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The content of raffinose oligosaccharides in legumes and their importance for animals

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ABSTRACT. The interest of consumers, as well as the food and feed industry in legumes is constantly increasing. However, the use of legume seeds is limited by the fact that they contain various non-nutritive compounds such as raffinose family oligosaccharides (RFOs). On the other hand, RFO compounds are considered prebiotic substances improving health and growth of organisms. Our study consisted in the long-term monitoring of RFO contents in seeds of several legumes cultivated in Poland. The seeds of pea (25 cultivars), faba bean (5 cultivars), and lupins (41 cultivars) were harvested between 2013 and 2019, whereas soybean seeds (27 cultivars) were harvested between 2015 and 2019. The analysis showed that the content of RFOs in soybean seeds ranged from 33.75 to 69.30 mg per g dry matter (DM), 57.23 to 130.38 mg/g DM in lupin seeds – 52.03 to 80.60 mg/g DM in pea seeds, and from 32.15 to 65.17 mg/g DM in faba bean seeds. Yellow lupin seeds had the highest total RFO contents in DM, whereas faba bean and soybean seeds showed the lowest RFO contents. Stachyose was the dominant oligosaccharide in all soybean and lupin seeds, while verbascose was the dominant oligosaccharide in most pea and faba bean seeds. Some pea cultivars contained more stachyose than verbascose. Crop species, cultivar, growing environment, and processing methods determine the suitability of individual seeds for the feed and food industry.

Introduction

In recent years, there has been a growing interest in the use of legume seeds for human and animal nutrition for various reasons. Independence from the import of genetically modified soybean products, feed quality and price play an important role in animal nutrition (Święcicki et al., 2020). Legume seeds are a rich source of nutrients for humans, thus their consumption prevents various diseases of civilization, such as hypertension, type 2 diabetes, hyperlipidaemia, and overweight (Conti et al., 2021). Legumes can fix atmospheric nitrogen, improve soil physical condition, require lower energy input (Rubiales and Mikic, 2015) and perfectly complement crop rotation.

It is well known that legumes also contain antinutrients (Gulewicz et al., 2014), such as trypsin inhibitors, lectins, phytic acid, tannins, quinolizidine alkaloids, saponins, and raffinose family oligosaccharides (RFOs). They have a diverse structure, and their activity and concentration in seeds are species-specific. Chemically, these compounds are sucrose homologues with (1→6) glycosidic linkages of galactose molecules. RFOs perform critical physiological functions. They increase drought and low temperature resistance, are responsible for respiration during germination, and extend the shelf life of legumes (Gu et al., 2018). Although RFOs are considered antinutrients for animals (Banti, 2021), recently these compounds have been recognized as functional substances with prebiotic activity (Amorim et al., 2020). They play a dual function because they contain (1→6) glycosidic bonds that are not digested in the gastrointestinal tract of monogastric animals due to the lack of α-1-6 galactosidase. Undigested carbohydrates enter the distal part of the ileum, where harmful bacteria produce gases such as carbon dioxide, hydrogen, and methane. This causes digestive disorders, discomfort, and consequently reduces the nutritional effect of food and feed (Kaczmarek et al., 2014; Rubiales and Mikic, 2015; Zartl et al., 2018). Many researchers observed that the elimination of oligosaccharides from legume seeds exerted positive nutritional effects on animals (Kasprowicz-Potocka et al., 2013; 2015; Zhang et al., 2019; Zaworska-Zakrzewska et al., 2020). On the other hand, authors of various studies also indicated that RFOs might be treated as bioactive compounds with nutritive properties (Gulewicz et al., 2014; Zartl et al., 2018; Abdel-Latif et al., 2020; Amorim et al., 2020; Karimi et al., 2020; Conti et al., 2021). The fact that RFOs cannot be digested in the upper gastrointestinal tract has sparked scientific interest in these compounds as potential prebiotics. *In vivo* and *in vitro* research showed that RFOs supported the growth of probiotic bacteria, exerted no adverse effects on the immune system, increased the concentration of short-chain fatty acids (SCFAs) in the caecum of rats, and when administered *in ovo*, improved the slaughter parameters of chickens (Bednarczyk et al., 2011). Fernando et al. (2010) found that raffinose could modulate intestinal microbial composition and promote intestinal health in humans. Moreover, dietary RFO supplementation significantly reduced the production of odour compounds by modulating the caecal microbial community (Zeng et al., 2021). A single dose of RFOs delivered *in ovo* on day 12 of egg incubation beneficially modulated chick body systems and did not affect optimal performance (Stadnicka et al., 2020).

RFOs sourced from legume seeds can be considered a promising prebiotic for animals and humans, provided that their extraction technology is scaled up for supplementation of large feed quantities. This article presents the results of long-term research on the content of oligosaccharides in the seeds of domestic legumes grown in Poland. The authors also recommend the best legume species and cultivars for animals, as well as the RFO extraction technology.

Material and methods

Seeds

Soybean seeds (*Glycine max* L.), seeds of white lupin (*Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.), narrow-leafed lupin (*Lupinus angustifolius* L.), pea seeds (*Pisum sativum* L.), and faba bean seeds (*Vicia faba* L.) used to measure the content of oligosaccharides of the raffinose family came from the following breeding stations: Agro Youmis, Saaten Union, Saatbau, Danko, Prograin ZIA, EURALIS, Poznań University of Life Sciences, and the HR Strzelce IHAR Group (Poland). Pea, faba bean, and lupin seeds were harvested between 2013 and 2019, while soybean seeds were harvested between 2015 and 2019. After harvesting, the seeds were cold-stored at a temperature of +4 °C. Measurements were always carried out after harvest in triplicate.

