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Fatty Acid Methyl Ester Profile from *Lupinus* in the Identification of Sweet and Bitter Species from this Gender with Oil of *Lupinus Uncinatus* Schlecht Seeds

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Abstract

This paper describes the implementation of a strategy to develop identification of *Lupinus* cultivars with fatty acid methyl esters relation about alkaloids. The genus *Lupinus* is abundant in Mexico, *Lupinus uncinatus* is an endemic plant of Mexico, and this has not been studied in terms of chemical composition and information about length, width and weight from seeds. This work is focused on analyzing the content of fatty acids methyl esters from *L. uncinatus* seeds. Where it was determined percentage oil as well as the concentration of twenty two fatty acid methyl esters and their corresponding equivalence TUFA (Total Saturated Fatty Acid), MUFA (Monounsaturated Fatty Acids) and PUFA (Polyunsaturated Fatty Acid). As a result of the analysis of lupine seeds, it was observed that the behavior of sweet spices versus bitter species has an ongoing relationship, with a higher content of PUFA (bitter) to higher MUFA (sweet) and measures of seeds.

Keywords: Lupinus sweet; Lupinus bitter; Oil composition; Methyl palmitate; Trans-9-elaidic methyl ester; Cis-9-oleic methyl ester; Trans-9,12-octadecadienoic acid methyl ester; Cis-9,12-octadecadienoic

Introduction

Lupine (*Lupinus* L.) is a universal plant with numerous useful properties. It may be used both as fodder and for soil fertilization. As fodder, low-alkaloid lupine species such as yellow fodder lupine (*Lupinus luteus* L.) and narrow-leaved forage lupine (*Lupinus angustifolius* L.) are used. Of course, lupines produce alkaloids not in order to supply them to man or animals. Various alkaloids function in plants as insecticides, herbicides, fungicides or pest protectors [1]. Lupinus seeds are characterized by high protein content. Therefore, attention is particularly focused on the quantity and quality of protein (the amino acid profile) due to the potential use of these in the diet of humans and animals. However, much less attention has been focused on the content and quality of Lupinus oil. Unlike protein whose level varies in a wide range (30-50%) depending on a particular variety, the oil content is considerably lower (5-10%) [2]. On the other hand *Lupinus uncinatus* Schlecht grown in Sierra Nevada at Mexico State over the mountain Tláloc, between 2932-2994 masl (meters above sea level) and slope of 2-23%. It is an annual, biannual, or perennial plant, more than 1 m tall, herbaceous, of hollow stems, stipules 7 to 9 mm long, petiole of 1.5-12.5 cm length, 5 to 8 leaflets 3 to 4 cm long and 6 to 8 mm wide, the fruits are dehiscent pods 4.5 to 5.5 cm long, containing 9 to 49 pods per branch and 1 to 7 mature seed per pod, flowering starts since March and the first mature seed appear in the middle of May. It is found in the soil with pH 6.3, 3.32% soil Organic Matter content, 0.13 % total Nitrogen, 14.7 mg kg^{-1}soil Phosphorus and 1.46 $cmolg^{-1}$ soil Potassium [3]. This study analyses the chemical composition and variability between the oils from seeds of wild *L. uncinatus* collected in Sierra Nevada at Mexico State over the mountain Tláloc.

Material and Methods

Plant material

Seeds of *L. uncinatus* Schlecht was collected in May, 2009. The study area is located on the oriental slope of the Sierra Nevada in Tláloc mountain in Mexico State, Mexico, between 19° 23 “43” y 19° 28 “37” North and between 98° 42 “51” and 98° 48 “12”.

Image hardware and software

A flatbed scanner (Xerox phaser 6110) and Phaser 6110 MFP software was used for image acquisition, ADOBE PHOTOSHOP 9.0 software was used to ensure identical orientation of the specimen. The digital image analysis was carried out by standalone version of IMAGN J 1.40g, a program developed at the United States National Institute of Health (freely available at http://rsb.info.nih.gov/ij/ or http://www.scioncorp.com/pages/download_now.htm). A standard personal computer was used for image analysis.

