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Chemical Quality Evaluation of Damaged Jojoba Seeds (*Simmondsia chinensis*)

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Abstract The objective of the research was to characterize the quality of damaged and undamaged jojoba seeds. The study was performed on jojoba seeds grown in La Rioja, Argentina. Proximal composition, fatty acid composition, acid value, peroxide value, conjugated dienes and trienes and protein electrophoresis profiles were determined in undamaged (JS) and damaged jojoba seeds (DJS). The fat content (wax) was lower in DJS (39.11%) than in JS (50.82%). The values of acid, peroxide, conjugated dienes and trienes were higher in DJS than in JS. No difference in fatty acid composition was observed between DJS and JS. The protein content was not significantly different between JS and DJS. However, DJS had lower soluble protein values. In the electrophoresis profiles, the band located at 50 kDa disappeared in DJS and the intensity of the band located at 25 kDa decreased. The deterioration process in jojoba kernels significantly affects the chemical quality of their proteins and waxes.

Keywords Jojoba · *Simmondsia chinensis* · Wax · Oxidation · Protein

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Introduction

Jojoba, *Simmondsia chinensis*, which originated from the desert regions of Mexico and the USA, is cultivated in various arid and semi arid areas [1]. Commercial production of jojoba in Argentina is favored in the semi arid regions of the provinces of La Rioja and Catamarca. There are about 720 ha of jojoba plantations in La Rioja. Argentina is a mayor exporter of jojoba wax.

Jojoba wax is extracted by pressing the seeds several times. About 870 tonne of wax and 1,300 tonne of expeller, a material remaining after expeller pressing of wax, are generated annually in Argentina. Jojoba wax has various applications. It has traditionally been used for cosmetic products. Currently, this wax is also used as a lubricant additive [1, 2]. The International Jojoba Export Council [2] defined several universal quality standards for jojoba wax. Wax quality is affected by several factors related to the extraction process and/or storage conditions [1]. The main degradation reactions are hydrolysis, chemical or enzymatic oxidation, polymerization and decomposition.

Flo et al. [3] reported information about methods for the elimination of appetite suppressant compounds (simmondsin) found in residual cakes and jojoba seeds. Jojoba residues without appetite suppressant compounds constitute a good raw material for animal feed.

Due to the fact that these seeds have a high percentage of wax and proteins, they represent valuable raw material for various industries such as the jojoba wax producer and the animal food manufacturer, respectively [3, 4]. For these industries, good quality of these raw materials is a fundamental goal. Therefore, physical and chemical integrity of jojoba seeds will affect the quality of jojoba wax and expeller. About 7% damaged jojoba seeds are detected annually in Argentina. The chemical quality parameters of

damaged jojoba seeds were unknown. Because of this, the objective of the present research was to characterize the quality of damaged jojoba seeds in comparison with undamaged seeds from La Rioja, Argentina.

Materials and Methods

Plant Material

Seeds were obtained from Bañado de los Pantanos, located at 67°14′W longitude, 28°36′S latitude and 865 m above sea level near to Aimogasta in La Rioja province, Argentina. Bañado de los Pantanos is an arid zone with 70 mm average annual rainfalls. The medium, maximum and minimum annual temperatures are 20 °C, 46 °C (registered in January), and −4 °C (registered in July), respectively. Bañado de los Pantanos has a period of 240 days without frost. Winds blow from the south and southeast up to 80–90 km/h.

The jojoba seeds were collected from the field for Agrinsa Agroindustrial S. A., Bañado de los Pantanos, La Rioja, Argentina in 2005. Five kilograms of the collected seeds were separated into two categories: damaged (DJS) and undamaged (JS) jojoba seeds. Those rotten and partially decomposed seeds and/or those seeds with internal dark color were considered DJS and the other ones were classified as JS.