Oligosaccharide contents

For chemical analysis, representative seed samples were ground and passed through a 0.5-mm sieve. High-pressure gas chromatography was used to measure RFO contents according to the procedure described by Lahuta et al. (2018). Seeds (1 g) were pulverized in a MM200 mixer mill (Retsch, Germany) for 2 min at a frequency of 22 Hz. Carbohydrates were extracted from pulverized material (40–45 mg each, in triplicate) with 900 µl of 50% aqueous ethanol solution containing xylitol (100 µg) as an internal standard. After heating at 90 °C for 30 min (with continuous shaking at 300 rpm), the samples were centrifuged (21 000 *g* for 30 min at 4 °C) and 400 µl of homogenate was deionized with the mixture (300 µl) of Dowex ion exchange resins for 45 min (with shaking at 1 250 rpm). After centrifugation, a portion of the clear extract (200 µl) was concentrated (in 2-ml gas chromatography vials containing glass inserts) in a rotary evaporator until dryness. Dry residues were derivatised with a mixture of TMSI (trimethylsilyl-imidazole) and pyridine (1:1, v/v) at 80 °C for 45 min. Carbohydrate TMS-derivatives were separated in a ZB-1 capillary column (15 m length, 0.25 mm diameter, 0.1 µm thickness; Phenomenex, Torrance, CA, USA) in a GC 2010 gas chromatograph (Shimadzu, Kyoto, Japan). The temperature of the injector and detector (flame ionization detector) was 325 and 350 °C, respectively. The column was heated from 150 to 350 °C at different rates of temperature increase. Helium was used as the carrier gas. The content of oligosaccharides was expressed as mg per seed dry matter (DM).

Statistical analysis

The SAS Enterprise Guide 9.1 (Cary, NC, USA) software was used for statistical analysis. The values were expressed as means ± standard deviation (SD) for DM (mg/g) and as means for percentages (%). Only total RFO content was analysed using one-way analysis of variance. Duncan's test was used to compare differences between means with high-range statistical domain at $P \leq 0.05$.

Results

The tables show the results of measurements of the total content of raffinose oligosaccharides (TRFOs) and the percentage of individual oligosaccharides in soybean seeds (Table 1), lupin seeds – white lupin, yellow lupin, and narrow-leafed lupin (Table 2), pea seeds (Table 3), and faba bean seeds (Table 4).

The average RFO contents in soybean seeds amounted to 50.5 mg per 1 g DM (from 33.75 to 69.3 mg/g), lupin seeds – 84.9 mg/g DM (from 57.23 to 130.38 mg/g), pea seeds – 65.6 mg/g DM (from 52.03 to 80.60 mg/g), and faba bean seeds – 50.1 mg/g DM (from 32.15 to 65.17 mg/g). Yellow lupin seeds had the highest total RFO contents in DM – 112.6 mg/g on average. The lowest total RFO contents was found in faba bean and soybean seeds – approx. 50 mg/g DM on average.

The following soybean cultivars had significantly the highest ($P \leq 0.05$) total RFO contents (approx. 69 mg/g DM): Paradis, Augusta, and Protina.

Table 1. Total content of raffinose family oligosaccharides and the percentage of individual saccharides in soybean (*Glycine max*) seeds, mg/g DM

Cultivar	Total RFO	Raffinose	Stachyose	Verbascose
Abelina (n = 3)	50.9 ± 3.08cF	8.6 ± 0.45	40.6 ± 2.36	1.7 ± 0.23
% TRFOs	100.00	16.87	79.73	3.40
Aldana (n = 5)	51.2 ± 3.21cF	10.6 ± 0.78	38.3 ± 1.44	2.3 ± 0.88
% TRFOs	100.00	20.71	74.88	4.40
Aligator (n = 2)	53.7 ± 2.56cF	9.1 ± 0.74	42.9 ± 2.41	1.7 ± 0.22
% TRFOs	100.00	16.89	79.96	3.16
Amandine (n = 2)	40.7 ± 2.33dI	7.8 ± 2.36	31.3 ± 1.54	1.5 ± 0.05
% TRFOs	100.00	19.24	77.00	3.76
Annushka (n = 4)	58.1 ± 3.24bG	9.8 ± 0.84	46.9 ± 2.57	1.5 ± 0.09
% TRFOs	100.00	16.85	80.64	2.51
Augusta (n = 5)	**69.3 ± 4.23aF**	**12.3 ± 1.01**	55.0 ± 3.02	2.0 ± 0.11
% TRFOs	100.00	17.74	79.33	2.93
Brunensis (n = 3)	50.6 ± 4.11dF	8.7 ± 0.41	39.7 ± 0.21	2.2 ± 0.09
% TRFOs	100.00	17.16	78.53	4.31
Caroline (n = 2)	43.8 ± 2.88dI	9.1 ± 0.77	33.3 ± 2.41	1.4 ± 0.14
% TRFOs	100.00	20.83	76.04	3.13
Erica (n = 6)	50.6 ± 4.37dG	9.1 ± 0.74	40.1 ± 3.14	1.4 ± 0.07
% TRFOs	100.00	18.00	79.33	2.67
ES Commandor (n = 2)	**33.8 ± 2.09fK**	7.7 ± 0.39	25.1 ± 0.21	1.0 ± 0.07
% TRFOs	100.00	22.76	74.37	2.87
ESG152 (Favor) (n = 2)	44.6 ± 1.23dI	11.7 ± 0.59	31.1 ± 2.61	1.8 ± 0.18
% TRFOs	100.00	26.25	69.78	3.97
ESG1711 (Governor) (n = 2)	39.3 ± 2.99dI	10.2 ± 0.99	28.4 ± 0.21	0.7 ± 0.08
% TRFOs	100.00	25.93	72.19	1.88
Lajna (n = 2)	35.0 ± 3.01dK	8.9 ± 0.77	25.3 ± 2.19	0.8 ± 0.09
% TRFOs	100.00	25.41	72.22	2.37
Lissabon (n = 3)	42.6 ± 3.99dI	10.6 ± 0.91	31.1 ± 2.32	0.9 ± 0.88
% TRFOs	100.00	24.94	72.95	2.10
Madlen (n = 4)	57.2 ± 0.44cG	9.5 ± 0.87	46.2 ± 3.63	1.5 ± 0.14
% TRFOs	100.00	16.55	80.82	2.63
Mavka (n = 3)	58.5 ± 0.49cG	7.9 ± 0.56	48.8 ± 4.12	1.9 ± 0.21
% TRFOs	100.00	13.45	83.32	3.23
Maya (n = 2)	43.5 ± 3.11dI	6.1 ± 0.59	36.1 ± 3.44	1.2 ± 0.11
% TRFOs	100.00	14.12	83.05	2.83
Meridian (n = 3)	40.1 ± 3.34dI	8.2 ± 0.67	30.4 ± 3.19	1.5 ± 0.08
% TRFOs	100.00	20.44	75.87	3.69
Merlin (n = 4)	62.6 ± 5.69bF	9.0 ± 0.36	51.7 ± 1.07	1.9 ± 0.12
% TRFOs	100.00	14.39	82.61	3.00
Moravians (n = 2)	**34.2 ± 1.22fK**	6.4 ± 0.27	27.1 ± 1.86	0.7 ± 0.04
% TRFOs	100.00	18.67	79.17	2.16
Naya (n = 2)	49.6 ± 2.33dG	7.5 ± 0.29	38.8 ± 1.78	3.2 ± 0.17
% TRFOs	100.00	15.16	78.30	6.54

