Annals of the University of Oradea, Fascicle: Ecotoxicology, Animal Husbandry and Food Science and Technology, Vol. XIX/B 2020
Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara, Vol.XIX/B 2020

ASSESSMENT OF FATTY ACID COMPOSITION AND NUTRITIONAL VALUE OF FATS IN WHITE LUPINE SEEDS FROM LOW-ALKALOID VARIETIES

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Abstract

The aim of the research carried out was to establish by means of chromatography the fatty acid profile of white lupine seeds with low alkaloid content, Amiga variety, obtained in the specific agroclimatic conditions of Transylvania ($47^0$17'03"N$23^0$40'34"E). Oleic acid (C18:1 n-9) (48.8%), which is a monounsaturated fatty acid, is the main fatty acid in the fat composition of the white lupine seeds analyzed. Among the polyunsaturated fatty acid, linoleic acid (C18:2 n-6) (20.12%) followed by linolenic acid (C18: 3 n-3) (9.98%) stands out. Lupine seeds fat shows an optimal omega-6:omega- 3 fatty acid ratio for human health, if considering recommended ratios ranging between 1/1 and 1/4. The high content of polyunsaturated fatty acid indicates that white lupine can be a potential source of fat, which can favorably influence the fatty acid profile of fats in animal agri-food products, with a sanogenous effect for consumers. Moreover, the high content of linoleic acid and linolenic acid highlight lupine seeds a good source of essential fatty acid for human and animal nutrition.

Key words: white lupine, FAME, gas chromatography, omega-6/omega-3.

INTRODUCTION

Old varieties of white lupine, due to their high alkaloid content (2-3%) have a limited use in animal feed. Recently, productive lupine varieties free of alkaloid (less than 0.002%) have been created through breeding works, so that they can be used as main protein source both in monogastric animal feed (pigs and poultry) and in human nutrition.

Lupine seeds contain 90-110 g fat/kg DM and have a high content of omega-3 and omega-6 series fatty acid. If this quality of lupine fats would be transferred to meat and eggs fats, it would lead to the improvement of their sanogenic lipid indices with beneficial effects for consumers (especially on the cardiovascular system). This increases the quality and competitiveness of the product obtained. Some researchers consider that in the future, white lupine seeds fat content could be increased by improvements works, so that they became comparable to soya beans related to protein and fat content (Voisin *et al.*, 2014; Faligowska and Szukała 2015, Reckling *et al.*, 2016).

In general, fats quality depends on the fatty acid profile and content, and also on the ratios of the main fatty acids (Rybinski *et al.,* 2017). In the case of polyunsaturated fatty acids (PUFA), the ratio between omega-6 and omega-3 series fatty acid is very important and especially the ratio between linoleic acid (C18:2 n-6, the main representative of omega-6 fatty acid) and linolenic acid (C18:3 n-3, the main representative of omega-3 fatty acid) both in animal nutrition, but especially in human nutrition (Suchy *et al.*, 2008).

The purpose of the research was to establish lupine seeds fatty acid profile by means of chromatography technique.

MATERIAL AND METHOD

For the studies, white lupine seeds with low alkaloid content, Amiga variety, obtained in the specific agroclimatic conditions of Transylvania (47017'03"N23040'34"E) were used, accordingly to the culture technology recommended by Joordens Zaden BV (Netherlands), from where seeds were purchased to set up the white lupine culture.

The elementary sampling and the achievement of the homogenized raw sample was done according to the norms provided in the standards SR ISO 6498/2012 and STAS 21/3-73. The raw sample was homogenized and cleaned up of impurities in the laboratory, and then minced using the Laboratory mill type Grindomix GM 200. The quarter-end method, described by Salajan *et al.* (1999), was used to obtain the laboratory sample from the homogenized raw sample, which was then stored in tightly sealed glass jars at room temperature until use (Sujak et al., 2006).

