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Effect of the extraction and bleaching processes on jojoba (*Simmondsia chinensis*) wax quality

The aim of the work was to evaluate the effect of extraction method and bleaching on the quality of jojoba waxes from Argentina. Jojoba waxes obtained by cold pressing, Golden wax (expeller-pressed wax) and Lite wax (bleached wax) were analyzed using standard methods adopted as recommended practices by the American Oil Chemists Society. Physical parameters, fatty acid and alcohol compositions were unchanged among waxes. Cold-pressed wax was noteworthy by its lower peroxide value, higher amounts of tocopherol and total phenol contents. Accordingly, it presented the best oxidative stability. Bleaching caused a strong decrease in both tocopherol and phenol contents; consequently the bleached wax showed poor oxidative stability.

Keywords: Jojoba waxes, extractive methods, refining.

1 Introduction

Jojoba (*Simmondsia chinensis*) is an evergreen shrub that is native to northern México and the Southwestern United States. In Argentina, the commercial production of jojoba is a comparatively recent agricultural activity, especially in semiarid regions of La Rioja and Catamarca provinces that are considered marginal areas for conventional crops.

The jojoba plant has economic value because 50% of its seeds are composed of a light yellow, odorless wax ester commonly referred to as jojoba oil. It is a narrow mixture of straight-chain esters, composed mainly of monounsaturated C_{18}, C_{20}, C_{22} and C_{24} acids and alcohols, and particularly two ester molecules containing 40 and 42 carbon atoms which make up to 80% of the oil. Interest in jojoba oil, stems from its unusual properties which differ from all other known seed oils. It is extensively used in the cosmetic industry for its dermatological properties. Other uses include pharmaceuticals, lubricants, foam control agents, plasticizers and foods [1-4].

As is the case for other crops, the jojoba industry faces the challenge of finding ways to improve the productivity and the quality of its products. A good quality jojoba wax is usually defined as having minimal non-ester components and oxidation products, but antioxidants such as tocopherols are desirable.

Different methods similar to those applied to other oilseeds have been used for extraction of jojoba oil [1, 5, 6]. Jojoba seed wax is usually extracted by a pressure system (expeller press). The quality of oils produced from the pressing operation is important. For instance, direct use of a screw-press for pressing the seeds may alter the quality of waxes because of the rise in barrel temperature during the procedure. Jojoba oil does not usually require de-gumming, neutralizing and deodorizing steps. However, a bleaching process is sometimes needed to remove undesirable substances such as pigments, as well as traces of heavy metals and autoxidation products. Although the bleaching step is applied to improve some aspects of jojoba oil quality, in oils other than jojoba it has been observed that bleaching may decrease tocopherol levels [7, 8].

The work presented here, examines the influence of extraction method and bleaching on the quality of jojoba waxes from Argentina.

2 Materials and methods

2.1 Plant material and experimental design

Jojoba seeds were collected from commercial plantations at Aimogasta, La Rioja province, Argentina. Three samples (5 kg each) were picked by hand at optimal stage of ripeness. After homogenization and cleaning, each sample was divided into two portions. One portion was ex-

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tracted using a manually operated hydraulic press (cold-pressed wax), and the other by a screw-press (Golden wax). Seeds contained about 4% moisture. The following experimental procedures were carried out:

a) Cold-pressed wax: a manually operated pilot-plant hydraulic press with variable load was used. For each run, 100 g of whole seeds were placed in a cylindrical container and subjected to the press load (600 kg/cm^2). Barrel temperature during the run (23 °C) was measured by a thermocouple inserted into the barrel wall near the discharge terminus.

b) Golden wax: an industrial screw-press (expeller press) was used. It was operated at 15 rpm. Barrel temperature during the run (60 °C ± 5 °C) was measured as above.

c) Lite wax (bleached wax): In this procedure, jojoba wax obtained from expeller press, as previously described, was placed in a Büchner flask attached to a vacuum line and heated at 110 °C. Subsequently, 3% of an acid-activated bleaching clay (Tonsil Supreme 167) was added and vacuum applied (30 hPa). The mixture was stirred vigorously for 35 min, and the clear, bleached wax decanted.

2.2 Wax analyses

Physical and chemical analyses and oxidative stability of waxes were determined according to standard methods of the AOCS [9]. Total phenol content was quantified by the method of *Cliffe* et al. [10].

