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Report

Oxidative Stability and Antioxidant Activity of Crude Jojoba Oil

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The seeds of jojoba [*Simmondsia chinensis* (Link) Schneider] contain a unique oil known as jojoba oil. It mainly consists of liquid wax monoesters with structures similar to human sebum wax and is popular as a cosmetic ingredient. We previously observed that the oxidative stability and antioxidant activity of crude jojoba oil are higher than those of many other vegetable oils. In this study, these two parameters were determined and compared among crude jojoba oils from different companies, countries, and years of production, together with deodorized or refined oils. Oxidative stability and antioxidant activity vary among crude oils, and there was a negligible correlation between these two parameters. Both deodorized and refined oils showed lower antioxidant activities than crude oils. In addition to wax esters, triglycerides and squalene are other major oil components of human sebum. To compare the autooxidation of jojoba oil (wax ester), olive oil (triglyceride), and squalene, we measured their acid, peroxide, and carbonyl values after heat treatment at 60°C for 60 d. The acid value did not change in jojoba oil but increased in the other ones. In addition, the peroxide and carbonyl values were the lowest in jojoba oil following heat treatment. These results suggest that jojoba oil was the most stable in terms of autooxidation among these three investigated oils. Finally, we determined the cytotoxicity of olive and jojoba oils in human epidermal cells, and concluded that they were non-toxic after heating at 60°C for 30 or 60 d.

Key words antioxidant activity, epidermal cells, jojoba oil, oxidative stability, sebum, wax monoester

INTRODUCTION

Oxidative stability is an important factor in cosmetic, food, and industrial applications. It can be defined as the induction time, which is the time required to create secondary products.[1] The poor oxidative stability of vegetable oils prevents them from meeting the rigorous specifications for cosmetic and industrial applications.[2] In addition, antioxidant activity is a useful property of cosmetic and edible vegetable oils, with some minor components being important natural antioxidants.[3]

Jojoba [*Simmondsia chinensis* (Link) Schneider] is a shrub cultivated in arid regions. It produces seeds that contain "jojoba oil", a bright-yellow liquid wax monoesters of fatty alcohols (mainly C20:1 and C22:1) and fatty acids (mainly C20:1) with an average content of approximately half of the seed weight.[4,5] Jojoba oil is a unique seed oil, because the main components of seed oils except jojoba are triglycerides. Wax esters, triglycerides, and squalene are the main lipid components of human sebum,[6] and the structures of jojoba wax monoesters resemble those of human sebum. This makes jojoba oil a popular cosmetic skin moisturizer.[7]

Recently, we compared the oxidative stability and antioxidant activity of jojoba oil with those of other oils.[8] The oxidative stability of refined jojoba oil was the highest among all the oils, and that of the crude form was also higher than 90% of the investigated oils. In contrast, the antioxidant activity of refined oil was lower than that of most oils, whereas the crude form had higher antioxidant activity than two-thirds of the oil samples. These results suggest that crude jojoba oil contains oxidatively stable major components, as well as minor components with antioxidant activity. In the present study, oxidative stability and antioxidant activity were compared among crude jojoba oils from different companies, countries, and years since manufacture, as well as with deodorized and refined oils. We also compared the autooxidation of jojoba oil, olive oil, and squalene using the acid, peroxide, and carbonyl values. The cytotoxicity of oxidized olive and jojoba oils in human epidermal cells was also investigated.