Image acquisition and analysis

The seed was placed on the scanner bed with the vertical side down with about 1 cm space between the specimens to avoid seed to seed contact. Grey image of seeds was acquired with the scanner software. The scanner resolution was 300 dpi. Images were stored in tiff format and exported to the PHOTOSHOP program for possible corrections regarding the orientation. Subsequently the IMAGEN J program was used for feature extraction. The test was for 25 seeds [4]. Seeds were

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weighed to determine the number of seeds per gram by counting out replicates of 100 seeds and placing them on a balance until 1.0 g of seeds are obtained.

Analysis oils

Material: HPLC grade hexane and methanol were purchased from Baker (Deventer, Nertherlands). Potassium hydroxide in methanol was freshly prepared, dissolving 11.2 g in 100 mL of methanol (2 N). The following fatty acid methyl esters (FAMEs) were purchased from Sigma Aldrich (St Louis, MO); Methyl butyrate (C4:0, 99.9% purity), methyl hexanoate (C6:0, 99.7%), methyl octanoate (C8:0, 99.9%), methyl decanoate (C10:0, 99.9%), methyl undecanoate (C11:0, 99.5%), methyl laurate (C12:0, 99.8%), methyl tridecanoate (C13:0, 99.4%), methyl myristate (C14:0, 99.7%), myristoleic acid methyl ester (C14:1, 99.9%), methyl pentanodecanoate (C15:0, 99.6%), cis-10-pentadecenoic acid methyl ester (C15:1, 99.0%), methyl palmitate (C16:0, 99.9%), methyl palmitoleate (C16:1, 99.7%), methyl heptadecanoate (C17:0, 99.9%), cis-10-heptadecenoic (C17:1, 99.9%), methyl stearate (C18:0, 99.9%), trans-9-elaidic methyl ester (trans-C18:1, 96.9%), cis-9-oleic methyl ester (cis-C18:1, 99.9%), trans-9,12-octadecadienoic acid methyl ester (trans-C18:2,99.6%), cis-9,12-octadecadienoic (cis-C18:2, 99.6%), methyl arachidate (C20:0, 99.9%), gamma-linolenic acid methyl ester (C18:3n6, 99.5%), cis-11-eicosenoic (C20:1, 99.9%), methyl linoleate (C18:3n3, 99.9%), methyl heneicosanoate (C21:0, 99.5%), cis-11,14-eicosadienoic acid methyl ester (C20:2, 99.9%), cis-8,11,14-eicosatrienoic acid methyl ester (C20:3n6, 99.1%), methyl erucate (C22:1n9, 99.7%), cis-11,14,17-eicosatrienoic acid methyl ester (C20:3n3, 99.2%), cis-5,8,11,14-eicosatetraenoic acid methyl ester (C20:4n6, 99.3%), methyl behenate (C22:0, 99.8%), cis-5,8,11,14, 17-eicosapentaenoic acid methyl ester (C20:5, 99.9%), methyl tricosanoate (C23:0, 99.9%), cis-13,16-docosadienoic acid methyl ester (C22:2, 99.9%), methyl lignocerate (C24:0, 99.8%), methyl nervonate (C24:1, 99.9%) and cis-4,7, 10, 13, 16, 19-docosahexaenoic acid methyl ester (C22:6, 99.7%).

Extraction of the crude oil

L. uncinatus samples (24.0 g) were dried for 24 hours at 90°C in a ventilated oven (Thomas Scientific) and then ground in a blender (Oster) grinder. Oil was extracted from ground *L. unicinatus* samples using the Soxhlet technique in an Avanti 2050 Soxtec from Foss Tecator (Denmark). Samples were weighed (2.5 g) into Whatman #4 filter paper, which was then folded and placed into cellulose extraction thimbles. Hexane (60 mL) was placed in aluminum extraction cups and the cups were placed in the position. Hexane was boiled for 40 min with the thimble in the hexane. Then the thimble was raised and the hexane continued to boil for 60 min (called the rinse phase). After rinsing, the remaining hexane was removed for 30 min. Then the cups with the oil were drying for 20 min. After the extraction process, the oil was transferred to amber vials covered with cap and placed in a freezer.

