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DETOXIFICATION OF JOJOBA (*Simmondsia chinensis*) MEAL WITH WATER .

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ABSTRACT

Detoxification of jojoba (*Simmondsia chinensis*) meal was carried out using deionized water. The extraction was done by (a) ultrasound generator (US) at 20 kHz frequency for stirring the mixture for 5, 15, 30, 45, and 60 min. interval at 30°C and / or (b) reducing the particle size of jojoba meal into 0.125 mm using centrifugal mill, before stirring with deionized water for 12 and 24 hrs at ambient temperature (20±2°c). The obtained extracts were separated by TLC and HPLC techniques. TLC technique separated only the simmondsin and simmondsin trans ferulate of raw jojoba meal into 7 components . Extending the duration of US treatment from 5 to 60 min at 30 °C reduced the color intensity of the separated spots on TLC plate. Water extraction with agitation for 24 hrs after reducing the particle size of jojoba meal into 0.125 mm led to complete disappearing of the different simmondsin compounds from the TLC plate.

The HPLC conditions used in this study, led to a good separation of the different trans simmondsin ferulates and their cis isomers. Extending the time of US treatment increased from the reduction percent of both simmondsin and simmondsin ferulates of jojoba meal. Nearly, most of these compounds were removed from micronized jojoba meal after 24 hrs of stirring in deionized water.

Keywords: Jojoba seeds, jojoba meal, simmondsin, simmondsin ferulates, detoxification, HPLC, TLC, ultrasound, micronization, deionized water .

INTRODUCTION

Jojoba nut (*Simmondsia chinensis*) is a new oilseed crop, grown in the arid and semi-arid lands southwestern United State and in the other countries. It produces highly marketable unusual oil, 50 – 60 % of seed weight. The oil comprises of esters of long chain mono-unsaturated acids and alcohols (Abbott et al., 1991). The protein content of meal after oil extraction was ranged from 25 to 30 %, high crude fiber, and toxic components for rats and some animals. It contains 6 – 11 % simmondsin , a cyano methylene cyclohexyl glucoside, (Cokelaere et al., 1992) in addition to low levels of simmondsin analogues namely; simmondsin 2 -trans ferulate; simmondsin 3-trans ferulate ; 4-demethyl simmondsin; 5-didemethyl simmondsin ; 4,5 didemethyl simmondsin; 4-demethyl simmondsin 2-trans ferulate and 5 demethyl simmondsin 2-trans ferulate (Van Boven et al., 1994). Biological tests by Cokelaere et al. (1996) on rats revealed that simmondsin 2-trans ferulate had two-third the activity of simmondsin, corresponding to an activity that is equimolar with that of simmondsin . Hydrolysis of simmondsin 2-trans ferulate to simmondsin occurs in the gastro intestinal system. The action mechanism of simmondsin in the gastro intestinal can be due to, the central

or a local action of this component in the gastro intestinal system, and / or to aglycon formed by enzymatic deconjugation in the gut. Recently, Flo et al. (1998) postulated that simmondsin and simmondsin 2-trans ferulate were active as a toxic and food intake inhibitors. Demethyl simmondsin, and didemethyl simmondsin showed no toxic properties.

Numbers of solvent extraction, heat, chemical, and biological treatments were tried to detoxify the jojoba meal (Verbiscar et al., 1981; Maga, 1984). Generally, the development of such methods will help to upgrade the economic value of this crop and extend from its utilization in a new trend. Therefore, the specific goal of this study is to suggest a new procedure to remove simmondsin and its related toxicants from jojoba meal using water extraction, with ultrasound stirring and/or reducing the particle size of jojoba meal to 0.125 mm before stirring with water, for different periods. The changes in simmondsin and its derivatives were monitored with TLC and HPLC techniques.

MATERIALS AND METHODS

Materials:

1- **Jojoba seeds** (*Simmondsia chinensis*):They were brought from Jenin, West bank, Palestine in 2001.