MATERIALS AND METHODS

Oils Nine commercially-available crude jojoba oils with bright-yellow color purchased in 2021 (A-I) and one oil purchased in 2017 (J) were shown in Table 1. A is Golden Jojoba Oil (Ease-Aroma Shop, Aichi, Japan), B is Jojoba Oil Virgin (Country of Origin: Israel) (Tree of Life Co., Ltd., Tokyo, Japan), C is Organic Jojoba (Country of Origin: Australia) (The Jojoba Company, Sydney, Australia), D is Premium Cold Pressed Jojoba Oil (Country of Origin: Israel) (Natural Orchestra, Tokyo, Japan), E is Jojoba Oil (Lot 0200701, Country of Origin: USA) (US Organic Group Corp, NJ, USA), F is Golden Jojoba Oil (Country of Origin: Israel) (Keinz, Kanagawa, Japan), G is 100% Jojoba Oil Gold Organic (Country of Origin: Peru) (Nanzen, Tokyo, Japan), H is Golden Jojoba Oil (Utataneya, Osaka, Japan), I is Organic Career Oil Jojoba Golden (Daily Aroma Japan, Tokyo, Japan), and J is Organic Jojoba Gold (Country of Origin: Argentina) (Inca Oil, Lima, Peru). Lot numbers were not indicated in oils other than E. Crude jojoba oils, which were cold pressed and filtered at the Egyptian Natural Oil Company (Sharqia, Egypt) from 2017 to 2020,

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Table 1. Results of Oxidative Stability and Antioxidant Activity Tests

Jojoba Oils	Induction period at 60ºC (d)		ORAC value (μmol TE/L)*	
	Average	S.D.	Average	S.D.
Crude oils**				
A	220.0	18.2	779.0	71.5
B (Israel)	198.3	5.9	1486.6	164.5
C (Australia)	197.7	10.2	950.7	288.6
D (Israel)	153.7	32.9	948.8	234.1
E (USA)	151.7	19.0	572.4	60.7
F (Israel)	145.0	11.2	626.9	144.0
G (Peru)	143.7	14.4	935.4	122.1
H	106.0	8.2	680.6	141.7
I	79.7	5.8	2795.6	765.9
J (Argentina)	51.3	4.0	1071.7	81.5
2017 (Egypt)	88.0	8.2	1083.9	232.2
2018 (Egypt)	77.0	6.9	1033.5	175.9
2019 Lot 1 (Egypt)	153.3	23.1	402.5	60.1
2019 Lot 2 (Egypt)	80.3	27.2	452.5	71.4
2020 Lot 1 (Egypt)	99.7	12.6	1759.5	222.8
2020 Lot 2 (Egypt)***	101.3	17.6	1115.0	117.2
2020 Lot 2 (Egypt), 60ºC 30 d treated****	81.7	17.2	1211.2	136.4
2020 Lot 2 (Egypt), 60ºC 60 d treated****	66.7	9.2	1032.4	166.2
Deodorized oil	131.3	24.3	332.9	61.6
Refined oil***	228.7	16.6	31.2	17.0

*TE, Trolox Equivalent.
**A-J are commercially-available crude jojoba oils. A-I were purchased in 2021. Countries of origin were shown in parentheses when available. Figures in Egyptian oils are years of manufacture.
***Tsuchimoto *et al.*, in press.
****One hundred grams of oils were heated in 200 mL beakers.

were obtained from Simmond Co., Ltd. (Osaka, Japan). Oil 2020 Lot 2 (Table 1) was produced using seeds obtained from a farm different from other Egyptian oils. Deodorized jojoba oil was supplied by Saraya Co., Ltd. (Osaka, Japan). Refined jojoba and olive oils were purchased from Ryohin Keikaku Co., Ltd. (Tokyo, Japan). Extra virgin olive oil (BOSCO), as a crude olive oil, and purified squalene (Tokyo Chemical Industry) were also obtained to examine autooxidation using heat treatment.

Chemicals Chemicals used for L-ORAC method have been described previously.[8] 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (Dojindo Laboratories) was used for the MTT assay.

Oxidative Stability Based on the Weighing Method The stability test of the autoxidation of cosmetic oils was performed by the weighting method, as described previously.[8] All experiments were performed in triplicate.

Antioxidant Activity Determined Using the L-ORAC Method The antioxidant capacity of each oil sample was evaluated using the L-ORAC measurement method as described previously.[8] All experiments were performed in six or more replicates.