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Table 1. continued

Cultivar	Total RFO	Raffinose	Stachyose	Verbascose
Paradis (n = 2)	**69.4 ± 6.01[aF]**	8.1 ± 0.78	60.3 ± 4.33	1.0 ± 0.09
% TRFOs	100.00	11.70	86.82	1.48
Petrina (n = 3)	59.0 ± 2.99[bG]	9.3 ± 0.42	47.1 ± 2.23	2.6 ± 1.47
% TRFOs	100.00	15.82	79.81	4.36
Protina (n = 2)	**68.3 ± 5.01[aF]**	6.4 ± 0.54	60.2 ± 3.48	1.8 ± 0.73
% TRFOs	100.00	9.30	88.14	2.56
Silesia (n = 4)	48.9 ± 3.21[dH]	8.6 ± 0.78	37.1 ± 3.06	3.2 ± 0.23
% TRFOs	100.00	17.54	75.85	6.60
Sirelia (n = 2)	55.0 ± 4.71[cG]	7.3 ± 0.29	45.9 ± 2.78	1.8 ± 0.08
% TRFOs	100.00	13.36	83.42	3.22
Solena (n = 2)	52.4 ± 3.89[dG]	8.1 ± 0.49	41.8 ± 0.39	2.5 ± 0.18
% TRFOs	100.00	15.52	79.71	4.77
Soybean, mean	**50.5 ± 8.13**	**8.8 ± 1.13**	**40.0 ± 7.94**	**1.7 ± 0.51**
% TRFOs, mean	**100.00**	**18.01**	**78.66**	**3.35**

DM – dry matter, RFOs – raffinose family oligosaccharides, TRFOs – total content of raffinose family oligosaccharides; the results are expressed as mean ± standard deviation (SD); [a-I, A-K] – means within a column with different superscripts are significantly different at $P < 0.05$; the values marked with the same letters do not differ significantly at $P > 0.05$; lowercase letters refer to the results in the table (for individual species); uppercase letters refer to all results

Table 2. Total content of raffinose family oligosaccharides and the percentage of individual saccharides in white lupin *(Lupinus albus* L.), yellow lupin (*Lupinus luteus* L.), and narrow-leafed lupin (*Lupinus angustifolius* L.) seeds (mg/g DM)

Species	Cultivar	Total RFO	Raffinose	Stachyose	Verbascose
White lupin	Boros (n = 7)	107.3 ± 11.95[cC]	8.0 ± 2.37	84.3 ± 10.18	15.1 ± 4.60
	% TRFOs	100.00	7.41	78.53	14.06
	Butan (n = 6)	105.3 ± 12.53[cC]	7.6 ± 2.26	84.7 ± 12.11	13.0 ± 2.94
	% TRFOs	100.00	7.23	80.47	12.30
	White lupin mean	**106.3 ± 1.00**	**7.8 ± 0.18**	**84.5 ± 0.23**	**14.0 ± 1.06**
	% TRFOs, mean	**100.00**	**7.32**	**79.50**	**13.18**
Yellow lupin	Baryt (n = 6)	124.3 ± 12.43[aA]	13.0 ± 1.95	60.0 ± 1.69	51.2 ± 6.76
	% TRFOs	100.00	10.48	48.30	41.22
	Bursztyn (n = 5)	**129.0 ± 10.58[aA]**	11.0 ± 1.72	67.8 ± 3.85	50.2 ± 4.43
	% TRFOs	100.00	8.56	52.52	38.92
	Dukat (n = 3)	116.0 ± 8.58[bB]	**13.0 ± 1.43**	54.7 ± 4.89	48.3 ± 4.36
	% TRFOs	100.00	11.21	47.12	41.67
	Lord (n = 6)	105.0 ± 4.43[cC]	10.4 ± 0.97	60.4 ± 6.25	42.9 ± 4.31
	% TRFOs	100.00	9.85	50.27	39.88
	Mister (n = 7)	105.0 ± 9.33[cC]	9.6 ± 0.89	57.0 ± 5.67	38.4 ± 3.89
	% TRFOs	100.00	9.15	54.29	36.56
	Parys (n = 4)	100.9 ± 3.68[dC]	11.8 ± 0.73	46.2 ± 0.89	43.0 ± 2.94
	% TRFOs	100.00	11.65	45.78	42.58
	Perkoz (n = 7)	**130.4 ± 7.54[aA]**	**13.2 ± 1.71**	63.7 ± 5.51	54..0 ± 2.61
	% TRFOs	100.00	10.04	49.21	40.75
	Poster (n = 3)	93.2 ± 1.21[eD]	11.7 ± 0.56	46.8 ± 0.57	34.7 ± 0.34
	% TRFOs	100.00	12.55	50.21	37.23
	Puma (n = 6)	107.8 ± 4.77[cC]	8.8 ± 0.27	52.2 ± 2.50	46.9 ± 2.54
	% TRFOs	100.00	8.15	48.37	43.49
	Taper (n = 4)	114.0 ± 3.80[bB]	12.3 ± 0.89	63.4 ± 1.66	38.5 ± 1.81
	% TRFOs	100.00	10.76	55.54	33.70
	Bojar (n = 3)	**68.7 ± 1.54[gF]**	10.0 ± 0.27	44.6 ± 2.58	14.0 ± 3.80
	% TRFOs	100.00	14.84	64.90	20.26
	Yellow lupin, mean	**112.6 ± 10.16**	**11.5 ± 1.21**	**57.2 ± 5.84**	**44.8 ± 5.31**
	% TRFOs, mean	**100.00**	**10.24**	**50.16**	**39.60**
Narrow-leaved lupin	Bolero (n = 6)	77.6 ± 3.96[fE]	9.4 ± 0.72	36.9 ± 1.06	31.4 ± 1.62
	% TRFOs	100.00	12.09	47.53	40.38
	Boros (n = 3)	**57.2 ± 1.52[hG]**	8.9 ± 0.21	30.4 ± 0.99	17.9 ± 0.84
	% TRFOs	100.00	15.52	53.15	31.33
	Boruta (n = 5)	74.6 ± 1.36[fE]	8.9 ± 0.45	43.6 ± 0.72	22.1 ± 0.64
	% TRFOs	100.00	11.93	58.38	29.68