2- **Jojoba meal**: The cleaned seeds were first boiled for 25 min in boiling water, cooled and manual dehulled. The dehulled seeds were dried in an air oven at 105 °C for 2 hrs then ground to pass through 60 mesh sieve and defatted in large Soxhlet apparatus for 24 hrs using diethyl ether. The resulted meal was desolventized in an air oven at 60 °C.

3- **Detoxified jojoba meal**: Removing of simmondsin and simmondsin derivatives were carried out as following;

a) **Ultrasound treatment**: Deionized water was used to extract the simmondsin and its derivatives using 1:10 (w/v) meal to water ratio in small glass flasks. The flasks were subjected for agitation in an ultrasound generator at 30 °C for 5, 15, 30, 45, and 60 min at 20 kHz frequency to increase the detoxification process.

b) **Micronization or reducing the meal particle size method**: Jojoba meal was first micronized used centrifugal mill, 0.125 mm, type Z M-100, Retch producer, Germany, then mixed with deionized water in glass flasks as described above. A polytetrafluoroethylene (PTEF) coated magnetic stir bar was added to each flask. The flasks were sealed with PTEF lined caps before subjecting for extraction on digital programmable hot plat stirrer to control the extraction time for 12 and 24 hrs, at ambient temperature (20 ± 2 °C).

4- **Reagents and other materials**: Simmondsin, 4-demethyl simmondsin, didemethyl simmondsin, simmondsin 2-trans ferulate, simmondsin 3-trans ferulate, 5- demethyl simmondsin 2-trans ferulate and 4- demethyl simmondsin 2-trans ferulate were brought from the laboratory of Toxicology, Katholieke Universteit Leuven, Belgium and used as reference. The cis isomers of these components were prepared by

exposure of the trans isomers to 365 nm, long UV radiation for 15 min (Van Boven et al., 1995). All used solvents in this study were HPLC grade.

Methods:

1-Extraction of simmondsin and simmondsin derivatives from raw and detoxified jojoba meal: One gram of jojoba meal was extracted with 50 ml pure methanol using a rotary mixer (Lapin, Belgium) with 100 ml glass extraction tubes as described by Van Boven et al. (1996). After rotating the tubes for 30 min, the solvent layer was separated from the meal by centrifugation. The obtained extracts were diluted to 100 times with methanol prior to HPLC analysis after passing through nylon filter.

2- Thin layer chromatography (TLC): TLC was performed on silica gel plates (polygram Sil. G/UV 254-Machery-Nagel, Germany) with a mixture of 30:70 v/v of methanol and chloroform as a developing solvent. The spots were localized by long wave length UV radiation at 365 nm.

3-High Performance Liquid Chromatography (HPLC): A model Knauer – Eurochrom 2000 high-performance liquid chromatography equipped with a knaure variable wave length UV-Vis. detector, knaure, Berlin, Germany and operated at 218 nm was used. The extracts were applied by a 20-µl injector loop. The separated system was consisted of lichrosorb c18 Column, 25x 0.4 cm, with a mixture of water and methanol, 70:30 v/v, at flow rate of 1 ml/min as an eluent after deaerating with helium. This system allows to separate both cis and trans isomers of simmondsin ferulate (Van Boven et al., 1996). Peak areas and retention times were determined with a D-2500 Merek-Hitachi chromato-integrator

RESULTS AND DISCUSSIONS

1- Thin layer chromatography (TLC).

The used TLC system in this study separated the different simmondsin and simmondsin trans ferulate. The prepared cis simmondsin derivatives were separated at the same R_f value of trans ones (Table 1). Therefore, this method did not able to differentiate between cis and trans isomers of the different simmondsin ferulates. Generally, simmondsin and simmondsin ferulate derivatives appeared as violet spots under long wavelength UV-radiation, 365 nm, as seen from Fig. 1. According to the results of TLC separation, the color intensity of the separated spots of the seven different simmondsin and simmondsin trans ferulates were generally reduced with extending the duration of ultrasound stirring from 5 to 60 min at 30°C (Fig. 1). In contrast, water extraction with agitation for 24 hrs after reducing the particle size of jojoba meal to 0.125 mm led to a complete disappearance of the different simmondsin compounds from the TLC plate (Fig. 1). Shortening stirring period from 24 to 12 hrs lowered greatly from the color intensity of the TLC separated simmondsin and simmondsin ferulates spots comparing with ultrasound treatment (Fig. 1).