Autooxidation Using Heat Treatment One hundred grams of oils in 200 mL beakers were heated at 60°C for 60 d in in an electric incubator (Drying Oven DRA430DA, ADVANTEC). The crude jojoba oil used for this experiment was the 2020 Lot 2 sample (Table 1). The acid, peroxide, and carbonyl values of the oils with or without heat treatment were determined at Japan Food Research Laboratories (Tokyo, Japan). Acid and carbonyl values were measured in accordance with the standard method for the analysis of fats, oils, and related materials as prescribed by the Japan Oil Chemists' Society.[9] The acetic acid – chloroform method was used for the peroxide value measurement. All experiments were performed in triplicate.

Cytotoxicity Small sample volumes were taken from jojoba and olive oils for autooxidation analysis after 30 and 60 d of heat treatment. Next, 50 μL of each sample was applied to LabCyte EPI-MODEL cells (Japan Tissue Engineering) that were preincubated at 37°C for 24 h in an incubator (SCA-80DS, Aztech) under 5% CO_2 concentration and then incubated for 24 h under the same conditions. The oil was then washed away using phosphate-buffered saline, and the cells were incubated again for 24 h under the same conditions. The cytotoxicity of oils was evaluated using MTT assay following the manufacturer's instructions. Oils were considered non-toxic when the living cell rates were more than 0.5. All experiments were performed in four replicates.

RESULTS AND DISCUSSION

Oxidative Stability of Crude Jojoba Oil The stability against autooxidation among 20 jojoba oils was determined using the weighing method: 10 commercially available crude oils, including those from different countries; eight crude oils having different years since manufacture that were produced in the same factory in Egypt; a deodorized oil; and a refined oil (Table 1). The induction period, defined as the number of days until the weight increased by 0.5% at 60°C, of each oil

was used to measure oxidative stability. The induction period of crude jojoba oil was between 51.3 and 220.0 d (Table 1), which was shorter than that of the refined form (228.7 d) but longer than those of the other 23 oils, including almond, argan, corn, cotton seed, and sunflower seed oils (1.0 – 29.0 d), which were measured using the same method.[8] The crude oil with the shortest induction period (J: 51.3 d) was the one purchased in 2017 and stored in a translucent plastic bottle for four years, whereas other crude oils were purchased in 2021 or stored in amber glass bottles or stainless-steel tanks. Crude oils produced in different years (2017–2020) at the same factory in Egypt were also compared. There was no or a weak correlation between the age of the oil and oxidative stability ($r = 0.293$). This may be attributed to the fact that the oils were stored in amber glass bottles or stainless-steel tanks; therefore, the effect of age deterioration would have been smaller than the difference in oil properties from years of production. When a crude oil (2020 Lot 2) was pretreated with heat, the induction period was decreased as expected. The induction period of a deodorized oil (131.3 d) was within the range of crude oil. This suggests that the effect of the deodorization process on oxidative stability was less than that of the purification process.

Antioxidant Activity of Crude Jojoba Oil The antioxidant activities were compared among the 20 jojoba oils using the lipophilic-oxygen radical absorbance capacity (L-ORAC) method (Table 1). The ORAC values of 18 crude oils varied (402.5 – 2795.6) but were higher than those of the deodorized and refined oils. Refined oil had the lowest ORAC value (31.2). These results support the notion that minor components of crude oil, which would have been reduced by the deodorization or purification processes, are involved in its antioxidant activity.[8] There was no or a weak correlation between the age of the oil and antioxidant activity ($r = 0.222$), and heat pretreatment did not decrease the activity. This suggests that the effect of age deterioration on the antioxidant activity of jojoba oil was minimal. Annual variation might have been caused by annual fluctuations in environmental conditions during cultivation. As 2020 Lot 1 and 2 were produced using seeds obtained from different farms, their ORAC value differences (1759.5 and 1115.0, respectively) may have resulted from the variation in environmental conditions between the growing areas. In addition to the above-mentioned factors, differences among crude oils from different companies may also be due to differences in the company-specific production processes. Despite of the variation in the ORAC values of crude jojoba oils described above, they were higher than those of 14 other kinds of oils, including those from cotton seeds, macadamia nuts, olives, palms, or sunflower seeds.[8]