Fatty acids and alcohols were analyzed by gas chromatography (GC). Briefly, waxes were subjected to alkaline saponification (1 N potassium hydroxide in methanol) and unsaponifiable matter extracted with n-hexane. Fatty acid methyl esters (FAMEs) were obtained using 1 N sulphuric acid in methanol and analyzed by GC according to Maestri and Guzmán [11]. Unsaponifiable material was fractionated on preparative thin-layer chromatography (TLC, silica gel 60 G, 0.5 mm) and developed with n-hexane: diethyl ether (50:50, v/v). After developing, the alcohol fraction was removed from the plate with chloroform and further purified by repeated preparative silica gel TLC for subsequent GC analysis. Identification of fatty acids and alcohols was carried out by GC-mass spectrometry (MS) [12, 13] and by comparison of retention times with those of reference compounds.

2.3 Statistical analyses

Statistical differences among jojoba waxes were estimated with the ANOVA test at the 5% level (P =0.05) of significance for all parameters evaluated. Whenever ANOVA indicated significant difference, a pair-wise comparison of means by least significant difference (LSD) was carried out [14].

3 Results

Physical and chemical parameters of jojoba waxes obtained with the tested methods (cold pressing, expeller press, and bleached wax) are shown in Tab. 1. No significant differences were found in boiling point, density, refraction index, viscosity, and unsaponifiable matter content.

The acid value of all analyzed samples was below 1, and fell within the value accepted by the International Jojoba Export Council. Waxes from expeller press had the greatest acid values (0.88 mg KOH/g), whereas the cold-pressed wax had the lowest (0.59 mg KOH/g). Peroxide values differed largely between waxes (0.39-5.19 meq O_2/kg), the lowest being obtained from cold-pressed wax.

The notable stability of jojoba wax has been attributed to its unusual composition [1], but it could be affected by the presence of natural antioxidants. Jojoba seed contains tocopherols and phenolic compounds with antioxidant activity, some of which are extracted together with the oil. Tocopherol contents and isomer distribution were consis-

Tab. 1. Physical and chemical characteristics of cold-pressed wax, expeller-pressed wax (Golden wax) and bleached wax (Lite wax) from jojoba seeds. Mean values ± standard deviations (n = 3). Means followed by the same letter within each row, are not significantly different at P = 0.05.

Parameters	Cold-pressed wax	Golden wax	Lite wax
Boiling point [°C]	$386.5^a \pm 0.5$	$386.5^a \pm 0.5$	$386.5^a \pm 0.5$
Density [g/ml] (25 °C)	$0.858^a \pm 0.001$	$0.857^a \pm 0.002$	$0.857^a \pm 0.001$
Refraction index (25 °C)	$1.447^a \pm 0.001$	$1.449^a \pm 0.008$	$1.447^a \pm 0.004$
Viscosity [cp] (25 °C)	$34.17^a \pm 0.04$	$35.02^a \pm 0.2$	$34.96^a \pm 0.2$
Acid value [mg KOH/g]	$0.59^a \pm 0.02$	$0.88^b \pm 0.04$	$0.82^b \pm 0.02$
Peroxide index [meq O_2/kg]	$0.39^a \pm 0.004$	$5.19^b \pm 0.004$	$4.79^c \pm 0.005$
Saponification index [mg KOH/g]	$101.25^a \pm 0.17$	$97.62^b \pm 0.05$	$99.05^b \pm 0.09$
Unsaponifiable matter [%]	$44.60^a \pm 1.04$	$44.40^a \pm 0.48$	$42.38^a \pm 1.12$

Tab. 2. Tocopherol and phenol contents of cold-pressed wax, expeller-pressed wax (Golden wax) and bleached wax (Lite wax) from jojoba seeds. Mean values ± standard deviations (n = 3). Means followed by the same letter within each row are not significantly different at P = 0.05.

