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CONSULTING

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Office of Food Additive Safety (HFS-200),
Food and Drug Administration,
5001 Campus Drive
College Park, MD 20740-3835

19 September 2022

New submission
Ref: GRAS Notice for Canola Concentrate for use as an ingredient in human food

To whom it concerns,

Pursuant to 21 CFR § 170 Subpart E consisting of § 170.203 through 170.285, NapiFeryn BioTech Sp. z o. o. (ul. Dubois 114/116, 93-465 Łódź 04103, Poland), through Pen & Tec Consulting S.L.U as its agent, hereby informs the United States Food and Drug Administration of the conclusion that Canola Concentrate (with trade name Raptein™30), is GRAS under the specific conditions of use as a food ingredient in food, on the basis of scientific procedures, and therefore, is not subject to the premarket approval requirements of the Federal Food, Drug and Cosmetic Act.

I hereby certify that the enclosed electronic files have been checked and found to be virus free.

Should you have any questions regarding this GRAS Notice at any point during the review process, you can reach out to the following contact persons:

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Yours sincerely,

Nicoleta Pasecinic
Regulatory Affairs Manager
Pen & Tec Consulting S.L.U.

Enclosures:
1. 2 CD´s containing the GRAS notice and appendices

FRONT PAGE

GRAS NOTICE OF

CANOLA CONCENTRATE (RAPESEED CONCENTRATE)
FOR USE AS AN INGREDIENT IN HUMAN FOOD

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1. Signed statements and certification

1.1. GRAS Notice submission

NapiFeryn BioTech Sp. z o. o. is hereby submitting a Generally Recognized As Safe (GRAS) Notice in accordance with 21 CFR part 170, subpart E.

1.2. Name and address of Notifier

NOTIFIER

Name:	NapiFeryn BioTech Sp. z o. o.
Address:	ul. Dubois 114/116
Postal code and city:	93-465 Łódź
Country:	Poland
Tel. no:	+48 503 542 475
E-mail:	regulatory@napiferyn.pl

NOTIFIER'S AGENT

Name:	Pen & Tec Consulting S.L.U.
Address:	Pl. Ausias March 1, 4th floor D01
Postal code and city:	08195 Sant Cugat del Vallès (Barcelona)
Country:	Spain
Tel. no:	+34 936 75 80 15

1.3. Name of the notified substance

The ingredient determined by NapiFeryn BioTech Sp. z o. o. (hereinafter 'NapiFeryn') to be GRAS is canola concentrate (rapeseed concentrate). The trade name of the notified canola concentrate is Raptein™30.

1.4. Intended conditions of use

NapiFeryn intends to use its canola concentrate (Raptein™30) in a variety of foods and beverages at use levels ranging from 5% to 100%. Canola concentrate (Raptein™30) can be incorporated into food products to provide nutritional value as a protein and/or fiber source and/or for its functional properties (e.g. thickener [21 CFR 170.3(o)(28)] or texturizer [21 CFR 170.3(o)(32)]). Canola concentrate is intended to be used in bakery products, breakfast cereals, grain products, pastas, bars, protein-fiber and nutritional powders, beverages and beverage bases non-alcoholic, milk products and dairy analogs, coffee and tea, meat product analogs, fat, oils and salad dressings, egg substitutes, soup and soup mixes, sauces, gravies, condiments and dips, nut products, snack foods, fruit and water ices, confections, candies and frostings, sweet sauces, toppings, and syrups, jams, jellies, gelatins, puddings, fillings, processed fruits, vegetables, legumes, batter/breadings, spice and seasoning mixes and processed meat and poultry. Food products that come under the U.S. Department of Agriculture's jurisdiction are also in the scope of this GRAS Notice. For use levels of canola concentrate, see Section 3.1 - Table 9 of this dossier.

1.5. Statutory basis for GRAS status

NapiFeryn has concluded that its canola concentrate (rapeseed concentrate), meeting the specifications described herein, is GRAS under its intended conditions of use through scientific procedures in accordance with 21 CFR § 170.30 (a) and (b).

1.6. Exemption from premarket approval

NapiFeryn has concluded that its canola concentrate is GRAS under the conditions of its intended use and therefore the notified substance is not subject to the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

1.7. Availability of information for FDA review

Napiferyn agrees to make available to the FDA the data and information that are the basis for NapiFeryn's conclusion of GRAS (upon request). Napiferyn agrees to the following procedures for making the data and information available to the FDA:

- upon FDA request, allow the FDA to review and copy the data and information during customary business hours at the address provided in Section 1.2, where data and information will be available to the FDA.

- upon FDA request, provide the FDA with a complete copy of the data and information either in an electronic format that is accessible for our evaluation or on paper.