Preparation of fatty acid methyl ester (FAMEs)

FAMEs were prepared by transmethylation using CH_3OK in CH_3OH according to the international standard ISO 5509 [5]. The mixture was purchased as a 100 mg neat mixture, containing C4 to C24 FAMEs (2 to 4% relative concentrations). The whole sample was diluted in 10 mL of hexane. Weigh 100 mg oil extracted from seeds of *L. uncinatus*. The sample was dissolved in 10 mL of hexane and 100 µL of 2 N potassium hydroxide in methanol was added. It was put in the vortex, and centrifuged. The clear supernatant solution is 10 mg/mL which was analyzed by CG-FID.

	Mean	SD	95% conf. Interval	
	(µ)	(σ)		
Length (mm)	6.13	0.621	5.88-6.38	n = 25
Width (mm)	4.74	5.463	4.52-4.95	n = 25
Weight (mg)	4.40	0.151	4.23-4.57	n = a 100 seeds sample

Table 1: Statistical parameters of *Lupinus uncinatus* Schlecht (expressed as Standard Deviation value "SD").

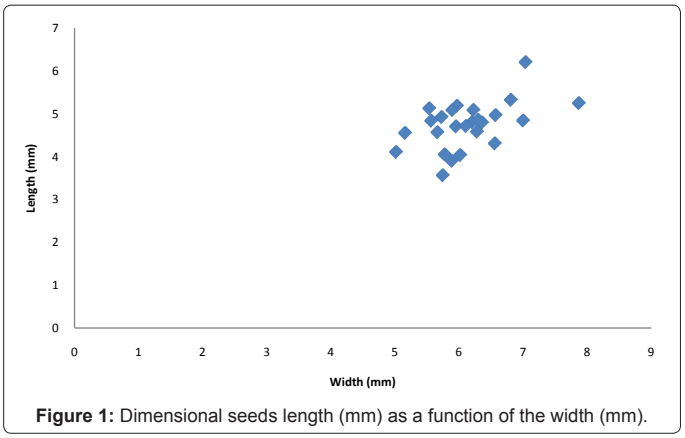

Figure 1: Dimensional seeds length (mm) as a function of the width (mm).

		Average	SD
	Formula	% oil	%
1	C8:0	0.10	0
2	C13:0	0.18	0.01
3	C14:0	0.05	0
4	C14:1	0.19	0
5	C15:0	0.04	0
6	C15:1	0.06	0.05
7	C16:0	13.12	0.89
8	C16:1	0.37	0.05
9	C17:0	0.05	0
10	C17:1	0.05	0.01
11	C18:0	6.77	0.47
12	C18:1 CIS	14.24	0.17
13	C18:1 TRANS	2.80	5.01
14	C18:2 CIS	50.59	3.96
15	C18:2 TRANS	0.31	0.56
16	C18:3n6	0.02	0.03
17	C18:3n3	7.81	0.09
18	C20:0	0.44	0.03
19	C20:1	0.17	0.02
20	C20:5	1.38	0.09
21	C22:2	0.04	0.04
22	C23:0	0.17	0.02
23	C24:0	1.05	0.01

Table 2: Fatty acid composition (expressed as Standard Deviation value "SD") of *Lupinus uncinatus* Schlecht.

