was the main component in all *M. glabra* individuals analyzed, independently of epicarp color. Unsaturated fatty acids were more abundant than saturated fatty acids. The data obtained may be useful to foster the use of the species, since the oils in its seeds may be better employed in food industry and other applications. The economically viable use of agribusiness waste is an important means to reduce environmental impact and open new alternatives to obtain products with greater added value, with environmental and economic benefits.

Key words: *Malpighia glabra*, acerola, seeds, total oils, fatty acids.

Resumo - (Composição de ácidos graxos das sementes de três genótipos de *Malpighia glabra* L. (Malpighiaceae)). *Malpighia glabra* (acerola) é uma planta originária das Antilhas e norte da América do Sul, bastante comercializada devido ao elevado teor de vitamina C dos seus frutos. O Brasil é o maior produtor, consumidor e exportador no mundo. O presente projeto tem como objetivo determinar o rendimento dos óleos totais e o perfil de ácidos graxos em sementes de três genótipos de *Malpighia glabra* (acerola). Os óleos totais foram extraídos com *n*-hexano e os ésteres metílicos de ácidos graxos foram analisados por CG/EM. A concentração total do óleo fixo das sementes variou de 63,33 ± 12,24 mg/g a 97,14 ± 31,47 mg/g de matéria seca. Os ácidos palmítico (C16: 0), esteárico (18:0), oleico (C18: 1) e linoleico (18:2) foram identificados. Todos os indivíduos da *Malpighia glabra* analisados apresentaram o ácido linoleico como majoritário, independente da cor do epicarpo. Os ácidos graxos insaturados foram mais abundantes do que os ácidos graxos saturados. Essas informações podem ser úteis para expandir a comercialização dessa espécie, pois indicam que o óleo das sementes dessa espécie pode ser mais bem aproveitado na indústria de alimentos e para outros fins. A valorização dos resíduos provenientes da agroindústria é uma boa alternativa para a redução do impacto ambiental bem como para obtenção de produtos com alto valor agregado, gerando benefícios ambientais e econômicos.

Palavras chave: *Malpighia glabra*, acerola, sementes, óleos totais, ácidos graxos.

Introdução

Malpighia glabra is a species native to Antilles and northern South America, popularly known as acerola, or Barbados' Cherry (UFRP 1985; Oliveira & Soares Filho 1998). The acerola fruit has considerable economic importance due to the high level of vitamin C it contains (Marino Neto 1986).

Brazil is the leading acerola producer, exporter and consumer market (Carvalho 2000). In Brazil, the area dedicated to acerola plantations exceeds 10.000 ha (Instituto Brasileiro de Geografia e Estatística, 2000). The Brazilian northeastern is the region with the largest crop area, mainly due to favorable climate and soil conditions (Paiva *et al.* 1999). The Japanese market is the largest importer of *M. glabra*, followed by the USA and European nations (Gonzaga Neto *et al.* 1999).

The agribusiness is responsible for most of the acerola production in the world. Acerola fruits are consumed *in natura* or as juices, preserves, jams, nutraceutical foods (pills) or food supplements, and as juice enrichment (Carpentieri-Pípolo *et al.* 2002).

Few reports have been published on the employment of other plant parts of this species. In general, the seeds are discarded in fruit processing (Dantas 1994). This poses serious environmental concerns, due to the large amount of acerola fruit waste being given this fate.

Recently, the reuse of agricultural byproducts has been the object of considerable scientific interest. Solís-Fuentes and Durán-de-Bazuá (2005) suggested that an alternative to solid waste management in agribusiness includes using byproducts as raw material in the extraction of final or intermediary commercially valuable components, such as vegetable oils.

In this sense, seeds represent important sources of lipids of considerable nutritional, industrial and pharmaceutical relevance. Triacylglycerols account for most constituents in vegetable oils. These compounds are important in seed germination, representing an important energy source. Most vegetable fatty acids belong to two groups, namely saturated fatty acids, and their unsaturated homologues. The predominant fatty acids in plant triacylglycerols are palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1), linoleic (C 18:2), and α-linolenic (C 18:3) (Bruneton 1993).