Chemical Analyses

After separation, samples of 100 g of seeds were ground until a material of uniform consistence was obtained. This material was used for the chemical analyses. Three samples from DJS and JS were examined for moisture, lipid, protein and ash. The seeds from each sample were selected at random. The moisture content was determined by method 27.005 [5]. The jojoba seeds were milled and oil was extracted for 16 h with petroleum ether (boiling range 30–60 °C) in a Soxhlet apparatus. The lipid percentage was determined by weight difference. Ash and nitrogen contents were determined according to AOAC methods 27.009 and 27.007, respectively [5]. Ash was obtained by incineration in a muffle furnace at 525 °C. The nitrogen content was estimated according to the Kjeldahl method and converted to protein percentage by using the conversion factor 6.25. The nitrogen-free extract (NFE) was quantified by difference using the following formula: NFE = 100 (% moisture + % ashes + % lipids + % proteins). NFE contained different kinds of carbohydrates such as sugars, fibers and starches.

Acid value (AV) and peroxide value (PV) analyses were performed according to AOAC methods 16.211 and 28.022, respectively [5]. AV and PV were expressed as mg potassium hydroxide per gram (KOH/g) and milliequivalents of active oxygen per kilogram of wax (mequiv O_2/kg), respectively. Conjugated dienes and trienes (CD and CT) were determined by dissolving 0.02 g of oil in 6 mL of *n*-hexane. The conjugated diene and triene absorbances were measured at 232 and 268 nm, respectively, in a spectrophotometer (UV–V Diode Array Spectrophotometer Hewlett Packard HP 8452 A, Palo Alto, CA, USA), using *n*-hexane as the blank. The results were reported as the sample extinction coefficient E (1%, 1 cm) [6].

Fatty Acid Composition

Fatty acid methyl esters were prepared with the jojoba wax by transmethylation with a 3% solution of sulfuric acid in methanol. The fatty acid methyl esters of total lipids were analyzed on a Hewlett Packard HP-6890 gas–liquid chromatograph (Palo Alto, CA, USA) equipped with a flame ionization detector. An HP-INNO-Wax capillary column (30 m × 0.32 mm × 0.5 nm, with 100% polar polyethylene glycol as stationary phases, Palo Alto, CA, USA) was used. Column temperature was programmed from 200 °C (held for 1 min) to 230 °C (20 °C min^{-1}). The injector temperature was 260 °C. The carrier gas (nitrogen) had a flow rate of 3.8 mL min^{-1}. The separated fatty acid methyl esters were identified by comparing their retention times with those of reference samples purchased from the Sigma Chemical Co., and quantitative fatty acids analysis was performed using an internal standard [7].

Extraction of Soluble Proteins

Sequential extraction of soluble proteins was performed using distilled water at pH 6.6 for albumin extraction. Globulins were obtained with a solution of 0.5 M sodium chloride at pH 7. Prolamines were extracted with 70% ethanol and glutelines were dissolved in borate buffer (0.02 M sodium borate and 0.2 M NaOH) at pH 10. In all cases, the defatted flour/solvent ratio was 1:20 w/v. The extraction time was 2 h (25 °C); the samples were shaken every 10 min. Afterward, they were centrifuged (13,000*g*, 25 °C, 10 min) and the supernatants were analyzed for their protein content using the Lowry method [8]. Solubility was expressed as mg per gram of total protein.

SDS-PAGE

For the extraction of total soluble proteins, the samples were suspended in 0.2 M phosphate buffer, at pH 7.4. Later