Table 1: R_f values and the occurrence of the different simmondsin and simmondsin ferulates in detoxified jojoba meal .

Compound	R_f values	ultrasound treated jojoba meal for					Micronized and stirred jojoba meal for	
		5	15	30	45	60(min)	12	24(hrs)
1) Trans simmondsin dervitavies								
simmondsin 3-trans ferulates	0.80	+++++	++++	+++	++	+	+	-
simmondsin 2-trans ferulates	0.62	+++++	++++	+++	++	+	+	-
5 demethyl simmondsin 2-trans ferulates	0.54	+++++	++++	+++	++	+	+	-
4 demethyl simmondsin 2-trans ferulates	0.49	+++++	++++	+++	++	+	+	-
2) simmondsin	0.42	+++++	++++	+++	++	+	+	-
3) 4 demethyl simmondsin	0.29	+++++	++++	+++	++	+	+	-
4) 4,5 demethyl simmondsin	0.14	+++++	++++	+++	++	+	+	-
5) cis simmondsin devitavies								
simmondsin 3-cis ferulate	0.80	N.D	N.D	N.D	N.D	N.D	N.D	N.D
simmondsin 2-cis ferulate	0.62	N.D	N.D	N.D	N.D	N.D	N.D	N.D
5 demethyl simmondsin 2-cis ferulates	0.54	N.D	N.D	N.D	N.D	N.D	N.D	N.D
4 demethyl simmondsin 2-cis ferulates	0.49	N.D	N.D	N.D	N.D	N.D	N.D	N.D

(+++++) very high intensity color spots
(++++)high intensity color spots
(+++) moderate intensity color spots
(++) low intensity color spots
(+) very low intensity color spots

(-) No color spots
(N.D) not detected

2-High performance liquid chromatography (HPLC).

Van Boven et al. (1996) mentioned that jojoba meal contains only the trans isomers of simmondsin ferulate and during extraction, these isomers can easily transform to cis isomers by UV-light. As illustrated from Fig. 2, the selected conditions of HPLC in this study led to a good separation of the different trans simmondsin ferulates and their cis isomers. Nevertheless, overlapping between some isomers was observed. The retention times of the different trans and cis ferulate were recorded in Table 2.

According to the results of TLC analysis, the detoxified jojoba meal treated with ultrasound for 30, 45, and 60 min at 30 °C in deionized water in addition to that stirring for 24 hrs in same water after micronizing the particle size of jojoba meal to 0.125 mm were subjected for HPLC analysis. The following could be concluded from the results in Fig. 3 and Table 2;

1- according to their concentration in raw jojoba meal in the following descending order, 5 demethyl simmondsin 2-trans ferulate, simmondsin 3-trans ferulate, 4 demethyl simmondsin, simmondsin 3-cis ferulate, simmondsin, 4 demethyl simmondsin 2-trans ferulate, simmondsin 2-cis ferulate, 5 demethyl simmondsin 2-cis ferulate, simmondsin 2-trans ferulate, 4 demethyl simmondsin 2-cis ferulate, and 5 demethyl simmondsin, respectively.
2- Extending the time of ultrasound treatment increased from the reduction percent of both simmondsin and simmondsin ferulates compounds of jojoba meal.
3- Nearly most of the simmondsin and simmondsin ferulate were removed from the micronized jojoba meal after 24 hrs of stirring in deionized water.
4- The reduction percentage of cis isomers simmondsin ferulates was relatively more and rapid comparing with trans isomers compounds.
5- The detection of both 4-demethyl simmondsin and 5-didemethyl simmondsin compounds were observed only in the detoxified samples which subjected for long period of agitation. According to Van Boven et al. (1996) using a mixture of 70:30 v/v water and methanol eluted both of demethyl simmondsin and didemethyl simmondsin in front of the chromatogram.