The specialized literature lists numerous reports on the chemical composition of pulp and juices of *M. glabra*, though little has been published on the reuse of their seeds. The present study analyzes total oil yield and fatty acid composition of seeds of three *M. glabra* genotypes obtained in the same cultivation area.

Materials and Methods

Malpighia glabra samples were obtained in the Sítio Sete Irmãos, a farm in the municipality of Indaiatuba, state of São Paulo (SP), Brazil (23º05"25'; 47º13"05'), distant approximately 32 km from the city of Campinas, SP. In the area, roughly 4,000 phenotypically variable acerola plants are grown. Based mainly on epicarp color, 14 *M. glabra* trees were pinpointed for fruit sampling, care taken to represent three different genotypes, as follows: genotype A (yellow epicarp, 3 individuals), genotype B (light red epicarp, 5 individuals), and genotype C (bright red epicarp, 6 individuals). In total, 30 fruit were collected from each tree, in the period between January and March 2002, considering the fructification season.

Pulp and seeds of the collected fruits were manually separated. For each tree, seeds were dried in a stove at 60ºC and then mashed. Of this dried seed mass, 1 g samples were retrieved and used in oil extraction procedures. Oils were extracted in Soxhlet using *n*-hexane for 6 h, in the dark. Extracts thus obtained were dried in a rotatory evaporator under low pressure at 40 °C. Then, extracts were transferred to previously weighed vials and stored in desiccators upon constant mass. Oil yields were calculated on a dry matter basis as mg/g.

Triglycerides were saponified in 10 mL of 10% KOH methanol solution under reflux for 2 h. Acidification with 10% HCL to pH 4.0 – 5.0 ensued. Four chloroform extraction procedures were conducted to remove fatty acids (Grunwald & Endless 1988). The extracts thus obtained were pooled and the remaining solvents removed under low pressure.

Methyl esters of fatty acids were obtained using a diazomethane ether solution and were concentrated to dryness under N_2 (Vogel 1971). The fatty acid methyl esters (1 µL in ether) were analysed by GC/MS performed with a HP 5890 ser. II Plus GC coupled to a HP 5989B MS. An Agilent Ultra 1 capillary column (25m x 0.32mm x 0.17 µm film thickness) coated with 100% dimethylpolysiloxane was used as the stationary phase. Helium was the carrier gas at 1.2 mL/min flow rate. The column temperature program was: 120^{o}C (1 min), a ramp of 10^{o}C/min to 280^{o}C, and then 280^{o}C isothermically (10 min). The injector temperature was 300^{o}C. MS were taken at 70 eV, source temperature 200ºC, quadrupole temperature 100ºC. Fatty acids were characterized by comparing retention times with standards, and mass spectra were matched against the HP Wiley 275 library.

Results and Discussion

Tab. 1 presents total oil yield and fatty acid composition of the three *M. glabra* genotypes sampled in the present study. Fixed oil yields ranged between 63.33 ± 12.24 mg/g (genotype C, accounting for nearly 6% of dry matter content), and 97.14 ± 31.47 mg/g (genotype A, nearly 10%). The values obtained are considerably higher than previous data for acerola seeds, of 39.2 mg/g (Aguiar *et al.* 2010).

The seed oil of *M. glabra* was shown to contain palmitic (C 16:0), stearic (C 18:0), oleic (C 18:1) and linoleic (C 18:2) acids. Except for stearic acid, the other components are the most common lipid constituents in seeds. These fatty acids are present in several widely traded crops, such as *Arachis hypogaea* (peanut), *Helianthus annuus* (sunflower), *Theobroma cacao* (cocoa), *Glycine max* (soy), and *Zea mays* (maize) (Bruneton 1993).

In the present work, the main fatty acid differs from data previously reported. Our data show linolenic acid, followed by oleic acid (genotype B) or palmitic acid (genotypes A and C). Aguiar *et al.* (2010) reported oleic acid as the major component, followed by linolenic and palmitic acids.

