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Short communication

Changes in the fatty acid composition in bitter *Lupinus* species depend on the debittering process

Carolina A. Curti[a,*], Ramiro N. Curti[b], Norberto Bonini[c], Adriana N. Ramón[c,d]

[a] Instituto de Investigaciones para la Industria Química (INIQUI), Facultad de Ciencias Exactas, Universidad Nacional de Salta – CONICET, Salta, Argentina
[b] Laboratorio de Investigaciones Botánicas (LABIBO), Facultad Ciencias Naturales Sede Regional Sur, Universidad Nacional de Salta – CONICET, Salta, Argentina
[c] Instituto de Investigaciones para la Industria Química (INIQUI), Facultad de Ciencias Exactas, Universidad Nacional de Salta, Salta, Argentina
[d] Cátedra de Ciencia y Tecnología de Alimentos, Laboratorio de Alimentos, Facultad de Ciencias de la Salud, Universidad Nacional de Salta, Salta, Argentina

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ABSTRACT

The evaluation of changes in the fatty acid composition in *Lupinus* species after the debittering process is crucial to determine their nutritional implications. The aim of this study was to evaluate changes in the fatty acid composition in *Lupinus albus* and *L. mutabilis* after the debittering process. *Lupinus* species showed different fatty acid compositions which changed depending on the debittering process applied. The debittering process changed the monounsaturated and polyunsaturated fatty acids in *L. albus*, whereas in *L. mutabilis* it changed the *w*-6/*w*-3 ratio. However, the total saturated fatty acid content remained stable in both species after the debittering process. The changes in *L. albus* were associated with the fatty acid desaturation and a conversion into unsaturated fatty acids, whereas in *L. mutabilis* with the lipid peroxidation by decreasing the linoleic acid content. Nutritional implications of these changes in the fatty acid composition are discussed.

1. Introduction

The debittering process is a necessary step which removes toxic and bitter alkaloids from legume seeds and antinutritional factors such as saponins, tannins and phytates to ensure a safe human consumption (Carvajal-Larenas, Linnemann, Nout, Koziol, & van Boekel, 2015; Mohammed, Mohamed, Yagoub, Mohamed, & Babiker, 2017; Patterson, 2017). This process generally consists in soaking seeds from 10 to 18 h in a wide range of water temperature, followed by cooking and washing stages which depend upon pulse genotype, cultivar and growing environment (Patterson, 2017). *Lupinus* species such as *Lupinus albus* (white lupin) and *L. mutabilis* (tarwi or Andean lupin) are legumes with high nutritional value (33.9–43.3 g $100\,g^{-1}$ of proteins and 5–19 g $100\,g^{-1}$ of fats) and differences in the fatty acid composition between these species have been observed. The oleic acid was the main fatty acid found in *L. albus*, whereas in *L. mutabilis* the oleic and linoleic acids were the major fatty acids determined. In addition, the eicosenoic and erucic acids were only found in *L. albus* (Boschin, D'Agostina, Annicchiarico, & Arnoldi, 2008; Carvajal-Larenas et al., 2015).

Lupinus species contain between 1 and 4 g $100\,g^{-1}$ of toxic alkaloids which must be removed prior to consumption (Carvajal-Larenas et al., 2015; Van de Noort, 2016). The traditional debittering process applied in *L. mutabilis* includes a soaking stage of seeds for 18 h, followed by a cooking step (0.5–6 h) to inactivate the germination capacity of seeds, their enzymes (lipase, lipoxygenase), to eliminate occurring microorganisms and to reduce the loss of proteins through their coagulation (Carvajal-larenas, Nout, Van Boekel, Koziol, & Linnemann, 2013). The process is completed with a washing step for several days in which most of the alkaloids are removed (Carvajal-larenas et al., 2013). The cooking and washing stages in other *Lupinus* species differ due to the anti-nutrient and alkaloid contents, in *L. albus* the normal aqueous debittering process uses water at 25–50 °C (Erbas, 2010; Mohammed et al., 2017).