No or a Weak Correlation between the Oxidative Stability and Antioxidant Activity Results of oxidative stability of 20 jojoba oils and antioxidant activity were displayed in a dot plot (Fig. 1), together with the aforementioned characteristics of 28 other kinds of oils, including almond, argan, corn, cotton seed, macadamia nuts, olive, palm, or sunflower seed oils.[8] The plot showed that high oxidative stability and high antioxidant activity coexist in crude jojoba oils, unlike in other types of oils. The correlation coefficient between the oxidative stability and antioxidant activity of crude jojoba oils was 0.251, suggesting that there was no or a weak correlation between them. This supports the notion that wax esters primarily contribute to the oxidative stability of crude jojoba oil, and minor components mainly contribute to its antioxidant activity.[8]

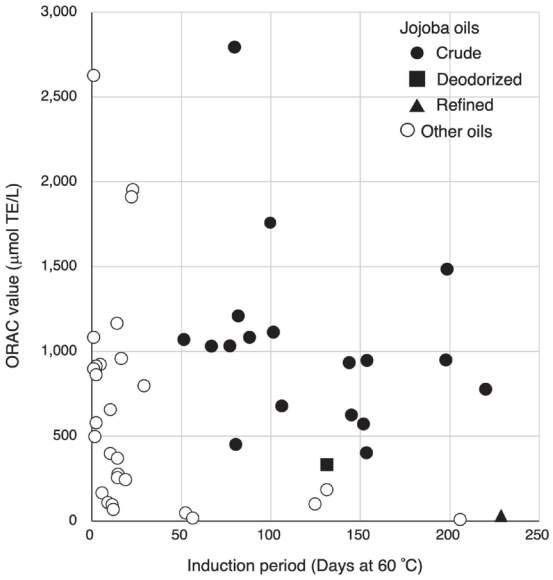

Fig. 1. Dot Plot of Oxidative Stability and Antioxidant Activity

Oxidative stability (induction period at 60°C) and antioxidant activity (ORAC value) of crude, deodorized, and refined jojoba oils were plotted together with those of other oils. TE, Trolox Equivalent.

Stability to Autooxidation Wax esters, triglycerides and squalene are the three major oil components of human sebum.[6] To compare the autooxidation of jojoba oil (wax ester), olive oil (triglyceride) and squalene, we measured their acid, peroxide, and carbonyl values after heat treatment at 60°C for 60 d (Fig. 2). Crude and refined jojoba and olive oils were used, and the investigated squalene was the purified form. After heat treatment, the acid value did not change in jojoba oil but increased in the other oils, especially about 39.3-fold in squalene, which displayed the highest acid value. The peroxide and carbonyl values were the lowest in the crude and refined jojoba oils after the heating. Moreover, the rates of increase of the two values were the lowest for jojoba oils. These results suggest that jojoba oil is the most stable to autooxidation among these three investigated oils. In contrast, squalene exhibited the highest acid and carbonyl values after heating. The peroxide value of squalene was lower than that of olive oil because the peroxide value finally decrease as lipid oxidation progresses.[10] These results suggest that autooxidation was more pronounced in squalene.

Cytotoxicity of the Oxidized Oils We then examined the cytotoxicity of olive and jojoba oils in cultured human epidermal cells using an MTT assay. After heat treatment at 60°C for 30 or 60 d, both crude and refined oils were applied to the cells, which were the same samples used to measure acid, peroxide, and carboxyl values (Fig. 2). Although significant differences in living rates were observed between 30 and 60 d in oils other than refined jojoba, they were between 1.0 and 1.2 in all cases (Fig. 3). These results suggest that the oxidized jojoba and olive oils were not cytotoxic, even though the peroxide value of the refined olive oil was more than 80 after the heat treatment.