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Table 2. continued

Species	Cultivar	Total RFO	Raffinose	Stachyose	Verbascose
	Dalbor (n = 7)	69.8 ± 0.97^{fF}	9.0 ± 0.95	38.6 ± 0.47	22.2 ± 0.68
	% TRFOs	100.00	12.95	55.31	31.74
	Graf (n = 4)	70.9 ± 3.06^{fE}	8.3 ± 0.31	46.3 ± 3.03	16.3 ± 0.29
	% TRFOs	100.00	11.66	65.39	22.95
	Heros (n = 2)	71.1 ± 4.55^{fE}	8.8 ± 0.19	43.3 ± 1.87	19.1 ± 1.07
	% TRFOs	100.00	12.38	60.84	26.78
	Homer (n = 3)	77.1 ± 1.45^{fE}	8.6 ± 0.65	47.1 ± 0.83	21.5 ± 0.98
	% TRFOs	100.00	11.18	61.00	27.81
	Jowisz (n = 5)	75.7 ± 5.63^{fE}	9.2 ± 0.95	38.7 ± 1.45	27.9 ± 1.02
	% TRFOs	100.00	12.09	51.12	36.79
	Kadryl (n = 3)	75.5 ± 5.41^{fE}	9.5 ± 1.35	45.9 ± 2.35	20.0 ± 1.70
	% TRFOs	100.00	12.63	60.85	26.52
	Kalif (n = 2)	72.8 ± 4.91^{fE}	**11.3 ± 1.44**	43.1 ± 4.18	18.4 ± 0.28
	% TRFOs	100.00	15.54	59.20	25.26
	Karo (n = 6)	79.8 ± 4.76^{fE}	10.7 ± 0.47	49.4 ± 2.29	19.7 ± 1.01
	% TRFOs	100.00	13.36	61.96	24.67
	Koral (n = 6)	68.2 ± 1.40^{gF}	8.6 ± 0.54	41.8 ± 3.71	17.8 ± 2.32
	% TRFOs	100.00	12.58	52.41	22.29
	Kurant (n = 3)	74.4 ± 0.73^{fE}	9.2 ± 0.24	44.5 ± 0.49	20.8 ± 0.38
	% TRFOs	100.00	12.30	59.80	27.89
	Lazur (n = 4)	77.1 ± 4.24^{fE}	9.0 ± 0.41	45.5 ± 2.54	22.5 ± 1.01
	% TRFOs	100.00	11.68	59.06	29.26
	Neron (n = 2)	**94.9 ± 3.48^{eD}**	**11.2 ± 1.11**	50.3 ± 1.23	33.4 ± 1.24
	% TRFOs	100.00	11.80	53.00	35.19
	Neptun (n = 6)	83.2 ± 1.83^{fE}	10.7 ± 0.86	48.7 ± 0.44	23.8 ± 0.23
	% TRFOs	100.00	12.80	58.54	28.65
	Oskar (n = 5)	68.5 ± 3.12^{gF}	7.9 ± 0.14	41.6 ± 1.10	19.1 ± 1.88
	% TRFOs	100.00	11.52	60.65	27.83
	Regent (n = 8)	74.0 ± 6.8^{3fE}	10.4 ± 1.47	39.7 ± 4.59	23.9 ± 4.24
	% TRFOs	100.00	14.02	53.62	32.36
	Roland (n = 4)	71.1 ± 6.97^{fE}	8.1 ± 0.83	41.2 ± 3.86	21.9 ± 0.98
	% TRFOs	100.00	11.43	57.86	30.72
	Rumba (n = 6)	76.5 ± 1.96^{fE}	8.9 ± 0.69	46.9 ± 3.56	20.7 ± 1.12
	% TRFOs	100.00	11.69	61.25	27.06
	Salsa (n = 3)	76.0 ± 2.34^{fE}	9.7 ± 0.87	47.6 ± 3.71	18.8 ± 0.95
	% TRFOs	100.00	12.70	62.59	24.71
	Samba (n = 6)	63.8 ± 2.17^{gF}	8.1 ± 0.64	35.0 ± 2.11	20.7 ± 1.01
	% TRFOs	100.00	12.67	54.95	32.38
	Sonet (n = 8)	68.3 ± 1.82^{gF}	7.5 ± 0.51	43.2 ± 1.74	17.6 ± 1.32
	% TRFOs	100.00	11.04	63.21	25.75
	Szot (n = 5)	60.8 ± 2.22^{hF}	7.2 ± 0.53	29.1 ± 1.24	24.5 ± 1.01
	% TRFOs	100.00	11.86	42.58	35.85
	Tango (n = 7)	79.9 ± 3.81^{fE}	9.6 ± 0.27	48.3 ± 2.62	22.0 ± 1.61
	% TRFOs	100.00	12.01	60.49	27.50
	Tytan (n = 6)	84.0 ± 4.89^{fE}	10.3 ± 1.32	48.1 ± 2.33	25.6 ± 1.89
	% TRFOs	100.00	12.31	57.25	30.44
	Wars (n = 5)	66.3 ± 5.91^{gF}	10.3 ± 1.62	38.4 ± 2.01	17.6 ± 1.79
	% TRFOs	100.00	15.57	45.74	20.89
	Zeus (n = 7)	83.6 ± 7.59^{fE}	9.9 ± 0.11	51.5 ± 3.25	22.2 ± 1.11
	% TRFOs	100.00	11.79	61.61	26.60
	Narrow-leaved lupin, mean	**73.8 ± 5.68**	**9.3 ± 0.85**	**43.1 ± 4.31**	**21.5 ± 3.04**
	% TRFOs	**100.00**	**12.56**	**58.32**	**29.08**
Total lupins, mean		**84.9 ± 16.09**	**9.5 ± 1.25**	**48.5 ± 8.22**	**26.8 ± 9.38**
% TRFOs, mean		**100.00**	**11.15**	**57.19**	**31.58**