The debittering process can induce changes in the chemical composition of lupins by decreasing the soluble carbohydrate and fibre contents (Carvajal-Larenas et al., 2015; Erbas, 2010), however it increases the protein content and might either increase or decrease the fat contents (Carvajal-Larenas et al., 2015; Erbas, 2010). Little is known about whether these changes in fat contents in *Lupinus* species after the debittering process would affect their fatty acid compositions. In *L. mutabilis* and *L. albus*, changes in the fatty acid composition and relationships between fatty acids were associated with the genotype (Villacrés, Pástor, Quelal, Zambrano, & Morales, 2013; Yorgancilar & Bilgiçli, 2014). The changes in both *Lupinus* species were observed in major fatty acids such as the oleic, linoleic and palmitic acid as well as in the polyunsaturated/saturated fatty acid ratio (Villacrés et al., 2013)

* Corresponding author at: Avenida Bolivia 5150 (4400) Salta, Capital, Argentina.
E-mail address: ccurti@unsa.edu.ar (C.A. Curti).

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Table 1
Fatty acid compositions (expressed as g 100 g^{-1} of total fatty acid methyl ester identified) of raw and debittered *Lupinus mutabilis* and *L. albus*. LSD values for each test are shown.

	Lupinus albus			*Lupinus mutabilis*		
	Raw	Debittered[†]	LSD	Raw	Debittered[‡]	LSD
Saturated fatty acids (SFA)						
Palmitic acid (C16:0)	6.3 ± 0.3[a]	6.4 ± 0.6[a]	1.03	8.2 ± 0.2[a]	8.2 ± 0.01[a]	0.26
Stearic acid (C18:0)	1.7 ± 0.1[a]	1.5 ± 0.1[b]	0.14	5.6 ± 0.03[a]	5.7 ± 0.01[b]	0.06
Arachidic acid (C20:0)	1.0 ± 0.04[a]	1.0 ± 0.04[a]	0.08	0.6 ± 0.03[a]	0.7 ± 0.2[a]	0.38
Behenic acid (C22:0)	3.3 ± 0.1[a]	3.5 ± 0.1[a]	0.28	0.7 ± 0.04[a]	0.7 ± 0.01[a]	0.05
Total SFA	12.3 ± 0.2[a]	12.4 ± 0.5[a]	0.89	15.1 ± 0.2[a]	15.4 ± 0.2[a]	0.49
Monounsaturated fatty acids (MUFA)						
Oleic acid (C18:1 *w*-9)	56.1 ± 0.3[a]	60.1 ± 0.4[b]	0.78	56.3 ± 0.5[a]	56.7 ± 0.1[a]	0.81
Eicosenoic acid (C20:1 *w*-9)	3.9 ± 0.2[a]	4.2 ± 0.2[a]	0.37	nd[*]	nd[*]	
Erucic acid (C22:1 *w*-9)	1.6 ± 0.2[a]	1.8 ± 0.1[a]	0.34	nd[*]	nd[*]	
Total MUFA	61.5 ± 0.4[a]	66.1 ± 0.1[b]	0.63	56.3 ± 0.5[a]	56.7 ± 0.1[a]	0.81
Polyunsaturated fatty acids (PUFA)						
Linoleic acid (C18:2 *w*-6)	18.4 ± 0.4[a]	19.8 ± 0.2[b]	0.80	26.1 ± 0.5[a]	25.0 ± 0.1[b]	0.80
Linolenic acid (C18:3 *w*-3)	7.8 ± 0.1[a]	8.2 ± 0.2[b]	0.28	2.5 ± 0.1[a]	2.8 ± 0.2[a]	0.39
Total PUFA	26.2 ± 0.4[a]	28.0 ± 0.01[b]	0.68	28.6 ± 0.6[a]	28.0 ± 0.2[a]	1.02
PUFA/SFA	2.1 ± 0.1[a]	2.3 ± 0.1[a]	0.18	1.9 ± 0.1[a]	1.8 ± 0.04[a]	0.11
w-6/w-3 ratio	2.4 ± 0.1[a]	2.4 ± 0.1[a]	0.16	10.3 ± 0.3[a]	8.8 ± 0.6[b]	1.16
Atherogenic index	0.07 ± 0.004[a]	0.1 ± 0.01[a]	0.01	0.1 ± 0.002[a]	0.1 ± 0.0002[a]	0.003

Means ± standard deviation followed by a different letter between columns for each species are significantly different ($P < 0.05$).
[†] Debittered *L. albus* after Fontanari et al. (2012).
[‡] Debittered *L. mutabilis* afterCarvajal-Larenas et al. (2013).
[*] nd: not detected.