According to above findings, water extraction was effective for removing simmondsin. It is also considered low cost and short time processing. The main disadvantages of such process are the losses of the water soluble compounds of jojoba meal and the drying of the wet detoxified meal to avoid microbial infection. Maga (1984) stated that chemical means used for decomposing the simmondsin and simmondsin ferulates, such as treating with 10 N aqueous ammonia for 30 days and ammonical hydrogen peroxide for 8 days decreased the nutritional quality of jojoba meal protein. In addition, Cotageorge et al. (1979) showed that a significant reduction in simmondsin when jojoba seeds were germinated for 5 days.

Table 2: HPLC retention time (min), peak area as percentage of total peaks area and reduction percent of simmondsin and simmondsin ferulate of raw and detoxified jojoba meal.

Compound	Retention time (min)	Peak area as % of total peaks area of raw jojoba meal	Reduction % of simmondsin and simmondsin ferulates			
			Ultrasound treated jojoba meal for			Micronized jojoba meal for 24 hrs
			30	45	60 (min)	
simmondsin	4.6	10.11	77.16	92.34	97.49	98.32
simmondsin 3-trans ferulate	8.4	15.16	64.84	86.02	91.67	94.80
simmondsin 3-cis ferulate	9.2	12.63	68.61	92.28	91.44	98.03
4 demethyl simmondsin 2-trans ferulate	10.6	8.66	71.58	92.56	93.13	99.08
5 demethyl simmondsin 2-trans ferulate	11.2	19.49	64.30	86.13	90.10	97.83
5 demethyl simmondsin 2-cis ferulate	12.8	4.33	65.96	94.46	97.34	100.00
4 demethyl simmondsin 2-cis ferulate	13.8	2.89	69.65	93.12	94.54	100.00
simmondsin 2-trans ferulate	14.2	3.62	67.99	N.D	96.21	100.00
simmondsin 2-cis ferulate	15.0	7.58	68.65	89.42	94.46	99.23
4 demethyl simmondsin	15.6	14.80	N.D	N.D	94.31	98.47
5 demethyl simmondsin	16.6	0.73	N.D	N.D	N.D	N.D

(N.D) not detected

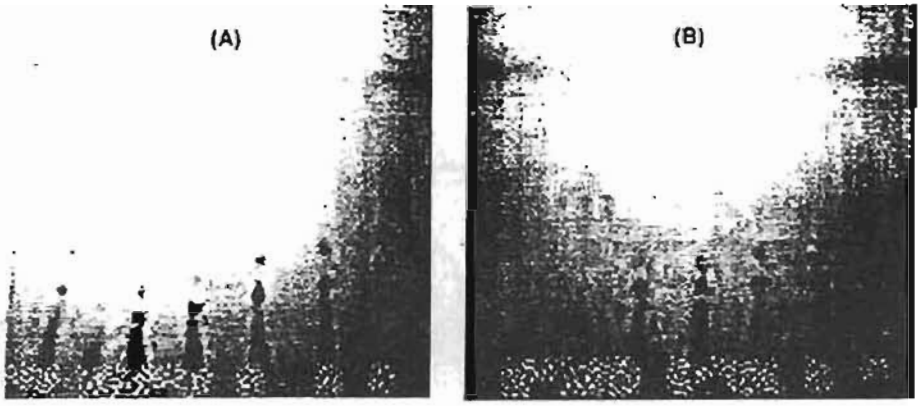

Fig. 1 : TLC separation of simmondsin and simmondsin ferulate from raw (A) and detoxified jojoba meal (B).

The numbers from1 to 5 indicate ultrasound treated jojoba meal for 5, 15, 30, 45, and 60 min, respectively. Meanwhile, the last two numbers (6 and 7) point to micronize and stirring jojoba meal for 12 and 24 hrs, in order .