the samples were mixed with cold acetone (ratio 1:5), incubated at −20 °C for 2 h, and centrifuged at 14,000*g* for 20 min [9]. The pellet (0.004 g) was resuspended in Tris–HCl buffer (100 μL), pH 6.8, 80 mM, containing 2% SDS (w/v) and 0.1 M β-mercaptoethanol and then boiled for 2 min. Then, aliquots (40 μL) of protein samples were loaded into each lane. The electrophoretic conditions were the following: SDS-PAGE gel containing 10% (w/v) acrylamide, Tris–glycine at pH 8.3 was the running buffer and current of 20 mA [10]. The electrophoresis was carried out with Minislab equipment (Model 28575-00, San Francisco, CA, USA). The proteins were fixed with 10% (w/v) trichloroacetic acid and stained with Coomassie Brillant Blue G-250. For the purpose of comparison, protein with known molecular weight (molecular weigh markers: WM), Bio Rad, Prestained SDS-PAGE Standards, Broad Range (Catalog # 161-0318, Hercules, CA, USA) were analyzed under identical electrophoresis conditions. The molecular weights (kDa) were the following: 5.73 (aprotinin), 18.53 (lysozyme), 28.49 (soybean trypsin inhibitor), 35.96 (carbonic anhydrase), 52.98 (egg albumin), 96.37 (bovine serum albumin), 111 (β-galactosidase) and 198 (myosin).

Densitometry Analysis

The electropherograms were scanned using an HP PSC 1410 densitometer and analyzed using the Scion software (Scion Image for Windows, 2000–2001 Scion Corporation, alpha 4.0.3.2). Presence, molecular weight and protein band intensity were compared.

Statistical Analysis

All chemical analyses were performed in triplicate. The data were analyzed using the InfoStat software, version 2006p.2 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba). Means and standard deviations were calculated. The analysis of variance ($\alpha = 0.05$) and the LSD test were performed to find significant differences between means.

Results and Discussion

Chemical analysis from DJS and JS are presented in Table 1. The highest fat content was found in JS (50.82%). Similar values were observed by other authors in jojoba seeds [1, 2, 11]. DJS (39.11%) had lower fat content than JS. This difference was significant ($P < 0.01$).

The protein percentages were not significantly different between DJS and JS. Jojoba seeds from America had the following protein contents: 14.90% [1], 15.00% [2] and 15.20% [11]. In this study, the protein percentages in DJS (18.40%) and JS (18.03%) were higher than the values reported by other researchers. Katsube [12] reported that physical deterioration of soybean seeds had no effect on protein content. Park et al. [13] observed that soy seeds severely infected with *Cercospora kikuchii* did not differ in protein content compared to uninfected ones. In this study, damaged seeds of jojoba with different degrees of deterioration did not affect protein content.

Significant differences were found between DJS and JS in ash percentages. Higher ash percentage in damaged jojoba seeds could be attributed to foreign material (dust, sand, etc.) adhering to the kernels. The ash contents in JS and DJS were higher than the values reported by other authors [1, 14].

The NFE content in DJS (35.25%) was significantly higher than in JS (24.32%). This result could be attributed to the minor wax content in DJS. The NFE value in JS was lower than other jojoba varieties reported by Wisniak [1].

Patil et al. [15] studied the effect of fungi on the lipid composition of soybean during the deterioration process. They reported that the lipid content of the seed decreased gradually, but a proportional increase in protein content was noted due to the biological utilization of fat by the fungi for conversion into protein. In this research, the oil content of the seeds in DJS decreased and NFE and ash percentage increased proportionally. However, the protein content did not change. Probably, some of the DJS could be affected by a development of fungi that utilized the lipids resulted in lower oil contents.

Table 1 Percent composition of damaged (DJS) and undamaged (JS) whole jojoba seeds

Samples	Moisture	Lipid	Protein	Ash	NFE
Dry weight[a] (%)					
JS	4.60 ± 0.64	50.82 ± 1.11 b	18.03 ± 1.43	2.23 ± 0.07 a	24.32 ± 2.43 a
DJS	3.87 ± 0.60	39.11 ± 0.79 a	18.40 ± 0.67	3.37 ± 0.28 b	35.25 ± 2.03 b
Anova	NS	$P \leq 0.01$	NS	$P \leq 0.03$	$P \leq 0.03$

NS not significant

[a] Mean value ± SD ($n = 3$). Different letters in the same column indicate significant differences ($P \leq 0.05$)