The fatty acid profile of fats in white lupine seeds was assessed by using the chromatographic gas method, which involves the conversion of sample fatty acid into methyl esters and their separation on chromatographic column, followed by set up of the fatty acid methyl esters ratio (FAME) from the determined fat.

Fat extraction from the sample was performed according to the method described by Folch *et al.* (1957), using as a solvent a mixture of chloroform-methanol (2:1, v/v). After sample was mixed with solvent and homogenized, the mixture was passed through a separatory funnel, and the filtrate was left to settle for 24 hours. The lower phase containing chloroform was used to dissolve the fat from the sample, it was passed into a flask and brought to dryness by evaporating the chloroform using a rotary evaporator. The fatty acid passed in methyl esters throughout chemical reaction with boron trifluoride/methanol at 80°C for two hours in a closed Pyrex glass tube.

The fatty acids were determined using a Shimadzu GC-17 A gas chromatograph (GC) coupled with a FID (flame ionization detector)

detector and equipped with an Alltech AT-WAX column, 30 m long, 0.25 mm inside diameter and 0.25 µm thickness of the stationary phase (polyethylene glycol). Helium was used as carrier gas at a pressure of 149 kPa. The split ratio was 1:28. A 260^0C temperature was set for the injector and detector. The oven program was: 70^0C for 2 min., then it was raised to 150^0C with a gradient of 10^0C/min., and then a level of 3 min., next it was increased again to 235^0C with a gradient of 4^0C / min (fig. 1). After the gas chromatograph reached the programmed operating parameters, the FID detector for stearic acid was calibrated using tristearin chloroform solution and heptadecanoic acid (internal standard) (fig. 2), after which 0.5 µl hexane solution of fatty acid methyl esters (FAME) was injected using a Hamilton syringe.

The peaks area determination on gas chromatograms was made by using fatty acids authentic standards, achieved from Sigma Aldrich (St. Louis USA). The ratio of fatty acids in the analyzed sample fat was determined by relating the area of fatty acid of the sample to the area of the dilution standard, the result being expressed for each fatty acid as a percentage of fatty acid methyl esters (FAME).

RESULTS AND DISCUSSION

Table 1 data regarding fatty acid composition of fats in lupine seeds, shows that oleic acid (C18:1 n-9) was the main fatty acid, followed by linoleic acid (C18:2 n-6) and then α-linolenic acid (C18:3 n-3).

Each fatty acid concentration was influenced by variety and agroclimatic conditions, but on average, the predominant fatty acid in white lupine seeds are oleic, linoleic and linolenic acid, which is also ascertained by Jezierny *et al.* (2010), Rusníková *et al.* (2013) and Mierliță *et al.* (2018).

Saturated fatty acid content in lupine seeds is relatively low (12.12% of FAME - fatty acid methyl esters), which are represented by: palmitic (C16:0), stearic (C18:0), arachidic (C20 :0) and behenic (C22:0) acids.

Table 1

Fatty acid composition of white lupine seeds fat (% of FAME)

Specification	Own results			Bibliographic references *		
	Mean	(Min - Max)	SD	1	2	3
A. palmitic (C16:0)	6,10	(5,19 - 8,07)	0,43	8,57	4,30	5,86
A. palmitic (C16:1)	0,28	(0,22 - 0,39)	0,03	0,37	0,30	0,32
A. stearic (C18:0)	3,12	(2,54 - 3,87)	0,39	1,57	2,28	2,98
A. oleic (C18:1 n-9)	**48,80**	(41,24 -	2,43	54,29	43,90	47,65