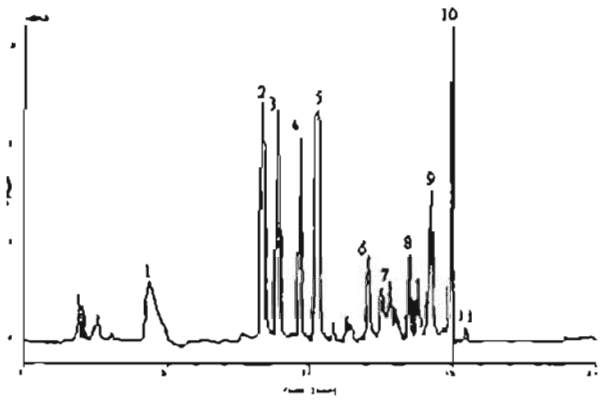

Fig. 2: HPLC chromatogram of raw jojoba meal.

The separated peaks were; (1) simmondsin, (2) simmondsin 3-*trans* ferulate, (3) simmondsin 3-cis ferulate, (4) 4 demethyl simmondsin 2-trans ferulate, (5) 5 demethyl simmondsin 2-trans ferulate, (6) 5 demethyl simmondsin 2-cis ferulate, (7) 4 demethyl simmondsin 2-cis ferulate, (8) simmondsin 2-trans ferulate, (9) simmondsin 2-cis ferulate, (10) 4 demethyl simmondsin, and (11) 5 demethyl simmondsin

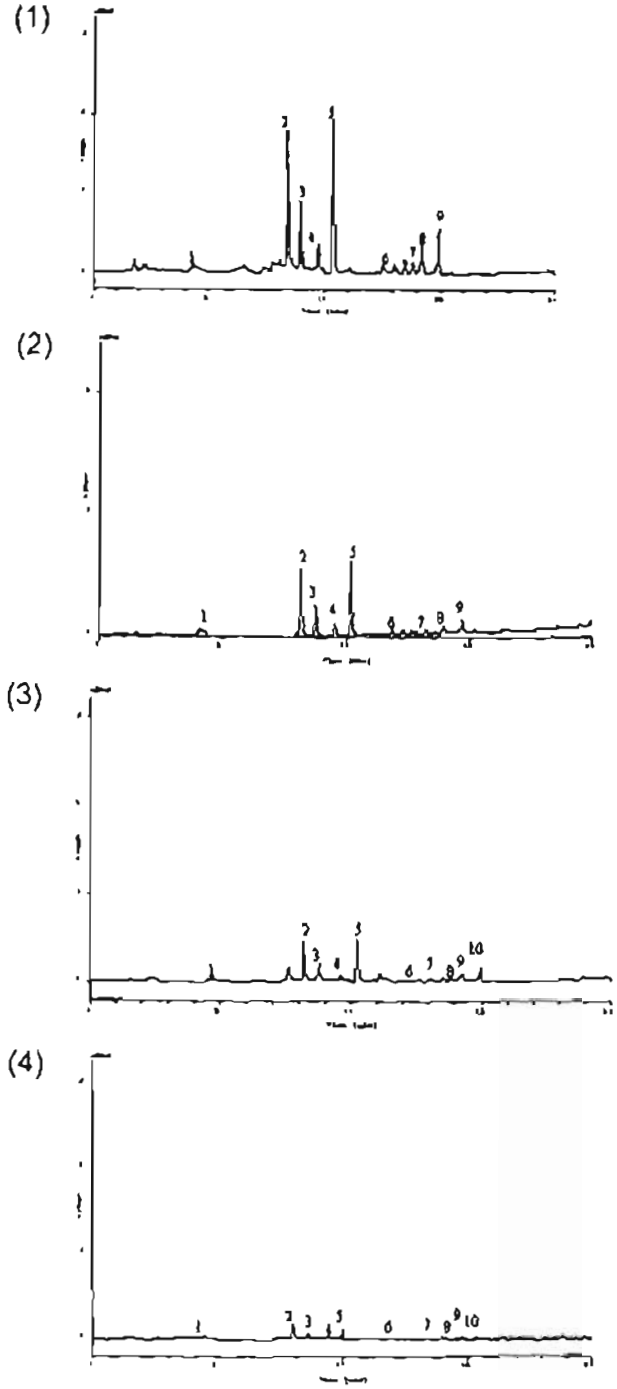

Fig. 3: HPLC chromatogram of detoxified jojoba meal treated with ultrasound for 30 min (1), 45 min (2), 60 min (3), micronized and stirred jojoba meal for 24 hrs (4) .

