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COLORIMETRIC VS. CHROMATOGRAPHIC ANALYSES OF ALKALOIDS IN LUPIN SEEDS

ABSTRACT

A characteristic trait of lupins is a production of alkaloids, which are a toxic and bitter taste compound of seeds. Due to the lack of fast, sensitive and inexpensive screening techniques to identify and reject high alkaloid plant material, development of suitable tools is important challenges for lupins breeding and seed production. The aim of this study was to compare two alkaloid content estimation methods in *Lupinus angustifolius* L. and *Lupinus albus* L.

During the Wagner's colorimetric test, which is recommended by the UPOV, seed halves were stained on four colors depending on the alkaloid content but only the level of 0.5% – 0.6% showed clear color change. Gas chromatography allowed accurate quantification and qualification of alkaloid content.

Since safe alkaloid content for consumption is 0.02% of seed dry weight, colorimetric method is less useful for dividing lupin cultivars into sweet and bitter, than gas chromatography but can be used as a screening technique.

Key words: gas chromatography; *Lupinus angustifolius* L.; *Lupinus albus* L.; sweet/bitter lupins; Wagner's colorimetric test

INTRODUCTION

The genus *Lupinus* covers four lupin crops: white lupin (*Lupinus albus* L.), narrow-leafed lupin (*Lupinus angustifolius* L.), yellow lupin (*Lupinus luteus* L.) distributed in the Mediterranean basin and Andean lupin (*Lupinus mutabilis* Sweet) originating from the South America. Despite their usage in crop rotation (N-fixation), fertilization, and ornamental, the main usage is as dry seeds for high protein content (up to 45%) and sometimes for oil

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content (up to 14%). Lupin domestication has a short history and the high-yielding cultivars available now are the result of the work of just three generations of breeders (Brummund & Święcicki, 2011) Alkaloids are the antinutritional compounds present in the lupin seeds. Their content can reach 2.88% of the seed dry weight (DW) in narrow-leafed lupin and even 12.73% of the seed DW in white lupin (Kamel *et al.* 2016; Kroc *et al.* 2016) (http://www.igr.poznan.pl/uploads/Lupinus angustifolius-1.pdf; http://www.igr.poznan.pl/uploads/biologiczne%20bazy%20danych%202016/The%20total%20alkaloid%20content%20and%20qualitative%20composition%20of%20%E2%80%A6.pdf)

First, lupins with less alkaloid content were selected and described in twenties/thirties of twenties century (Brummund and Święcicki, 2011; Święcicki *et al.* 2015). Alkaloid content in modern cultivar is e.g. 0.0089% of the seed DW in the narrow-leafed lupin cv. 'Lazur' (Synthesis of results of register trials, 2015). According to Cowling *et al.* (1998) a safe content of alkaloids for consumption is below 0.02% of the seed DW. Guidelines of the International Union for the Protection of New Varieties of Plants (UPOV) (UPOV Guidelines, 2004) divide lupin cultivars into bitter (high-alkaloid) and sweet (low-alkaloid) based on cheap Wagner's colorimetric test and suggest controls, bitter and sweet cultivars, for a given lupin crop (UPOV Guidelines 2004). This test is based on the color reaction of seed cotyledons to the presence of alkaloids. Therefore, the aim of study was to reveal the level of alkaloid content which results in a visible color reaction. Results will be useful for breeding selection and distinction, homogeneity and stability (DHS) description of the cultivars.

MATERIAL AND METHODS

Plant material

For two lupin crops, 20 accessions each with differentiated alkaloid content were selected (narrow-leafed lupin: from 0.007% to 1.50% in seed DW; white lupin: from 0.039% to 3.13% in seed DW), based on the Polish Lupin Collection Database (http://www.igr.poznan.pl/uploads/Lupinus%20angustifolius-1.pdf; http://www.igr.poznan.pl/uploads/biologiczne%20bazy%20danych%202016/The%20total%20alkaloid%20content%20and%20qualitative%20composition%20of%20%E2%80%A6.pdf), UPOV controls: narrow-leafed lupin, bitter cv. 'Azuro' (Wt 95941) and sweet cv. 'Bordako' (Wt 96192) and white lupin, bitter cv. 'Feli' (Wt 95531) and sweet cv. 'Nelly' (Wt 95480) were included in the study. Plants were grown for seed multiplication in the Plant Breeding Station at Wiatrowo (Poznan Plant Breeders Ltd.) during the vegetation season April–August 2015 and harvested in full maturity (water content 13%).

Gas chromatography

From each accession 100 g seeds were sampled. From each sample 10 g was milled for estimation of the total and quantitative composition of alkaloids according to the procedure described by Kamel *et al.* 2016.

Wagner's colorimetric test

The Wagner reagent was prepared in following way. First, 14 g of potassium iodide was dissolved in purified water 7 days before analyzes. Then, 10 g of iodine was added along with purified water up to 100 ml in a dark volumetric flask. The solution was maintained in dark till the analyzes. Before using for the tests, it was diluted five times.

For the colorimetric test, 10 seeds were cut into halves and placed on Petri dishes. Each half of the seed was plunged into the diluted Wagner reagent for 10 seconds and then in purified water for 5 seconds. Then a coloration of the lupin seed halves was observed.

Statistical analysis

The basic statistical characteristics describing quantitative composition of alkaloids in two lupin species were calculated. Also, an analysis of variance for complete randomized design with equal replications was conducted to study substantial differences between means for a percentage share of individual alkaloids.