(Yorgancilar & Bilgiçli, 2014). However, there are few studies aimed at evaluating the extent to which variation in the global fatty acid composition and fatty acid relationships are related to a variation in the debittering process applied.

This study analysed changes in the fatty acid composition in *Lupinus albus* and *L. mutabilis*. We expected to find changes in the fatty acid composition and relationships between fatty acids in *L. mutabilis* and *L. albus* depending on both the individual fatty acid composition and the debittering process applied.

2. Materials and methods

2.1. Plant materials and processing conditions

Lupinus albus (cv. Multitalia) and *L. mutabilis* samples were purchased in local markets of Salta (Argentina) and Sucre (Bolivia), respectively. Seeds were manually cleaned to remove foreign materials, broken and immature seeds. Raw seeds were milled to pass through a 250 μm aperture sieve and stored in air tight containers at 4 °C. *L. mutabilis* was debittered according to the commercial method described by Carvajal-Larenas et al. (2013). Briefly, seeds were soaked at 25 °C in a 1:3 (w/ v) ratio for 18 h with 3 water changes, then cooked in boiling water (98 °C) for 1 h and washed at 25 °C for 96 h.

L. albus seeds were debittered by soaking in water at 50 °C in a 1:3 w/v ratio for 18 h with 3 water changes, followed by washing with water at 50 °C for 96 h (Fontanari et al., 2012). Debittered *L. albus* and *L. mutabilis* samples were oven-dried at 60 °C for 10 h, then milled into a 250 μm powder and stored in air tight containers at 4 °C until analysis.

2.2. Fatty acid analysis

The fatty acid composition of raw and debittered *L. albus* and *L. mutabilis* samples were determined in triplicate according to the AOAC 969.33 method (AOAC, 1990) after the conversion of fatty acids into fatty acid methyl esters (FAME). The FAME were analysed by GC – IT/MS in a Clarus 680 gas chromatograph attached to a Clarus 600 mass spectrometer. The following chromatographic operation conditions were used: a capillary column ELITE WAX, DF: 0.25 μm, length: 30 m and ID: 0.32 mm, hydrogen as carrier gas, column temperature: 125 °C gradually increasing by 5 °C min^{-1}, feeder temperature: 220 °C, injection temperature: 200 °C and flame ionization detector temperature: 300 °C. The source temperature of the mass spectrometer was 180 °C, with a filament current of 2.47 and transfer line temperature of 200 °C. The concentrations of individual fatty acids were expressed as g 100 g^{-1} of the total fatty acid methyl esters identified. The total saturated fatty acids (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) as well as relationships between PUFA/SFA and *w*-6/*w*-3 were calculated. The atherogenic index (AI) was calculated according to the equation proposed by Ulbritch and Southgate (1991).

2.3. Statistical analysis

Results were expressed as mean values with standard deviations. A separate analysis for each species was carried out. We performed a paired *t*-test for each fatty acid content and their relationships. Means among treatments were compared based on the Least Significant Difference test (LSD). Pearson's correlation coefficients were used to calculate the magnitude and type of association between each pair of fatty acids. This analysis was performed for each species independently. A canonical variate analysis (CVA) was conducted to discriminate raw and debittered *Lupinus albus* and *L. mutabilis* observations. Quantitative variables considered were common individual fatty acids for both species. In addition, the Mahalanobis squared distances (D^2) were calculated to test if group's observations were statistically different from each other. Statistical analyses were performed by using Infostat software 2017 (Di Rienzo et al., 2017).

3. Results

Table 1 shows the fatty acid compositions of raw and debittered *L. albus* and *L. mutabilis*.

Significant differences were found in the stearic acid, oleic acid, linoleic acid, linolenic acid contents as well as the MUFA and PUFA contents between raw and debittered *L. albus*. Among these differences, the debittering process decreased only the stearic acid content, however it increased the content of all the other fatty acids mentioned above

Table 2
Pearson's coefficients of correlation among fatty acids in *L. albus*.