Parameters	Cold-pressed wax	Golden wax	Lite wax
α-Tocopherol [ppm]	$22.7^a \pm 0.04$	$20.3^b \pm 0.03$	$6.41^c \pm 0.03$
γ-Tocopherol [ppm]	$40.6^a \pm 0.10$	$36.1^b \pm 0.09$	$14.4^c \pm 0.07$
Total tocopherols [ppm]	$63.30^a \pm 0.6$	$56.40^b \pm 0.4$	$20.81^c \pm 0.5$
Total phenols [ppm galic acid]	$11.69^a \pm 0.63$	$7.80^b \pm 0.03$	$1.13^c \pm 0.01$

tent with the literature [1, 15]. γ-Tocopherol was the predominant tocopherol in all waxes, with a smaller amount of α-tocopherol (Tab. 2).

Total tocopherol content varied significantly among wax types (Tab. 2). There is no published literature regarding the effect of extraction conditions on jojoba wax tocopherol content. But *Prior* et al. [7] have found that a small increase in tocopherol content could be observed with an increase in extraction temperature. In the present work, tocopherol content was higher in cold-pressed wax (63.3 ppm). Extraction of waxes by an expeller-type press caused a reduction in tocopherols. Tocopherols are heat sensitive and may have been destroyed during pressing at temperatures near 60 °C or higher.

Bleaching resulted in the greatest loss of tocopherol content. Works reported by *Prior* et al. [7] and *Akoh* [16] with other seed oils show similar results. One explanation is the selective absorption of these non-ester components on the bleaching clay.

The amounts of total phenolic compounds in the waxes differed widely between extraction methods (Tab. 2). Cold-pressed wax displayed the highest values. Lower total phenol levels in wax extracted with the expeller press could be due to an oxidative process that turns phenols into quinones [17]. As with tocopherols, bleaching caused a strong decrease in total phenol content.

The differences in natural antioxidant content affected the oxidative stability of waxes (Fig. 1). During oxidation at 105 °C, the cold-pressed wax was found to be significantly reduced in peroxide values: its induction period (considered as the number of hours needed for the peroxide value of the sample to reach 20 meq O_2/kg oil) was much longer than that of the other waxes. The bleached wax showed the fastest increase in peroxide value and reached induction period at 24 h. Increased stability of the cold-pressed wax, in addition to its tocopherol and phenol contents, could have been due to reduced initial acidity and peroxide values.

Analyses of fatty acid composition are shown in Tab. 3. Nine fatty acids were identified. Jojoba waxes were found to be highly unsaturated, exceptionally rich in 11-*cis*-eicosenoic acid (C20:1). Mean values of fatty acids differed slightly depending on the method employed to obtain the wax, although the differences were not statistically significant in most cases. All types of wax studied had similar alcohol compositions: *ca.* 0.7% 9-*cis*-octadecenol, 41% 11-*cis*-eicosenol, 48% 13-*cis*-docosenol and 9% 15-*cis*-tetracosenol (Tab. 4). Thus, the extraction process would not be expected to influence the fatty acid and alcohol composition of jojoba wax.

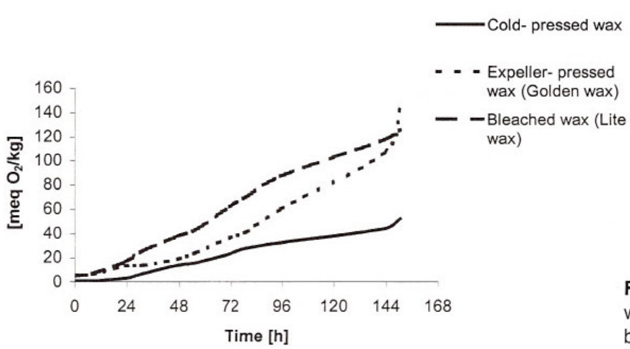

Fig. 1. Oxidative stability (105 °C) of cold-pressed wax, expeller-pressed wax (Golden wax) and bleached wax (Lite wax) from jojoba seeds.

Tab. 3. Fatty acid composition of cold-pressed wax, expeller-pressed wax (Golden wax) and bleached wax (Lite wax) from jojoba seeds. Mean values ± standard deviations (n = 3). Means followed by the same letter within each row are not significantly different at P = 0.05. Tr, trace <0.1%.