GC-FID analysis

The FAMEs were analyzed with an Agilent 7890A GC equipped with an FID. Automated split injection was performed using an Agilent 7683B Automatic sampler operated at 250°C. The instrumental and analytical conditions are based in norm UNE-EN ISO 5508:1996. An HP-INNOWax column (30 x 0320 mm id x 0.25 µm film thickness) (19091N-113Agilent) was used. The carrier gas was Helium (constant pressure approximately 230 kPa at 50°C, 33 cm/s at 50°C), the auxiliary

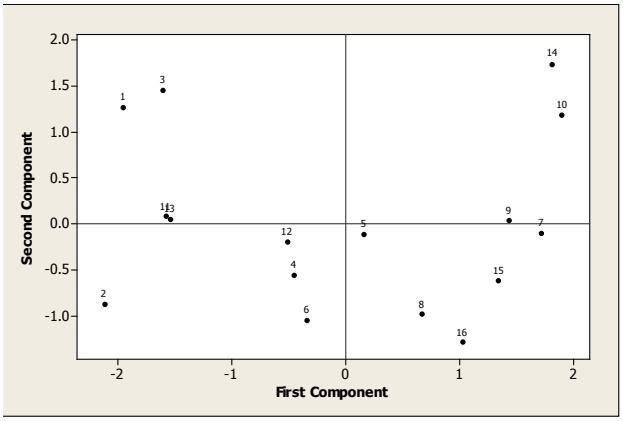

Figure 2a: Score plot of MUFA, TSFA, PUFA.

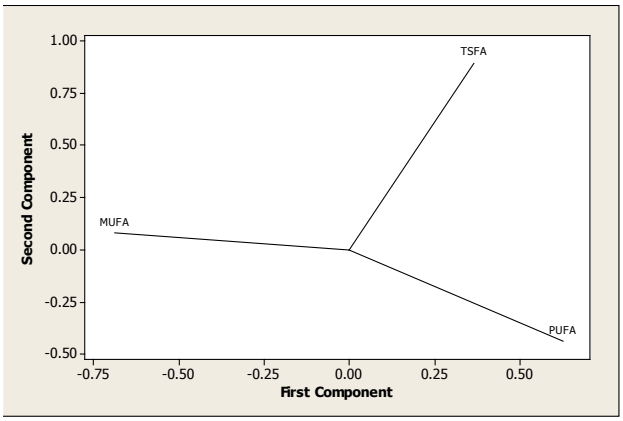

Figure 2b: Loading plot of MUFA, TSFA, PUFA.

gases were H_2 40 mL.min^{-1}, Air 450 mL.min^{-1} and Helium 30 mL.min^{-1}. Detector temperature was set at 280°C; the temperature program was: 2 min at 50°C, from 50°C to 220°C at 30°C/min then 20 min at 220°C, from 220°C to 255°C at 5°C/min then 5 min at 255°C. Analyses were processed with ChemStation Workstation software (Version REV. B.04.01 SP1). The analysis was performed at least in triplicate. Peaks were identified by comparison of retention times with standard compounds.

Results and Discussion

Sizes and measures of seeds length, width and weight of each specimen were measured (Table 1) and the average of each seed was calculated with length 6.13 mm, width 4.74 mm and weight 0.044 g. The length was plotted *versus* the width (Figure 1).

The oil content for individual sample combination ranged from 6.97% to 9.64% (mean 7.76 ± 1.11 standard deviation). These values appear to be in good agreement with literature data. The genus *Lupinus* typically contains 5-20% oil [5], Roth-Maier and Kirchgessner [6] reported that the oil levels in some varieties of white Lupin (*Lupinus albus*) are 7.6%. Uzun et al. [7] obtained 10.75% in two varieties of *Lupinus albus*, while Wilson et al. [8] reported *Lupinus albus* contained 9% oil, Boschin et al. [9] states that the seed of a variety of *Lupinus albus* L., contains about 9-14% oil. Other studies [10] report about *Lupinus albus* samples containing 14.7% oil. *Lupinus angustifolius* was reported with 6.4% oil and *Lupinus mutabilis* with 16-20% oil [8,10] agrees that *Lupinus mutabilis* contains about 20% comparison with soybean oil from dry seeds which can vary from 12% to 26%.