Qualitative variation in fatty acid profile of seeds has already been pointed out among cultivars of several species (Velasco *et al.* 1998, Ishikawa *et al.* 2001, Orhan *et al.* 2002). These differences may be related to genetic variability, geographic localization, environmental or cultivated conditions of growth (Silva *et al.* 2001, Abdala *et al.* 2002). Since the seed samples used by Aguiar *et al.* (2010) were obtained at Bragantina (state of Pará – North of Brazil), very far from Indaiatuba (state of São Paulo – Southest of Brazil), where the sample of the present study were harvested, geographic variation could account for the distinct profiles.

Tab. 1. Total oil yield (mg/g dry matter), fatty acids composition (relative percentage) and ratio of saturated to unsaturated fatty acids of three *Malpighia glabra* genotypes.

Genotypes (number of individuals)	Yield (mg/g)	Fatty acid composition				
		Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	S/I
A (n = 3)	83.33 ± 32.14	21	18	14	47	0.63
		34	7	18	41	0.69
		24	8	21	47	0.47
B (n = 5)	97.14 ± 31.47	14	15	34	37	0.41
		16	15	32	37	0.45
		23	13	26	38	0.56
		24	13	26	37	0.43
		26	12	29	33	0.61
C (n = 6)	63.33 ± 12.24	24	15	10	51	0.63
		28	11	16	45	0.63
		34	7	5	54	0.69
		25	15	19	41	0.66
		27	12	13	48	0.63
		23	11	22	44	0.51

In the present study, linoleic acid was the main constituent in all *M. glabra* individuals analyzed, independently of epicarp color. Linoleic acid is classified as an essential fatty acid, since it is not biosynthesized by animals (including humans), being therefore considered an important nutrient in human diet. Linoleic acid acts as a precursor of some hormones, including the prostaglandins involved in blood pressure regulation and smooth muscle contraction (Engler *et al.* 1998). This fatty acid is important in several physiological processes in the prevention and treatment of cardiovascular disorders, atherosclerosis, thrombosis, hypertriglyceridemia, hypertension, diabetes, arthritis, several inflammatory conditions, and cancer (Uauy & Valenzuela 2000), as well as degenerative chronic diseases (Simopoulos 2000). Linoleic acid also plays a role in retina and brain functions (Bushman *et al.* 2004).

Although high levels of linoleic acid have been found in the seed oil of *M. glabra*, the high content of saturated fatty acids, consisting of palmitic and stearic acids, decreases its nutritional advantages. However, this oil could be useful for cosmetics formulations, biodiesel production, soap manufacture and other uses (Ferrari *et al.* 2004).

The quantitative and qualitative characterization of the oil extracted from acerola seeds may render more valuable the waste generated in the processing of this fruit for commercial concerns. The data obtained herein suggest that *M. glabra* should be employed as a byproduct.

The specialized literature reports on the several options as to using fruit processing byproducts as a commercial and environmental alternative to disposal. Vegetable oils, mainly those containing essential fatty acids as main constituents, are currently considered interesting raw materials for the production of fowl and swine feeds. These ingredients may increase the energy level of feeds, improving palatability and increasing liposoluble vitamin uptake (Pupa 2004). Also, Lousada Junior *et al.* (2005) analyzed acerola waste as feed supplement to ovine livestock. The aim was to verify the possibility to improve husbandry nutritional aspects related to low productivity, mainly during draughts typical of the climate in northeastern Brazil. The authors observed that this waste represents an important source of calcium, phosphor and non-fibrous carbohydrates — apart from the high levels of dry matter and protein — which meet the elementary requirements for good ruminant function.

Yet another application for agribusiness waste lies in the conversion of fatty acids obtained from vegetable waste into flavor enhancers. Almosnino & Belin (1991) described the biotransformation of linoleic acid obtained from apple waste into alcohols and aldehydes used in the cosmetic industry.

The present study shows that the oil extracted from *M. glabra* seeds is a good source of linoleic acid and may be useful to widen the spectrum of applications of this species. Increasing the value of agribusiness waste is a good alternative in mitigating environmental impacts and in obtaining added value products, side by side with economic and environmental benefits.

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