RESULTS AND DISCUSSION

The quantitative composition of the alkaloids showed clear differences between the lupin crops and was similar to the earlier investigations available in the Polish Lupin Collection (Kamel *et al.* 2016; Kroc *et al.* 2016) (Table 1; http://www.igr.poznan.pl/uploads/Lupinus%20angustifolius-1.pdf; http://www.igr.poznan.pl/uploads/biologiczne%20bazy%20danych%202016/The%20total%20alkaloid%20content%20and%20qualitative%20composition%20of%20%E2%80%A6.pdf). Four major alkaloids (abundance >1% of the total alkaloids) were identified in the narrow□leafed lupin: lupanine, 13-hydroxylupanine, angustifoline and isolupanine; but six were identified in the white lupin: lupanine, 13-hydroxylupanine, multiflorine and angustifoline, albine (mean content 11.88% in seed DW) and 11,12-seco-12,13-didehydromultiflorine (mean content 2.64% in seed DW), both absent in the narrow-leafed lupin (isolupanine is a minor alkaloid, abundance <1%).

The results of the statistical analysis performed to study differences between two species for quantitative content of alkaloids were given in Table 1. This analysis showed a substantially higher mean percentage share of 13-hydroxylupanine, angustifoline and isolupanine in the narrow-leafed lupin, but lupanine and multiflorine in the white lupin.

Table 1

Statistical characteristics of the narrow-leafed lupin (Nar) and white lupin (Whi) alkaloids and results of testing differences between means

Alkaloid	Lupin species	[%Values of total content]		Coefficient of variation [%]	Mean	Differences between means (Nar-Whi)
		Minimum	Maximum			
Lupanine	Nar	11.45	83.00	36.72	49.86	-13.63*
	Whi	30.69	84.93	19.97	63.49	
13-hydroxylupanine	Nar	8.87	65.52	46.67	28.41	18.78*
	Whi	3.08	22.77	52.25	9.64	
Angustifoline	Nar	4.80	25.12	31.25	15.01	11.02*
	Whi	1.11	9.28	50.33	3.99	
Isolupanine	Nar	0.73	14.25	83.81	3.98	3.30*
	Whi	0.30	2.86	92.68	0.68	
Multiflorine	Nar	0.01	2.6	107.38	0.64	-5.15*
	Whi	1.08	17.30	78.32	5.79	

* significant at $p<0.01$

Table 2.

A comparison of two methods of alkaloid content estimation in lupin seeds.

Narrow-leafed lupin			**White lupin**		
Accession number	**Total alkaloid content [% seed DW]**	**Color of seed half**	**Accession number**	**Total alkaloid content [% seed DW]**	**Color of seed half**
96126	0.0015	*	95449	0.0104	*
96225	0.0023	*	95472	0.0250	*
96164	0.0035	*	**95480**[a]	**0.0737**	*
96101	0.0044	*	95496	0.0755	*
96193	0.0062	*	95404	0.1203	**
96131	0.0071	*	95494	0.1337	**
95935	0.0098	*	95422	0.1582	**
96191	0.0146	*	95487	0.2867	**
96195	0.0173	*	95433	0.3649	**
96192[a]	**0.0192**	*	95507	0.4284	**
96114	0.0195	*	95174	0.5427	***
96182	0.0198	*	95168	0.6076	***
96212	0.0296	*	95176	0.6432	***
95927	0.0584	*	95476	0.6774	***
95928	0.0685	*	95486	0.7870	*
96199	0.0760	*	95443	0.9304	***
95916	0.1851	**	95242	1.0264	****
96110	0.4083	**	**95531**[b]	**1.0629**	***
95941[b]	**0.5643**	***	95457	1.1470	****
95719	0.7698	***	95232	1.1833	****
95932	0.7721	***	95208	1.3793	****
95714	0.9774	***	95503	1.7711	****

[a] sweet control [b] bitter control; Half seed color (cotyledons) after Wagner reagent treatment: * yellow, ** dark yellow, *** brown, **** dark brown

The most important aim of this study was to compare the results obtained by two methods, colorimetric and chromatographic (Table 2). The total alkaloid content was differentiated, in the narrow-leafed lupin accessions from 0.0015% to 0.9774% in seed DW (sweet control – 0.1920%, bitter control – 0.5643%) and in the white lupin from 0.0104% to 1.7711% (sweet control – 0.0737%, bitter control – 1.0629%). After the treatment with the Wagner reagent, seed halves (cotyledons) showed four colors: yellow, dark yellow, brown and dark brown. In both lupin crops a clear color change due to the presence of alkaloids is brown color with their content level 0.5%–0.6% in seed DW. So, the Wagner's colorimetric method does not reveal desirable low alkaloid content (0.02% in seed DW maximum), safe for feeding and as such is less useful for dividing lupin cultivars into sweet and bitter. But it can be used in breeding and seed production as an introductory screening technique allowing to select or reject bitter plant material. For further estimation and selection, the chromatographic method must be involved.

CONCLUSIONS

A decrease of alkaloid content in lupin plants is an important aim in breeding. Current challenges for lupins breeding and seed production include the development of fast, sensitive and inexpensive screening techniques to identify and reject high alkaloid plant material. The presented study is focused on comparison of two the most popular alkaloid estimation methods, Wagner's colorimetric test and gas chromatography. Our results show, that Wagner's test can be used only as an introductory screening technique, because clear color change can be observed only on the level of 0.5% – 0.6% of the total alkaloid content. Unfortunately, this is too little sensitivity, since the safe content for consumption amounts 0.02% of seed dry weight. It suggests, that colorimetric method is less useful for distinguishing of sweet lupin cultivars from bitter, than gas chromatography, which clearly determines a qualitative and quantitative alkaloid content. This information would be very helpful for lupin breeders worldwide.

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