The percent composition of the FAMEs in *L. uncinatus is* presented in Table 2. On average, FAMEs ranked in the following order of abundance: *cis+trans*

C18:2>C16:0>cis+transC18:1>C18:3n3>C18:0>C22:6>C24:0>C22:0+C20:5>C20:0>C17:1>C22:1n9=C22:2>C16:1>C13:0=C14:1>C8:13=C15:0>C15:1=C20:1>C23:0>C14:0=C17:0.

Concerning TSFA (Total Saturated Fatty Acid), the percentage is 24%; in respect to MUFA (Monounsaturated Fatty Acids) 17% and PUFA (Polyunsaturated Fatty Acid) are 58% and 1% coelute C22:0+C20:5 (Figure 2a, 2b).

Literature data for some species of lupines has been a different distribution between TSFA, MUFA and PUFA (Table 3).

On the other hand, data were analyzed for TSFA, MUFA and PUFA *L. unicinatus* against those reported in the literature, separating the data into two groups, bitter and sweet (Figure 3). In sweet group the MUFA were the highest concentration than TSFA and PUFA. In the case of bitter group the PUFA were the highest concentration than TSFA and PUFA. Based on the results of *L. uncinatus*, it could be predicted that is a species bitter waiting to checking alkaloid content by quantitative methods.

Sample number	Lupines	TSFA (%)	MUFA (%)	PUFA (%)	Reference
1	*L. albus* AB47	25	59	16	[9]
2	*L. albus* Biovar astra	12	59	29	[15]
3	*L. albus* cv. Luxe	27	55	18	[9,13]
4	*L. angustifolius*	18	40	43	[17,19]
5	*L. angustifolius*	22	32	45	[12]
6	*L. angustifollius Sweet*	15	35	45	[7]
7	*L. exaltus*	26	14	60	[14]
8	*L. luteus*	18	24	55	[17,19]
9	*L. mexicanus*	26	17	56	[14]
10	*L. montanus*	34	13	53	[14]
11	*L. mutabilis*	19	54	28	[14]
12	*L. mutabilis* bitter	20	41	40	[15]
13	*L. mutabilis* semi-sweet	19	54	29	[15]
14	*L. stipulates*	37	14	48	[14]
15	*L. uncinatus*	22	18	60	
16	*L. varius*	17	19	60	[11]

Table 3: Lupinus TSFA, MUFA and TUFA in percentage.

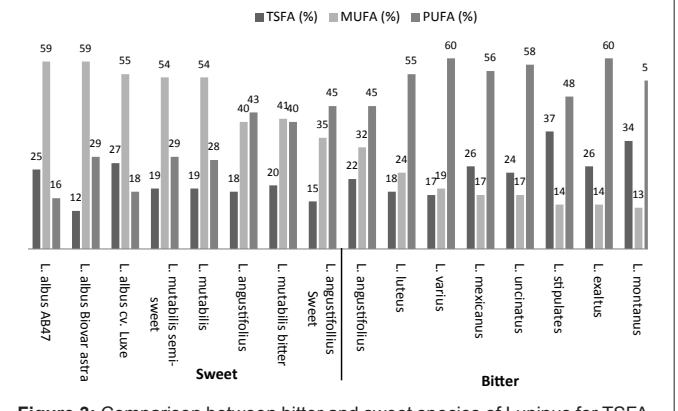

Figure 3: Comparison between bitter and sweet species of Lupinus for TSFA, MUFA and PUFA.

Conclusion

It was concluded that the fatty methyl ester profile is a signal to selection of the Lupinus bitter or sweet. In addition, it also will be synergistic effect in the development of plant because the oils are a primary metabolism, and then changes with the evolution of plant. With the signal is possible, the comparison in plants of this gender at different age. Therefore, further experimentation is required to determine the role of alkaloids with fatty methyl ester profile and in this way; the investigators can have a new rapid test of sweet or bitter Lupinus cheaper than test of alkaloids.

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