Verbiscar *et al.* (1981) found that nine of fifteen-tested *Lacobacillus spp* reduced simmondsin and simmondsin ferulates after 10 days of incubation on an autoclaved jojoba meal. Abbott et al. (1991) reported that solvent extractions by methanol, ethanol, 2-prpanol – H_2O (7:3 v/v) or butanol – water – acetic acid removed about one-third of the jojoba meal weight including simmondsin, simmondsin ferulate and sugars in addition to its negative economic effect due to the expensive of solvent.

CONCLUSION

The following could be concluded from the results of this research:

1- Jojoba meal contains only simmondsin and the trans isomers of simmondsin ferulates. The later compounds, can easily be transformed to cis isomers by UV light.
2- Simmondsin and simmondsin derivatives can be easily extracted from jojoba meal by water extraction. The proper conditions for removing most of these components were using 1:10 (w/v) jojoba mea to deionized water ratio with a continous stirring for 24 hrs at ambient temperature after micronize jojoba meal to 0.125 mm.
3- However, the used TLC technique in this study was unable to differentiate between trans and cis simmondsin ferulates, this technique can be used as a rapid method to monitor the detoxification of jojoba meal.
4- The used HPLC system eluted both demethyl simmondsin and didemethyl simmondsin in the front at the chromatogram. To overcome such disadvantage, starting the analysis with 10 % methanol, and programming to 40 % methanol can be suggested to include the simmondsin analogous in the HPLC analysis.

Note: Jojoba is one of the promising crops in Egypt. Recently, it is cultivated in severul region in Egypt: New Salhia, south of Sina, Sewa and Toshky

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إزالة سمية كسب بذور الجوجوبا (*Simmondsin chinensis*) بالماء

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استخدم الماء فى هذه الدراسة لازالة سمية كسب بذور الجوجوبا . و تم الاستخلاص بواسطة: (أ) مولد موجات فوق صوتية للتقليب لفترات ٥ ، ١٥ ، ٣٠ ، ٤٥ ، ٦٠ دقيقة على درجة حرارة ٣٠ °م . (ب) طاحونة لتصغير حجم حبيبات كسب الجوجوبا الى ٠,١٢٥ مم قبل التقليب مع الماء المقطر لمدة ١٢ ، ٢٤ ساعة على درجة حرارة الغرفة (٢٠±٢°م) .

واستخدم كل من تكنيك الفصل الكروماتوجرافى على الطبقة الرقيقة (TLC) ، وأيضا تكنيك التحليل الكروماتوجرافى السائل عالى الكفاءة (HPLC) لفصل والتعرف على المركبات السامة بالكسب . ولقد تم فصل سبعة من مركبات السيموندسين المختلفة، وفريولات ترانس السيموندسين من كسب بذور الجوجوبا الخام على لوح TLC . وانخفضت شدة لون المركبات المفصولة على TLC وذلك بزيادة فترة التقليب بالموجات فوق الصوتية من ٥ الى ٦٠ دقيقة، وأدى الاستخلاص بالماء مع التقليب بالماء لمدة ٢٤ ساعة بعد تصغير حجم حبيبات الكسب الى ٠,١٢٥ مم الى الاختفاء التام لمركبات السيموندسين المختلفة من على لوح TLC .

ولقد حدث فصل جيد لمركبات فريولات السيموندسين فى صورها المتيس والترانس باستخدام جهاز التحليل الكروماتوجرافى السائل عالى الكفاءة (HPLC) وذلك بزيادة فترات التقليب بالموجات فوق الصوتية ، مما أدى الى حدوث انخفاض فى النسبة المئوية لكل من مركبات السيموندسين ومركبات فريولات السيموندسين من كسب بذور الجوجوبا . ولقد حدث تخلص تام لمعظم هذه المركبات بعد ٢٤ ساعة من التقليب فى الماء بعد تصغير حبيبات الكسب الى ٠,١٢٥ مم .