		56,12)				
A. linoleic (C18:2 n-6)	**20,12**	(18,20 - 23,18)	1,96	14,92	22,19	19,97
A. α-linolenic (C18:3 n-3)	**9,98**	(6,43 - 11,51)	0,88	7,22	12,14	10,93
A. arachidic (C20:0)	1,07	(0,97 - 1,63)	0,32	0,81	0,99	0,90
A. gadoleic (C20:1 n-9)	5,51	(4,70 - 6,20)	0,47	4,14	8,14	6,82
A. behenic (C22:0)	1,83	(1,65 - 2,21)	0,24	2,73	ND	3,15
A. erucic (C22:1 n-9)	**1,34**	(0,97 - 1,38)	0,38	1,59	ND	1,42
Σ Saturated fatty A.	**12,12**	(9,71 - 14,08)	1,51	16,14	12,52	12,89
Σ Monounsaturated fatty A.	**55,93**	(51,43 - 60,37)	4,29	58,79	52,34	56,21
Σ Polyunsaturated fatty A.	**30,10**	(27,92 - 33,80)	3,21	25,07	35,14	30,90
n-6 / n-3	**2,02**	(1,22 - 3,71)	0,13	2,11	1,90	1,83
Polyunsaturated Index (PI)	**40,08**	-	-	29,36	46,47	41,83

*1- Andrzejewska *et al.*, 2016; 2 - Zraly *et al.*, 2007; Mierliță *et al.*, 2018.
FAME - fatty acid methyl esters; ND – not determined.

Monounsaturated fatty acid (MUFA) were the most common in fats structure of lupine seeds, representing 55.93% of the total fatty acid identified using chromatographic analysis (Fig. 1). The highest concentration was recorded in case of oleic acid (C18:1 n-9), which held 48.8% of FAME (fatty acid methyl esters). The results presented are generally consistent with the values obtained by Erbas *et al.* (2005), Uzun *et al.* (2007), and Rybinski *et al.* (2017). However, we should note that oleic acid concentration and implicitly monounsaturated fatty acid concentration in white lupine seeds was lower in our study, compared to those reported by Andrzejewska *et al.* (2016) (54.29% for C18:1 n-9 and 58.79% for MUFA, respectively), but higher than those refered by Zraly *et al.* (2007) in white lupine Amiga variety (43.9% in C18:1 n-9 and 52.34% in MUFA).

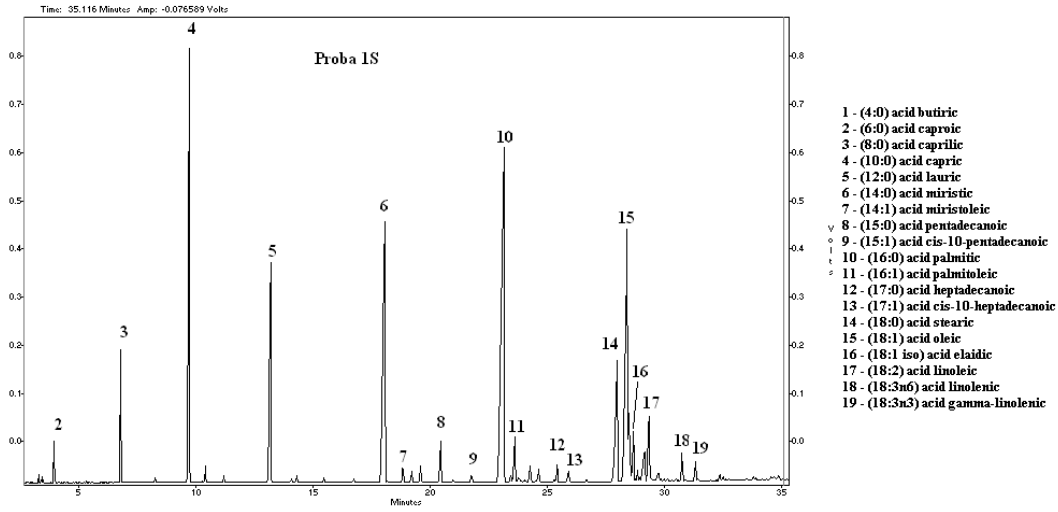

Fig. 1. Representative chromatogram of fatty acid in lupine seeds

The presence of erucic acid (C22:1) in food and feed is not desirable due to its toxic and antinutritional effects in humans and animals (Bhardwaj and Hamma, 2013). Our studies presented in the present paper, along with the data reported by Rybinski *et al.* (2017) is showing an advantage of white lupine, which is the presence of erucic acid in small amounts. The minimum and maximum values of erucic acid ranged from 0.97 to 1.38%.