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Behenic acid	Eicosenoic acid	Erucic acid
Palmitic acid	1								
Stearic acid	0.3	1							
Oleic acid	0.04	−0.6	1						
Linoleic acid	0.1	−0.8*	0.8*	1					
Linolenic acid	−0.2	−0.4	0.9**	0.6	1				
Arachidic acid	−0.3	0.5	−0.04	−0.4	0.3	1			
Behenic acid	−0.2	−0.2	0.7*	0.3	0.9**	0.6	1		
Eicosenoic acid	−0.3	−0.4	0.7	0.6	0.8*	0.4	0.8*	1	
Erucic acid	−0.2	−0.2	0.7*	0.4*	0.8	0.6	0.9**	0.9**	1

* Significant at 0.05 level.
** Significant at 0.01 level.

(Table 1).

Significant differences were found in the stearic and linoleic acid contents as well as the *w*-6/*w*-3 ratio between raw and debittered *L. mutabilis* (Table 1). The stearic acid content increased, whereas the linoleic acid content and the *w*-6/*w*-3 ratio decreased after the debittering process (Table 1).

Of the 36 correlation coefficients evaluated in *L. albus*, 11 showed significant associations (Table 2), whereas in *L. mutabilis* only two of the 21 correlation coefficients evaluated showed significant associations. Negative associations were found between stearic and linoleic acid content ($r = -0.9$; $P = 0.01$) and linoleic and linolenic acid contents ($r = -0.87$; $P = 0.03$) in *L. mutabilis*.

Results of the CVA are shown in Fig. 1. The first two functions were highly significant. The first function discriminated observations according to *Lupinus* species. All the fatty acids except for the oleic acid, showed highly significant canonical correlations ($0.8 < r < 0.9$). Palmitic, stearic and linoleic acids showed a positive correlation whereas linolenic, arachidic and behenic acids were negative correlated. The second function discriminated between raw and debittered *L. albus* in relation to a high oleic acid content ($r = 0.98$). According to the Mahalanobis distance (Table 3), no statistical differences were found between raw and debittered *L. mutabilis*. The greater variation was found between debittered *L. albus* and raw *L. mutabilis*, followed by *L. albus* and *L. mutabilis* debittered samples.

Table 3
Mahalanobis distances (D^2) between four groups of *Lupinus*.

Groups	RLA‡	DBLA	RLM	DBLM
RLA	0			
DBLA	3204**	0		
RLM	35496**	42175**	0	
DBLM	33078**	39557**	114.9	0

** D^2, that is, distances differing from zero at a 99% confidence interval.
‡ RLA: raw *Lupinus albus*; DBLA: debittered *Lupinus albus*; RLM: raw *Lupinus mutabilis* and DBLM: debittered *Lupinus mutabilis*.

4. Discussion

L. albus and *L. mutabilis* showed differences in their fatty acid compositions (Tables 1 and 3). Higher contents of linoleic, palmitic, stearic acids, SFA and *w*-6/*w*-3 ratio and AI were found in raw and debittered *L. mutabilis*, whereas higher contents of PUFA/SFA, behenic, linolenic and arachidic acids were found in *L. albus* (Table 1 and Fig. 1). The debittering process changed the fatty acid composition, however larger changes were observed in *L. albus* rather than in *L. mutabilis* (Fig. 1). In *L. albus*, the changes were observed in the oleic acid, linoleic, linolenic contents and consequently in the total MUFA and PUFA contents (Table 1 and Fig. 1). In addition, eicosenoic and erucic acids were only found in *L. albus* however no changes in these fatty acids