DM – dry matter, RFOs – raffinose family oligosaccharides, TRFOs – total content of raffinose family oligosaccharides; the results are expressed as mean ± standard deviation (SD); $^{a\text{-}f,\ A\text{-}F}$ – means within a column with different superscripts are significantly different at *P* < 0.05; the values marked with the same letters do not differ significantly at *P* > 0.05; lowercase letters refer to the results in the table (for individual species); uppercase letters refer to all results

Table 3. Total content of raffinose family oligosaccharides and the percentage of individual saccharides in pea (*Pisum sativum*) seeds (mg/g DM)

Cultivar	Total RFO	Raffinose	Stachyose	Verbascose
Akord (n = 4)	72.8 ± 3.56^{bE}	8.4 ± 0.62	23.3 ± 1.54	41.0 ± 3.11
% TRFOs	100.00	11.57	32.06	56.37
Arwena (n = 4)	**52.0 ± 4.23^{eG}**	6.7 ± 0.21	20.6 ± 0.45	24.8 ± 0.61
% TRFOs	100.00	12.77	39.60	47.63
Batuta (n = 7)	59.3 ± 2.18^{dG}	7.7 ± 0.24	23.3 ± 1.14	28.4 ± 1.81
% TRFOs	100.00	12.91	39.20	47.90
Cysterski (n = 8)	56.4 ± 3.21^{dG}	8.0 ± 0.42	23.1 ± 1.02	25.4 ± 0.98
% TRFOs	100.00	14.13	40.89	44.98
Eureka (n = 3)	77.3 ± 3.77^{bE}	9.0 ± 0.84	25.2 ± 1.61	43.1 ± 2.36
% TRFOs	100.00	11.63	44.67	76.46
Ezop (n = 6)	63.4 ± 2.22^{cF}	6.2 ± 1.02	24.8 ± 1.65	32.4 ± 1.25
% TRFOs	100.00	9.81	39.07	51.12
Hubal (n = 6)	66.8 ± 3.21^{cF}	10.0 ± 0.89	33.0 ± 1.02	23.8 ± 1.45
% TRFOs	100.00	14.96	49.37	35.67
Klif (n = 2)	76.9 ± 2.34^{bE}	12.0 ± 0.74	37.6 ± 1.28	27.3 ± 1.35
% TRFOs	100.00	15.57	48.92	35.52
Lasso (n = 6)	71.9 ± 3.65^{bE}	11.0 ± 0.99	38.8 ± 1.36	22.1 ± 1.10
% TRFOs	100.00	15.31	53.97	30.72
Mecenas (n = 5)	66.0 ± 2.36^{bF}	9.6 ± 0.84	26.4 ± 1.23	30.1 ± 1.66
% TRFOs	100.00	14.49	39.95	45.56
Medal (n = 2)	57.3 ± 2.41^{dG}	8.6 ± 0.79	18.9 ± 1.28	29.7 ± 1.62
% TRFOs	100.00	15.07	33.04	51.89
Medyk (n = 3)	57.1 ± 0.21^{dG}	7.8 ± 0.32	21.3 ± 0.21	28.0 ± 0.22
% TRFOs	100.00	13.67	37.30	49.03
Mentor (n = 4)	63.4 ± 3.12^{cF}	10.1 ± 0.81	29.0 ± 1.33	24.4 ± 1.47
% TRFOs	100.00	15.89	45.69	38.42
Milwa (n = 7)	62.4 ± 2.48^{cF}	7.5 ± 0.66	28.7 ± 0.97	26.2 ± 1.09
% TRFOs	100.00	11.99	46.06	41.95
Model (n = 6)	65.0 ± 4.89^{cF}	6.8 ± 1.56	23.9 ± 1.87	34.4 ± 2.88
% TRFOs	100.00	10.40	36.71	52.88
Olimp (n = 5)	**52.9 ± 3.01^{eG}**	9.1 ± 0.74	21.6 ± 1.56	22.3 ± 1.63
% TRFOs	100.00	17.19	40.79	42.02
Pomorska (n = 2)	75.8 ± 6.21^{bE}	12.0 ± 1.02	34.1 ± 3.04	29.6 ± 2.61
% TRFOs	100.00	15.87	45.03	39.10
Roch (n = 6)	63.3 ± 2.45^{cF}	8.1 ± 0.71	32.1 ± 1.32	23.1 ± 1.11
% TRFOs	100.00	12.79	50.74	36.47
Sokolik (n = 6)	59.0 ± 2.55^{dG}	7.8 ± 0.41	22.6 ± 0.79	28.6 ± 0.66
% TRFOs	100.00	13.21	38.26	48.53
Starski (n = 6)	74.4 ± 2.71^{bE}	9.5 ± 0.56	38.4 ± 1.78	26.5 ± 1.48
% TRFOs	100.00	12.76	51.62	35.62
Tarchalska (n = 7)	70.4 ± 4.21^{bE}	7.9 ± 0.65	25.5 ± 2.10	37.1 ± 3.01
% TRFOs	100.00	11.22	36.13	52.65
Turnia (n = 5)	57.8 ± 3.77^{dG}	10.0 ± 0.45	26.8 ± 1.24	21.0 ± 1.56
% TRFOs	100.00	17.30	46.41	36.29
Tytus (n = 4)	71.9 ± 2.89^{bE}	8.4 ± 0.68	23.4 ± 2.01	40.2 ± 3.44
% TRFOs	100.00	11.68	32.48	55.85
Wenus (n = 4)	65.0 ± 1.98^{bF}	6.7 ± 0.49	22.5 ± 1.54	35.8 ± 1.84
% TRFOs	100.00	10.29	34.65	55.06
Wiato (n = 3)	**80.6 ± 6.81^{aD}**	**13.2 ± 0.76**	47.2 ± 3.51	20.3 ± 1.77
% TRFOs	100.00	16.33	58.55	25.12
Total pea, mean	**65.6 ± 6.68**	**8.9 ± 1.42**	**27.7 ± 5.58**	**29.0 ± 5.06**
% TRFOs, mean	**100.00**	**13.55**	**42.45**	**45.31**