Fatty acids	Cold-pressed wax	Golden wax	Lite wax
		[% total fatty acids]	
9*cis*-Hexadecenoic	Tr	Tr	Tr
Hexadecanoic	$1.14^a \pm 0.03$	$0.91^b \pm 0.07$	$0.85^b \pm 0.05$
9*cis*-Octadecenoic	$9.28^a \pm 0.23$	$8.92^a \pm 0.30$	$8.40^a \pm 0.14$
Octadecanoic	Tr	Tr	Tr
11*cis*-Eicosenoic	$75.00^a \pm 0.70$	$75.70^a \pm 0.90$	$76.60^a \pm 0.90$
Eicosanoic	Tr	Tr	Tr
13*cis*-Docosenoic	$13.42^a \pm 0.25$	$13.45^a \pm 0.40$	$12.99^a \pm 0.30$
Docosanoic	Tr	Tr	Tr
15*cis*-Tetracosenoic	$1.16^a \pm 0.04$	$0.99^a \pm 0.23$	$1.11^a \pm 0.02$

Tab. 4. Fatty alcohol composition of cold-pressed wax, expeller-pressed wax (Golden wax) and bleached wax (Lite wax) from jojoba seeds. Mean values ± standard deviations (n = 3). Means followed by the same letter within each row are not significantly different at P = 0.05. Tr, trace <0.1%.

Fatty alcohols	Cold-pressed wax	Golden wax	Lite wax
		[% total fatty acids]	
Hexadecanol	Tr	Tr	Tr
9*cis*-Octadecenol	$0.71^a \pm 0.11$	$0.69^a \pm 0.09$	$0.79^a \pm 0.03$
Octadecanol	Tr	Tr	Tr
11*cis*-Eicosenol	$41.85^a \pm 0.38$	$41.61^a \pm 0.48$	$41.30^a \pm 0.29$
Eicosanol	Tr	Tr	Tr
13*cis*-Docosenol	$48.40^a \pm 0.23$	$48.33^a \pm 0.22$	$48.47^a \pm 0.11$
Docosanol	Tr	Tr	Tr
15*cis*-Tetracosenol	$9.02^a \pm 0.04$	$9.36^a \pm 0.17$	$9.41^a \pm 0.18$

4 Discussion

All physical characteristics and most of the chemical parameters evaluated were not affected by the conditions employed to obtain the jojoba wax. However, the extraction method (cold pressing and screw pressing) affected the acidity and peroxide values, the tocopherol and total phenol contents, and the oxidative stability of jojoba wax.

Expeller-pressed wax presented the highest values for both acid and peroxide indices, whereas tocopherol and total phenol contents were higher in cold-pressed wax. Accordingly, the latter presented the best oxidative stability.

It is known that extraction method and refining process have a strong effect on the composition and oxidative stability of oils [6-8, 18]. For example, crude soybean oil is less stable during processing, with the order of stability being crude > degummed > alkali refined > bleached oil. Other studies have also shown that crude oils are more stable than processed oils [8, 15, 19]. The stability of jojoba oil is determined by its composition but is dependent on the presence of minor components including tocopherols, phenolic compounds and metal ions, which are partially removed during processing. Bleaching removes pigments including chlorophylls and carotenoids, but the hydroperoxide level may also be reduced at this stage [5]. The acid-activated bleaching clay used in this work was an effective absorber of color bodies. However, it was less effective at removing peroxides. Tocopherols and phenolic compounds, which act as natural antioxidants, were starkly decreased by the bleaching step with total losses of 63% and 85%, respectively, compared to values obtained with Golden wax. These losses dramatically affected the oxidative stability of jojoba wax. The degree of reduction in tocopherols and phenolic compounds by bleaching was consistent with results reported for other oils [7, 8].

To summarize, results of the investigation presented and other related [1, 15] works, show that to obtain high quality jojoba wax, cold-pressing extraction is to be recommended. Extraction conditions (mainly temperature) and the bleaching process exert a considerable effect on the content of antioxidant components and, consequently, on the oxidative stability of jojoba wax.

Acknowledgements

These studies are cooperative investigations between Instituto de Ciencia y Tecnología de los Alimentos (ICTA) and Centro Regional de Investigaciones Científicas y Transferencia Tecnológica La Rioja (CRILAR). Financial support for this work was provided by CONICET, SECYT-UNC and *Agencia Córdoba Ciencia*. The authors express their gratitude *to Agrinsa Agroindustrial* S.A. for samples of jojoba seeds.

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[Received: May 16, 2003; accepted: August 26, 2003]