The white lupine seeds polyunsaturated fatty acid content (PUFA) rreached values between 27.92% and 33.8%, with a 30.1% average value of total fatty acid identified. PUFAs were represented by linoleic acid (C18:2 n-6) in a ratio of 20.12% and linolenic acid (C18:3 n-3) in a ratio of 9.98% of FAME (fig. 2).

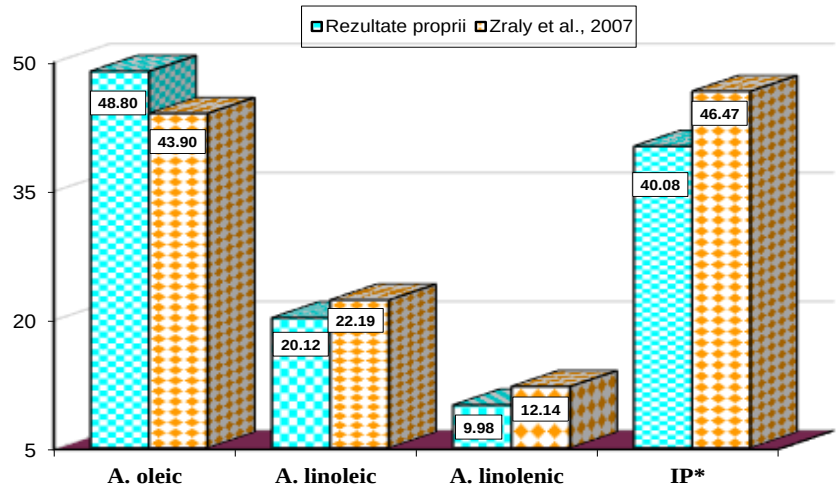

Fig. 12. Ratio of the main fatty acid and polyunsaturation index (IP) of white lupine seeds fats

Large range of variation of linolenic acid concentration (6.43 - 11.51%) indicates the possibility of selection for a high content of omega-3 fatty acid series of white lupine seeds. A large range of variability (5.6-12.8%) was also reported by Rybinski *et al.* (2017). Moreover, even data referenced in scientific publications related linolenic acid in fat content in white lupine seeds vary a lot: 7.22% linolenic acid, in the study performed by Andrzejewska *et al.* (2016) and 12.14% linolenic acid in the study carried out by Zraly *et al.* (2007).

Omega-6/omega-3 fatty acid ratio is very important in human nutrition and should be between 1:1 and 1:4 (Simopoulos, 2003). The white lupine seeds analyzed in this study meet this criterion, linoleic acid (n-6) and linolenic acid (n-3) ratio being 1: 2.02, with variation limits between 1: 1.22 and 1: 3.71, due to linolenic acid concentration large variation limits.

The present study shows high values of polyunsaturation index (PI) of fats in lupine seeds, which indicates that lupine contains a large amount of polyunsaturated fatty acid (PUFA). PUFA input in human diets is recommended for cardiovascular diseases prevention (Simopoulos, 2003), and on the other hand polyunsaturated fatty acid are precursors for long-chain omega-3 polyunsaturated fatty acid biosynthesis, specifically eicosanoids, which act as biological regulators of many cellular processes and immune system (Ijarotimi *et al.*, 2015).

CONCLUSIONS

The high content of polyunsaturated fatty acid indicates that white lupine can be a potential source of fat, which can favorably influence the fatty acid profile of animal agri-food products, with a sanogenic effect for consumers. Furthermore, linoleic acid and linolenic acid high content of lupine seeds indicate that it as a good essential fatty acid source for human and animal nutrition.

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