The fatty acid methyl ester composition of seed waxes is presented in Table 2. Jojoba waxes are rich in *cis*-11-eicosenoic acid (66.35%) followed by *cis*-9-octadecenoic (16.99%) and *cis*-13-docosenoic (14.24%) acids. The other detected fatty acids, *cis*-9-hexadecenoic, octadecanoic (18:0), eicosanoic (20:0) and docosanoic (22:0) acids were found in trace amounts (<0.1%). There were no significant differences in the fatty acid composition between DJS and JS. Tobares et al. [16] reported the fatty acid methyl esters composition in nine clones of jojoba seeds from Aimogasta, Argentina. Wisniak [1] studied the fatty acid composition in varieties from Arizona (USA) and Israel. In both studies, lower values of *cis*-9-tetracos-hexadecenoic, *cis*-9-octadecenoic and *cis*-13-docosenoic acids and higher values of *cis*-11-eicosenoic and -15-enoic acids, with respect to the results shown in this study, were reported.

The chemical quality of DJS and JS waxes is shown in Table 3. The AV of the waxes extracted from DJS (3.29 mg KOH/g) was significantly higher ($P < 0.0001$) than the waxes obtained from JS (0.65 mg KOH/g). This could be attributed to the degradation and hydrolysis of the waxes in damaged seeds. IJEC [2] allows an acid value in commercial wax of less than 1.0 mg KOH/g. Considering this AV level, the wax of DJS was out of the specified limit in this parameter.

The highest PV was exhibited by DJS (0.97 mequiv O_2/kg). PV in the waxes obtained from JS was not detected. IJEC [2] allows a peroxide value in commercial jojoba wax of less than 2 mequiv O_2/kg. DJS had acceptable quality for this parameter.

The CD and CT in the waxes extracted from DJS (1.99 and 0.38, respectively) were significantly higher than in the waxes obtained from JS (1.38 and none detected, respectively).

Wang et al. [17] reported that fungal damage caused by *Phomppsis* and *Cercospora kikuchii* had a devastating impact on soybean quality. The degradation processes observed in deteriorating jojoba seeds affected their chemical quality; increasing the AV, PV, CD, CT in waxes and decreasing the lipid content.

The results obtained from the sequential extraction of soluble proteins in JS and DJS are presented in the Table 4. Albumins and globulins were the highest protein fractions in JS and DJS. Shrestha et al. [18] detected that the region of maximum protein solubility was between pH 6 and 8, and the highest percentage was dissolved in water fraction corresponding to albumin and globulin fractions. In DJS, all fractions were significantly lower than in JS. The deterioration process in the seeds affected the protein solubility in all fractions. Particularly, the glutelin fraction had the greatest reduction in DJS.

The reduction in the amount of soluble protein fractions can be cause by protein denaturation. This process may produce (a) insoluble protein aggregation for interaction protein–protein, (b) increase of hydrophobic property in protein producing hydrophobic interaction lipid-protein, and/or (c) increase in the protein-fatty acid interaction favored by an increase of acidity that is generated by the free fatty acids [19]. The deterioration process occurred in DJS could produce protein denaturation; decreasing the

Table 2 Fatty acid composition of damaged (DJS) and undamaged (JS) jojoba seeds

	Fatty acid[a] (g/100 g)				
	16:0	18:1	20:1	22:1	24:1
JS	1.96 ± 0.36 a	16.99 ± 2.09 a	66.35 ± 1.28 a	14.24 ± 1.10 a	0.47 ± 0.07 a
DJS	1.75 ± 0.04 a	15.51 ± 0.19 a	67.63 ± 0.63 a	14.85 ± 0.51 a	0.26 ± 0.36 a
Anova	NS	NS	NS	NS	NS

NS not significant

[a] Mean value ± SD ($n = 3$). Different letters in the same column indicate significant differences ($P \leq 0.05$)

Table 3 Chemical quality from damaged (DJS) and undamaged (JS) jojoba seed waxes

Samples	Acid value[a] (mg KOH/g)	Peroxide value[a] (mequiv O_2/kg)	Conjugated dienes[a]	Conjugated trienes[a]
JS	0.65 ± 0.01 a	ND a	1.38 ± 0.02 a	ND a
DJS	3.29 ± 0.02 b	0.97 ± 0.04 b	1.99 ± 0.18 b	0.38 ± 0.09 b
Anova	$P \leq 0.0001$	$P \leq 0.001$	$P \leq 0.04$	$P \leq 0.03$