DM – dry matter, RFOs – raffinose family oligosaccharides, TRFOs – total content of raffinose family oligosaccharides; the results are expressed as mean ± standard deviation (SD); $^{a\text{-}f,\,A\text{-}F}$ – means within a column with different superscripts are significantly different at $P < 0.05$; the values marked with the same letters do not differ significantly at $P > 0.05$; lowercase letters refer to the results in the table (for individual species); uppercase letters refer to all results

Table 4. Total content of raffinose family oligosaccharides and the percentage of individual saccharides in faba bean (*Vicia faba)* seeds (mg/g DM)

Cultivar	Total RFO	Raffinose	Stachyose	Verbascose
Albus (n = 5)	**32.2 ± 1.66**^{cK}	1.5 ± 0.25	5.8 ± 0.41	24.8 ± 1.21
% TRFOs	100.00	4.64	18.10	77.26
Amigo (n = 5)	61.4 ± 3.22^{aF}	1.8 ± 0.21	8.1 ± 0.65	51.5 ± 3.84
% TRFOs	100.00	2.85	13.26	83.89
Amulet (n = 5)	45.1 ± 3.54^{bH}	**4.2 ± 0.23**	13.4 ± 1.01	27.6 ± 2.47
% TRFOs	100.00	9.27	29.61	61.12
Fernando (n = 4)	49.1 ± 3.06^{bH}	1.8 ± 0.11	7.3 ± 0.33	40.0 ± 3.32
% TRFOs	100.00	3.74	14.84	81.42
Granit (n = 4)	**65.2 ± 4.41**^{aF}	2.1 ± 0.32	10.2 ± 0.87	52.9 ± 2.83
% TRFOs	100.00	3.18	15.65	81.17
Olga (n = 5)	47.7 ± 3.44^{bH}	3.4 ± 0.21	10.3 ± 0.78	34.0 ± 1.81
% TRFOs	100.00	7.14	21.62	71.24
Faba bean, mean	**50.1 ± 3.22**	**2.5 ± 0.22**	**9.2 ± 0.67**	**38.5 ± 2.58**
% TRFOs, mean	**100.00**	**5.14**	**18.84**	**76.02**

DM – dry matter, RFOs – raffinose family oligosaccharides, TRFOs – total content of raffinose family oligosaccharides; the results are expressed as mean ± standard deviation (SD); $^{a-f, A-F}$ – means within a column with different superscripts are significantly different at $P < 0.05$; the values marked with the same letters do not differ significantly at $P > 0.05$; lowercase letters refer to the results in the table (for individual species); uppercase letters refer to all results

The lowest ($P \leq 0.05$) RFO contents (33–34 mg/g DM) was found in the cv. ES Commandor and Moravians. Stachyose was the dominant oligosaccharide in all soybean seeds – 76% TRFOs (range 71–83%), followed by raffinose (mean 18%; range 9–26%) and verbascose (mean 3%, range 2–7%).

Among lupins, the highest RFO contents was found in seeds of the cv. Bursztyn, Perkoz, and Baryt – approx. 130 mg/g DM ($P \leq 0.05$), whereas the lowest total RFO contents was determined in seeds of narrow-leaved lupin cv. Szot and Boros (60.81 and 57.23 mg/g, respectively) ($P \leq 0.05$). The mean RFO contents in white lupin seeds was approx. 106 mg/g DM, yellow lupin seeds – 112 mg/g, and 74 mg/g DM in narrow-leaved lupin seeds. Stachyose was the dominant saccharide in these lupins – overall, it constituted 57% of TRFOs. It was followed by verbascose (32%) and raffinose (11%). Lupin species differed significantly ($P \leq 0.05$) in terms of RFO content and structure (Figure 1). White lupin seeds contained the highest (80%) proportion of stachyose in TRFOs. It was lower in yellow (50%) and narrow-leaved lupin seeds (58%). Verbascose content amounted to 40% of TRFOs in yellow faba bean seeds and 29% in narrow-leaved lupin seeds. The lowest percentage of verbascose was recorded in yellow lupin seeds – approx. 10% of TRFOs. Raffinose content was 10% in yellow lupin seeds and 13% in narrow-leaved lupin seeds. The lowest content was found in white lupin seeds –7%.