1.8. Exemption from disclosure

None of data and information in Parts 2 through 7 of this GRAS Notice are exempt from disclosure under the Freedom of Information Act, 5 U.S.C. 552.

1.9. Certification

The undersigned certifies that to the best of our knowledge, this GRAS Notice is a complete, representative, and balanced submission that includes unfavorable information, as well as favorable information known to NapiFeryn and pertinent to the evaluation of the safety and GRAS use of the canola concentrate (Raptein™30), meeting appropriate specifications described herein, and used in accordance with current Good Manufacturing Practice (cGMP) in food.

1.10. FSIS/USDA – Use in Meat and Poultry Products

NapiFeryn BioTech intends to add canola concentrate (Raptein™30) to meat and poultry products that come under U.S. Department of Agriculture (USDA) jurisdiction (21 C.F.R. §170.270) and authorizes the FDA to send USDA any portion of this filing, which does not include any discussion of trade secrets.

1.11. Name and position of Signatory

Date and signature	Notifier's Agent
	Nicoleta Pasecinic
	Regulatory Affairs Manager
	Pen & Tec Consulting S.L.U.
	Pl. Ausias March 1, 4th floor D01
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- upon FDA request, provide the FDA with a complete copy of the data and information either in an electronic format that is accessible for our evaluation or on paper.

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1.11. Name and position of Signatory

Date and signature	Notifier's Agent
	Nikolaas Tilkin-Franssens
27 January 2023	Regulatory Affairs Manager
[redacted]	Pen & Tec Consulting S.L.U.
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2. Identity, method of manufacture, specifications and physical or technical effect

2.1. Data and information that identifies the notified substances

2.1.1. Description

Canola concentrate (rapeseed concentrate), which consists of 30-45% protein and 40-70% dietary fiber (dry matter basis) is the subject of this GRAS notification. The canola concentrate (rapeseed concentrate) ingredient is to be marketed under the trade name Raptein™30. The ingredient will be referred to as canola concentrate throughout this document.

This GRAS notification concerns a canola concentrate obtained from rapeseed (canola) press cake that is a by-product of rapeseed oil production. Rapeseed oil is produced by pressing the seeds of the rape plant from three varieties, *Brassica napus* and/or *Brassica juncea* and/or *Brassica rapa*. The rapeseed cake is composed of crushed seeds. The canola concentrate is mainly composed of protein (30-45% dry matter) and dietary fiber (40-70% dry matter).

Two major storage protein fractions in rapeseed are cruciferins and napins. Other proteins that are present in rapeseed are e.g. oil body proteins (Chmielewska et al., 2020). Cruciferins are globulins and are the major storage protein in the seed. Cruciferins are classified as 12S globulins (~300 kDa) based on their sedimentation coefficient. Cruciferins are salt soluble neutral glycoproteins (Nietzel et al., 2013). Napins belong to the albumin class. These are water soluble, low-molecular weight basic proteins classified as 2S or 1.7S proteins (Ren and Bewley, 1999; Chmielewska et al., 2020), representing 20-40% of the total seed protein, and having molecular weights in the range of 12–17 kDa (Stone et al., 2014; Von Der Haar et al., 2014). Napins are low molecular weight storage proteins composed of two disulfide-linked polypeptides (Tan et al., 2011). Predominant oil body proteins in rapeseed are oleosins with molecular mass ~ 18 – 25 kDa. Oil body proteins also include caleosins (~30 kDa) and steroleosins (~40 kDa) (Chmielewska et al., 2020).

To confirm the occurrence of mentioned proteins, SDS PAGE analysis was performed. Because native cruciferin is a multimeric protein, its subunits are observed as separated using that method. Similarly, napin has two main chains. Canola concentrate samples were analyzed in both reducing and non-reducing conditions (see Appendix 7). The results were similar for both options: 9-12 or 9-17 bands were identified in non-reducing and reducing conditions. The highest band intensity was observed for proteins with molecular weight of approximately 17 and 11 kDa. Independently, as part of the allergenicity tests, another SDS PAGE analysis was also performed. The banding pattern for Raptein™30 (run as reduced and non-reduced samples) showed the presence of proteins of about 100 kDa, 16-19 kDa and below 10 kDa (derived from the hydrolysis of larger protein and observed in the reduced samples). On the other hand, the intact mass spectrometry (LC-MS) results revealed signals only for 10.0 and 19.9 kDa (because of partial hydrolysis of samples). Furthermore, the following proteins were detected in the Raptein™30 samples as part of the allergenicity analysis: three cruciferins (11S globulins), napin-3 (1.7S seed storage proteins), oleosin S2-2 and squalene monooxygenase 1,2. The complete set of data is provided in the Appendix 4.