ND not detected

[a] Mean ± SD ($n = 3$). Different letters in the same column indicate significant differences ($P \leq 0.05$)

Table 4 Soluble protein fractions from damaged (DJS) and undamaged (JS) jojoba seeds

Sample[a] (mg/g)	Albumin fraction	Globulin fraction	Prolamine fraction	Glutelin fraction
JS	93.84 ± 12.24 b	72.96 ± 6.48 b	26.40 ± 6.54 b	61.08 ± 15.52 b
DJS	66.00 ± 10.81 a	47.44 ± 8.02 a	16.08 ± 4.04 a	19.31 ± 5.34 a
Anova	$P \leq 0.001$	$P \leq 0.0001$	$P \leq 0.007$	$P \leq 0.0001$

[a] Mean ± SD ($n = 3$). Different letters in the same column indicate significant differences ($P \leq 0.05$)

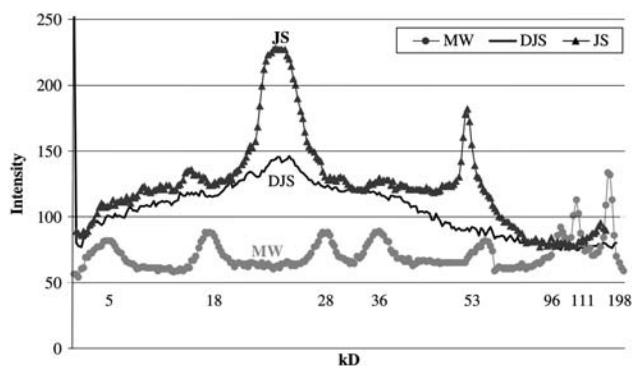

Fig. 1 Electropherograms of molecular weight markers (*MW*) and soluble proteins from damaged (*DJS*) and undamaged (*JS*) jojoba seeds

protein solubility without affecting the total protein content determined by the Kjeldahl method, which measures the organic nitrogen content [5].

The electropherograms of soluble proteins from JS and DJS, and molecular weight markers are presented in Fig. 1. In JS, there are two major bands around 25 and 50 kDa, and one minor band located near to 18 kDa. These two major bands were also observed by Wolf et al. [20] and Shrestha et al. [18]. In DJS, the soluble proteins showed only one band around 25 kDa. The other major band (50 kDa) and the minor band (18 kDa) were detected in JS and disappeared in the soluble protein of DJS. This effect is evidence of protein degradation due to seed deterioration process. This deterioration process in the seeds also affected the solubility of their proteins showing lower soluble protein content in DJS than in JS (Table 4). A similar effect was reported by Meriles et al. [21] regarding soluble protein of soybean infected with *Fusarium*, these authors observed protein degradation; furthermore, high-molecular-weight proteins were the most affected. The deterioration of the seeds clearly affects their chemical composition as well as the integrity and quality of proteins and waxes.

The results of the chemical composition of DJS showed a negative effect on the wax and protein components. In the wax, a significant decrease in content and quality was observed. With respect to the wax quality, an increase in acid and peroxide values and in conjugate dienes and trienes were observed. The protein content remained constant but the solubility decreased supporting the deterioration processes. Considering that Argentina produces 2,200 tonne of jojoba seeds, 150 tonne of them are damaged and have lprobably undergone deterioration. The consequence of this process is manifested in their quality which impact, negatively, on the industrial products derived from jojoba seeds.

Seeds that have better quality parameters will, in turn, produce a higher jojoba wax quality; implying lower costs for the refining process. Therefore, the use of a selection process to eliminate DJS before starting the wax production process is recommended due to the reduced quality of the wax and residual cake.

The use of DJS residues as ingredients for pet food or protein concentrates should be avoided due to the reduction in protein quality.