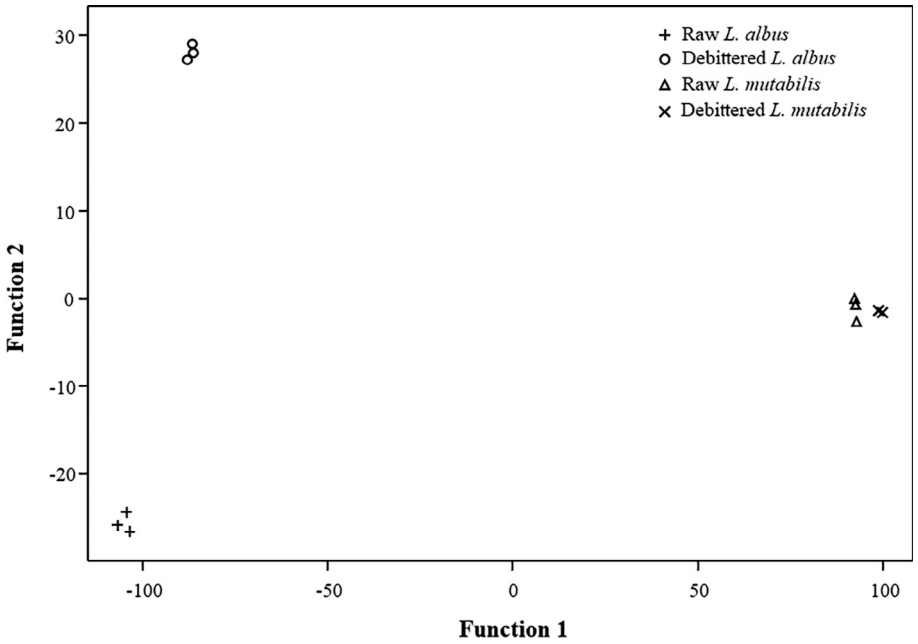

Fig. 1. Separation of *Lupinus* species by the first two canonical functions (Function 1 and Function 2) in relation to the debittering process applied.

were observed after the debittering process. In *L. mutabilis*, slightly changes were found in the stearic and linoleic acid contents as well as the *w*-6/*w*-3 ratio after the debittering process (Table 1). Accordingly, these observations were poorly discriminated in the CVA by showing a closer distance between them (Table 3).

Different changes in the fatty acid composition among *Lupinus* species seemed to be explained by their initial composition and the debittering process applied. These changes might be associated with a desaturation of the stearic acid internal bonds and a conversion into oleic and linoleic acids. According to our results, this mechanism could explain the decrease in the stearic acid content together with the increase in the oleic acid content (Table 1) as well as the high association found between the stearic acid and linoleic acid after the debittering process (Table 2). This mechanism was also used to explain changes in the fatty acid composition in naturally debittered olives (Aktas, Ozen, & Tokatli, 2014). On the other hand, the changes in the oleic, linoleic and linolenic acid contents in *L. albus* could also be associated with a co-existence of tocopherols in the food matrix (Yorgancilar & Bilgiçli, 2014). Tocopherols can prevent lipid peroxidation by acting as peroxide radical scavenging (Hall, Hillen, & Robinson, 2017; Traber & Atkinson, 2007). Similar results were observed in cooked lentils (Pal et al., 2017; Zhang et al., 2014).

In contrast, the cooking stage in *L. mutabilis* induced a fatty acid peroxidation and changed the linoleic acid content and its relationship with the linolenic acid content (Table 1). No substantial changes in the contents of saturated fatty acids were observed after the debittering process (Table 1). These results indicate that the majority of the SFA in *L. mutabilis* remained stable against peroxidation after cooking (Choe & Min, 2005).

Changes in the fatty acid composition after the debittering process in *L. albus* and *L. mutabilis* would have advantageous nutritional implications when consuming seeds in a context of a healthy diet. The oleic acid, MUFA and PUFA contents observed in debittered *L. albus* could be beneficial for chronic disease risk reduction (Hall et al., 2017). The MUFA and PUFA intakes has been associated to a reduction in the total and LDL cholesterol and serum triglycerides (Hall et al., 2017). In addition, a decrease in the *w*-6/*w*-3 ratio in *L. mutabilis* after the debittering process could be a beneficial fact related to the reduction in risk factors associated with coronary heart disease (Hall et al., 2017).

5. Conclusions

The debittering process is a necessary step that *Lupinus* species require to ensure a safe human consumption. Our results showed that the debittering process changed important nutritional components as fatty acids. These changes were larger in *L. albus* than in *L. mutabilis*. Changes in the fatty acid composition depended on the *Lupinus* species and the debittering process applied. While in *L. albus* the MUFA and PUFA contents changed, the *w*-6/*w*-3 ratio was modified in *L. mutabilis*. These changes must be considered when consuming *Lupinus* seeds in a context of a healthy diet.

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Conflict of interest

The authors declared no conflict of interest.

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