Herein, we showed that the oxidative stability and antioxidant activity of crude jojoba oils were variable but generally higher than those of many other oils. Moreover, there was no or a weak correlation between these two parameters. Hence, we can conclude that jojoba oil is stable against autooxidation,

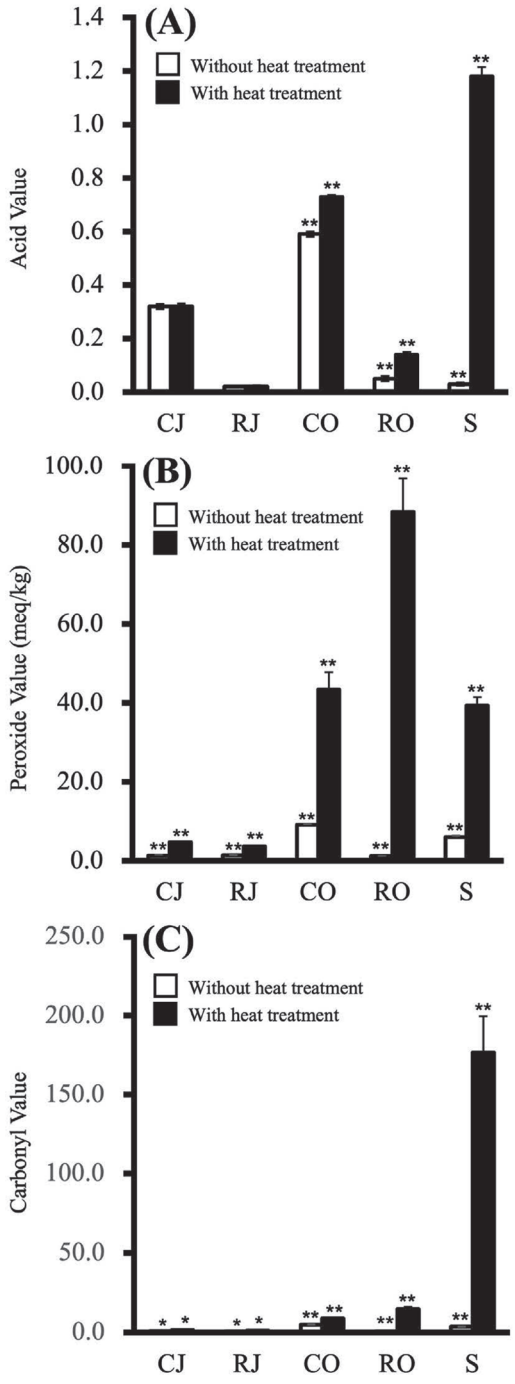

Fig. 2. Autooxidation of Oils

Acid (A), peroxide (B), and carboxyl (C) values were measured. Crude jojoba oil (CJ), refined jojoba oil (RJ), crude olive oil (CO), refined olive oil (RO), and squalene (S) with or without a heat treatment at 60°C for 60 d were used. Significant difference ($*P < 0.05$, $**P < 0.01$) between with and without heat treatment.

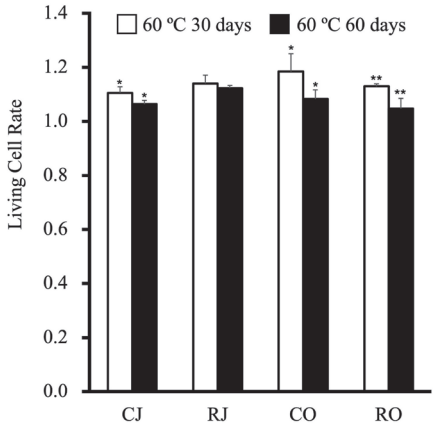

Fig. 3. Cytotoxicity Assay of Oils

Living rates of cells treated with crude jojoba oil (CJ), refined jojoba oil (RJ), crude olive oil (CO), and refined olive oil (RO) with a heat treatment at 60°C for 30 or 60 d are shown. Significant difference ($*P < 0.05$, $**P$ <0.01) between 30 and 60 d heat treatment.

has high antioxidant activity, and is non-toxic even after heating at 60°C for 60 d.

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Conflicts of interest Kiichi Fukui is a representative director, and Suguru Tsuchimoto is the director of Simmond Co., Ltd. This study was funded by Saraya Co., Ltd. to Kiichi Fukui, as well as Simmond Co., Ltd. and the Arid Land Research Center, Tottori University (No. 02C2023) to Suguru Tsuchimoto.

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