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References

1. Wisniak J (1987) The chemistry and technology of jojoba oil. AOCS Press, Champaign
2. International Jojoba Export Council (accessed April 2008) Jojoba Production Worldwide. Worldwide Jojoba Cultivation by Country. http://www.ijec.net
3. Flo G, Abbott T, Vermaut S, van Boven M, Daenens P, Decuypere E, Pedersen M, Cokelaere M (1997) Growth performance of rats fed with jojoba proteins. Possible correlations with trypsin inhibitory activity in jojoba proteins. J Agric Food Chem 45:4384–4387
4. Cornejo Ortiz (1999) Soy grain quality and its effect on industrial products and sub products. Oil Lipids 35:264–271
5. Horwitz W (1980) Official methods of analysis of the association of official analytical chemists, 13th edn. AOAC Press, Washington
6. COI (2001) Método de análisis, prueba espectrofotométrica en el ultravioleta. In: International Olive Oil Council (IOOC). Madrid Document COI/T 20/Doc no. 19/Rev 1
7. Grosso NR, Nepote V, Guzmán CA (2000) Chemical Composition of Some Wild Peanut Species (*Arachis* L.) Seeds. J Agric Food Chem 48:806–809
8. Lowry O, Rosebrough N, Lewis Farr A, Randall J (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275

9. Labuckas D, Maestri D, Perelló M, Martínez M, Lamarque A (2008) Phenolics from walnut (Juglans regia L.) kernels: antioxidant activity and interactions with proteins. Food Chem 107:607–612. doi:10.1016/j.foodchem.2007.08.051
10. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
11. Cappillino P, Kleiman R, Botti C (2003) Composition of Chilean jojoba seeds. Ind Crops Prod 17:177–182
12. Katsube T (1980) The effect of soybean purple blotch on growth yield and some chemical components of the seed. Annu Rep Soc Plant Prot N Japan 31:64–66
13. Park WM, Ko YH, Yoo YJ, Lee YL (1982) The change of peroxidase activity in the soy seed followed by infection with *Cercospora kikuchii*. Korean J Plant Prot 21:21–26
14. Perez-Gil F, Sanginés GL, Torreblanca RA, Grande ML, Carrasco JM (1989) Estudio sobre la composición quimica y contenido de factores antifisiologicos de la pasta residual de jojoba (*Simmondsia chinensis*). Arch Latinoam Nutr 39:591–600
15. Patil K, Shivamurthy S, Badami R (1986) Effect of fungi on the lipid composition of soybean during storage at different levels of humidity. Fette Seifen Anstrich 88:18–19
16. Tobares L, Frati M, Guzmán C, Maestri D (2004) Agronomical and chemical traits as descriptors for discrimination and selection of jojoba (*Simmondsia chinensis*) clones. Ind Crops and Prod 19:107–111
17. Wang D, Dowell FE, Ram MS, Schapaugh WT (2004) Classification of fungal-damaged soy seed using near-infrared spectroscopy. Int J Food Properties 7:75–82
18. Shrestha MK, Peri I, Smirnof P, Brik Y, Golan A (2002) Jojoba seed meal proteins associated with proteolytic and protease inhibitory activities. J Agric Food Chem 50:5670–5675
19. Cheftel J, Cuq J, Lorient D (1989) Proteínas Alimentarias, bioquímicas, propiedades funcionales, valor nutricional y modificaciones químicas. Ed Acribia, Zaragoza, España
20. Wolf W, Schaer M, Abbott T (1988) Protein extractability of defatted jojoba meals: Effect of pH and salt concentration. In: Baldwin A. R. (ed) Proceedings of the seventh International Conference on jojoba and its uses. America Chemists Society, Champaign
21. Meriles J, Lamarque A, Labuckas D, Maestri D (2004) Effect of fungal damage by *Fusarium* spp and *Diaporthe/Phomopsis* complex on protein quantity and quality of soybean seed. J Sci Food Agric 84:1594–1598