Among pea cultivars, the seeds of Arwena and Olimp had the lowest total RFO contents ($P \leq 0.05$) – 52–53 mg/g. The highest content was found ($P \leq 0.05$) in the cv. Wiato – 80 mg/g DM. The following RFO oligosaccharides were

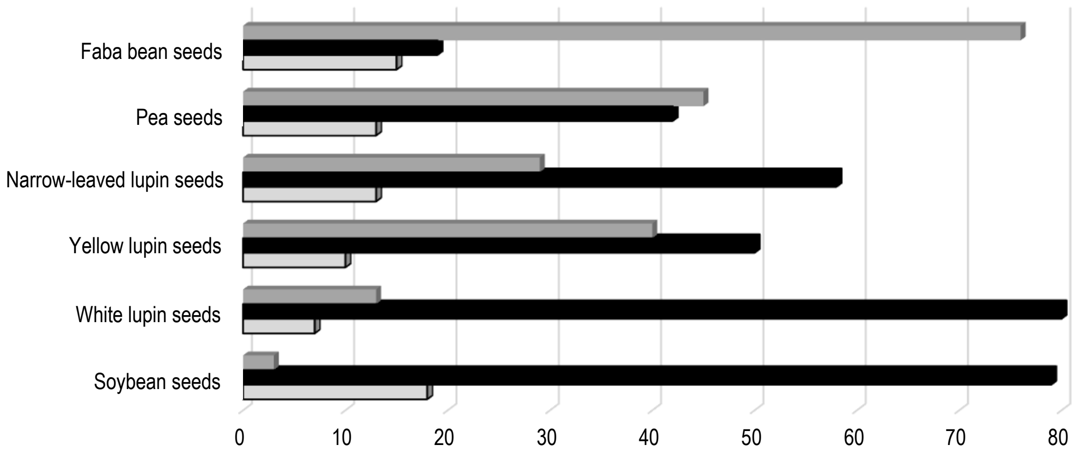

Figure 1. Content and structure of raffinose family oligosaccharides in legume seeds (%)

dominant: verbascose – 45% of TRFO content in pea seeds, stachyose – 42%, and raffinose – 13%. The percentage of stachyose in the cv. Hubal, Kliff, Lasso, Milwa, Wiato, Pomorska, Roch, Turnia, Mentor, and Starski was higher than that of verbascose.

Among *Vicia faba* seeds, the highest ($P \leq 0.05$) total RFO contents was found in the cv. Granit (65.2 mg/g DM) and Amigo (61.4 mg/g DM), whereas the lowest in Albus seeds – 32.2 mg/g ($P \leq 0.05$). The mean value of total RFOs was 50.1 mg/g. The dominant oligosaccharides were: verbascose – 76%, stachyose – 19%, and raffinose – 5% of total RFOs.

Discussion

The raffinose family oligosaccharides are an important component found mainly in seeds and other common storage organs of plants. It is the most widely distributed group of compounds in plants. RFOs have various functions in seeds, such as protecting cellular structures during desiccation and being a carbon reserve during germination. Moreover, RFOs are known for their wide range of chemical reactivity and molecular sizes (Gulewicz et al., 2014).

Analysis of raffinose family oligosaccharides should address issues such as the development of food products or plant breeding. Legumes are the main natural source of RFOs. Many factors are responsible for RFO concentrations in food and feed components, such as plant species and cultivar, growing environment, stress, and form of consumption (raw or processed) (Banti, 2021). According to Martınez-Villaluenga et al. (2005a), total RFO contents in different lupin cultivars ranged from 5.30 to 12.30%, and this range was consistent with our study. Kadlec et al. (2001) found that RFO contents in different pea seeds ranged from 2.3 to 9.6%, whereas in our study it varied from 5.3 to 8.0%. RFO contents in seeds of soybean cultivars grown in Poland and analysed in our study ranged from 33.7 to 69.3 mg per 1 g DM. Studies conducted in various soybean genotypes in India reported similar results (Kumar et al., 2010). Banti (2021) observed that the content of raffinose, stachyose, and verbascose in dry matter of lupin seeds amounted to 0.9–19, 5.2–86, and 35 mg/g, respectively; 6–14, 17.1–27.0, and 23.0 mg/g in pea seeds, respectively, 0.27–1.3, 0.9–2.5, 0.67–5.03 mg/g in faba bean seeds, respectively, while the content of raffinose and stachyose in soybean seeds was 0.67–2.56 mg/g, and 2.09–7.10 mg/g, respectively.

Stachyose was the dominant oligosaccharide in all soybean and lupin seeds analysed in our study. Verbascose was the dominant oligosaccharide in most pea and faba bean seeds. Stachyose content in some pea cultivars was higher than that of verbascose. : It could be due to the fact that Hubal, Kliff, Lasso, Milwa, Wiato, Pomorska, Roch, and Turnia are fodder pea cultivars. By contrast, Mentor and Starski are white-flowering edible pea cultivars, which are particularly resistant to environmental problems. The growing environment is a crucial aspect affecting the accumulation of raffinose family oligosaccharides. RFO synthesis levels are particularly high in an environment exposed to abiotic stress. Stachyose is considered an essential transport carbohydrate in various woody plants and legumes, a membrane stabilizer and stress tolerance mediator (Van den Ende, 2013). Moreover, RFO contents also depend on the genetic background. Vidal-Valverde et al. (2003) analysed variation in 18 pea accessions from a germplasm collection and found many differences associated with seed genetic background. Moreover, the latter authors observed correlations between the brown colour of the seed coat and the lowest content of verbascose and sucrose, and seed size and verbascose and total oligosaccharide contents. RFO contents in seeds is influenced by various factors, including genetic characteristics, selection methods, species and cultivar, environmental conditions, as well as the method of analysis (Banti, 2021).

The high concentration of RFOs in legumes limits their consumption and acceptance worldwide, especially in developed countries. This is because monogastric animals and humans lack α-galactosidase, which is required to hydrolyse α (1→6) glycosidic linkages. Undigested oligosaccharides in the ileum pass into the caecum and contribute to the production of gases during anaerobic bacterial metabolism. This causes stomach discomfort, abdominal rumblings, cramps, pain, and diarrhoea (Gulewicz et al., 2014). The antinutritional effects of RFOs in soybean meal (SBM) and lupins have been observed to reduce net dietary energy in poultry, rats, and pigs. This osmotic imbalance decreased nutrient absorption and protein utilisation (Kaczmarek et al., 2014). Researchers have analysed various processing methods (fermentation, germination, cooking, gamma radiations, etc.) and applied genetic manipulation techniques to reduce RFO content in non-ruminant diets (Kasprowicz-Potocka et al., 2013; 2015; Zhang et al., 2019; Zaworska-Zakrzewska et al., 2020). However, seed processing is costly as it requires a lot of energy, time, and sometimes special equipment (Zhang et al., 2019). Since soybean meal is a common source of protein for livestock, higher RFO concentrations in seeds are a significant problem in the efficient use of soybean as food and feed. Soybean RFOs are not eliminated by processing during SBM production and constitute approxim. 4–6% of DM.

It is likely that RFOs are responsible for increasing digesta viscosity, which interferes with the digestion of nutrients. Zeng et al. (2021) observed that dietary raffinose (0.2 and 0.5%) could reduce pig growth performance by decreasing feed intake and nutrient digestibility, while improving intestinal morphology and negatively affecting immunity. Reduction of oligosaccharide contents in soybean meals could increase the amount of soybean proteins in diets. Researchers have made numerous attempts to evaluate existing soybean germplasm and mutagenized materials to increase the contents of digestible carbohydrates and beneficial nutritional factors. Zhang et al. (2019) suggested that microbial α-galactosidase seemed to be a promising solution for RFO degradation. Qiu et al. (2015) identified and characterized a mutant of the soybean stachyose synthase gene controlling reduced stachyose content, which would benefit the soybean seed breeding programme in the future.

A diet based on faba bean and soybean seeds seems to be the most suitable for monogastric animals due to its RFO contents. In our study, RFO contents in the dry matter of seeds of 14 soybean cultivars and 4 faba bean cultivars did not exceed 5%. The seeds of the soybean cv. Commandor and Moravians, and the faba bean cultivar Albus showed particularly low RFO contents. The content of RFOs in white and yellow lupin seeds was almost 2-fold greater compared to narrow-leaved lupin seeds, whereas the cultivar Boros had a similar RFO contents to those of soybeans and faba beans. RFO contents in 8 cultivars of pea seeds ranged from 5 to 6%. Among the cultivated legumes, there are cultivars with lower oligosaccharide contents, which may positively affect the production performance of animals sensitive to too high RFO levels in the diet. Kaczmarek et al. (2014) reported that especially the AMEn value of lupin seeds was negatively correlated with the level of raffinose ($r = -0.72$; $P < 0.05$) and that the relationship between raffinose content and the AMEn value was linear ($P < 0.05$). The lowest percentage of raffinose in TRFOs was found in faba bean and white lupin seeds. Raffinose content in all the seeds tested ranged from 6 to 13 mg/g DM, but it did not exceed 2 mg/g DM in the faba bean cv. Albus, Amigo, and Fernando. The seeds of the soybean cultivar Moravians had both the lowest RFO and raffinose contents. These species and cultivars could primarily be used in animal feeds.

Raffinose and other RFO compounds cannot be digested by non-ruminants, but can be metabolized by colon bacteria, and thus they are considered prebiotic candidates (Fernando et al., 2010). The gut microbiota utilises prebiotic oligosaccharides to grow and produce lactate and short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. The prebiotic potential of RFOs has been reported in single and co-culture microbial models. Amorim et al. (2020) found that the addition of raffinose increased the production of SCFA and carbon dioxide, while reducing the final medium pH and ammonia concentration. Moreover, the presence of raffinose as a substrate increased the relative abundance of *Bifidobacterium* and *Lactobacillus* species, while decreasing Proteobacteria populations, including common pathogens, such as *Escherichia coli*. Fernando et al. (2010) also concluded that both the chickpea and raffinose diets modulated the gut microbiota of the subjects and had potentially beneficial effects, e.g. an increased abundance of *Bifidobacterium* spp. and a decrease in *Clostridium* clusters, including pathogenic and putrefactive bacteria. Other authors indicated that these functional oligosaccharides were also effective in alleviating chronic nutrition-related problems and were the main active ingredients responsible for hepatoprotective effects in plant products (Conti et al., 2021). Scientists have also reported their influence on mineral absorption, immune response, lipid and glucose homeostasis, and satiety regulation. Bednarczyk et al. (2011) found a prebiotic effect of *in ovo* RFO injection on growth performance. The latter authors concluded that it could replace antibiotic growth promoters as a non-antimicrobial enhancing additive. Abdel-Latif et al. (2020) observed that dietary raffinose increased growth performance and immune responses of Nile tilapias. These authors recommended a supplementation dose of 0.85–1.37 g raffinose per kg in the diet of this species. Karimi et al. (2020) suggested that including 1.0 or 2.0 g of raffinose per kg diet could promote immune competence and increase health indices in carp aquaculture. Martınez-Villaluenga et al. (2005b) found that *Bifidobacterium lactis* Bb-12 and *Lactobacillus acidophilus* could be used with the addition of RFOs for the production of fermented milk products in a mixed culture at a 1:1 ratio. They caused rapid growth, increased acidification and likely enhanced the probiotic effect of the final functional product. Zhu et al. (2020) observed that dietary supplementation of broilers with stachyose (0.6%) significantly reduced the production of odour compounds by modulating the caecal microbial community.

RFOs can be extracted from legume seeds, which have recently been considered an alternative protein source for feeding monogastric species. Martınez-Villaluenga et al. (2005b) isolated RFOs from *Lupinus albus* var. Multolupa, whereas Bednarczyk et al. (2011) and Stadnicka et al. (2020) isolated and purified RFOs from seeds of *Lupinus luteus* L. cv. Lord. Raffinose can also be produced from cheap and abundant agro-residues such as soybean waste, which is an excellent environmental and economic advantage (Fernando et al., 2010). In our study, yellow and white lupin seeds were the richest source of RFOs, with average contents exceeding 10% in seed dry matter. RFO contents in the seeds of the cv. Bursztyn, Perkoz, and Baryt was higher than 12%. Therefore, they could be used for the industrial production of prebiotic substances. The percentage of raffinose in the seeds of yellow and narrow-leaved lupin seeds was higher than in other legumes and exceeded 10% of total RFO contents.

Conclusions

Many years of observations showed that legume seeds with low RFO content (soybean, faba bean, pea) or lupin cultivars with the lowest content of oligosaccharides should be used in animal diets. RFOs could be isolated from lupin seeds, predominantly yellow and white, and used as a food or feed additive due to its prebiotic activity and modulation of microflora to improve growth, health, and reduce odour emissions.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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