Table 1 presents typical amino acid content in five lots of canola concentrate as well as average values. Complete data is included in the Appendix 1. Table 2. shows the amino acid profile of Raptein™30 with comparison to partially defatted rapeseed powder authorized by the European Commission as a novel food ingredient (EFSA, 2020) and previously GRAS-notified canola proteins (Puratein® and Supertein®) (FDA, 2010). Some similarities in the profiles can be noticed, in particular when comparing the canola concentrate (Raptein™30) and partially defatted rapeseed powder.

Table 1. Typical amino acid content in representative lots of canola concentrate (Raptein™30)

Amino acid [g/100g sample]	Lot number					Average	% of protein amino acids total (w/w protein)
	R-03#35	R-06#60	R-09#41	R-13#60	R-15#66		
Glycine	2.32	1.97	1.96	1.92	2.04	2.04	6.16
Alanine	2.09	1.78	1.75	1.66	1.81	1.82	5.49
Valine	2.42	2.03	2.03	1.90	2.09	2.09	6.32
Leucine	3.39	2.84	2.85	2.62	2.84	2.91	8.78
Isoleucine	1.89	1.53	1.55	1.44	1.58	1.60	4.82

Amino acid [g/100g sample]	Lot number					Average	% of protein amino acids total (w/w protein)
	R-03#35	R-06#60	R-09#41	R-13#60	R-15#66		
Serine	2.13	1.85	1.73	1.73	1.82	1.85	5.59
Threonine	2.30	1.97	1.82	1.72	1.97	1.96	5.90
Tyrosine	1.76	1.47	1.41	1.33	1.46	1.49	4.49
Aspartic Acid	3.66	2.75	2.53	2.48	2.72	2.83	8.54
Phenyl-alanine	1.97	1.57	1.54	1.48	1.56	1.62	4.90
Tryptophan	0.67	0.53	0.51	0.05	0.51	0.45	1.37
Proline	2.00	1.51	1.62	1.50	1.56	1.64	4.94
Methionine	0.88	0.70	0.71	0.66	0.75	0.74	2.24
Cysteine + Cystine	0.60	0.48	0.49	0.50	0.54	0.52	1.58
Lysine	2.69	2.09	2.11	2.00	2.10	2.20	6.63
Histidine	1.17	0.96	0.96	0.92	1.03	1.01	3.04
Arginine	2.64	2.28	2.21	2.23	2.35	2.34	7.07
Glutamic Acid	4.95	3.69	3.72	3.66	4.09	4.02	12.14
Hydroxy-proline	<0.05	<0.2	<0.2	<0.2	0.22	<0.05 - 0.22	-
Ornithine	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	-

Table 2. Amino acids profile of Raptein™30 with comparison to partially defatted rapeseed powder, Puratein® and Supertein®

Amino acids [g/100 g protein]	Raptein™30	Rapeseed powder*	Cruciferin-rich Puratein®**	Napin-rich Supertein®**
Aspartic acid + Asparagine	8.5	8.5 Asparagine	9.3	2.6

Threonine	5.9	5.3	3.7	3.2
Serine	5.6	5.0	4.1	3.3
Glutamic acid + Glutamine	12.1	16.6 Glutamine	19.8	24.6
Proline	4.9	6.1	5.8	9.2
Glycine	6.2	5.7	5.4	4.3
Alanine	5.5	5.1	4.2	4.0
Cysteine + Cystine	1.6	1.8	1.6 Cystine	4.5 Cystine
Valine	6.3	5.8	5.5	4.3
Methionine	2.2	2.1	1.9	2.4
Isoleucine	4.8	4.5	4.4	3.0
Leucine	8.8	8.1	8.2	6.0
Tyrosine	4.5	3.4	4.1	1.4
Phenylalanine	4.9	4.6	4.9	2.6
Histidine	3.0	2.8	2.5	3.6
Lysine	6.6	6.1	4.0	7.4
Arginine	7.1	6.6	7.2	5.8
Tryptophan	1.4	1.6	2.0	1.4

* Average values from five lots of partially defatted rapeseed powder (EFSA, 2020)
**Values are average of duplicate analyses (FDA, 2010)

Rapeseeds contain fiber fractions, which are mostly found in the hulls. Insoluble dietary fiber is a predominant fiber type in rapeseeds. This fraction is mostly composed of lignins, celluloses and usually some hemicelluloses. Apart from the hull, fiber fraction is also present in the seed kernels and it is these kernel fiber components that make up the majority of the fiber components in canola concentrate (Raptein™30). Soluble dietary fiber in rapeseed includes hemicelluloses, gums, pectins, mucilages and mixed β-glucans. Also, a wide range of indigestible constituents (such as glycoproteins) are associated with dietary fiber (Chmielewska et al., 2020; Joehnke et al., 2018).

According to the batch data in section 2.4, Raptein™30 contains on average 62.4 %DM of total dietary fiber (TDF), most of which is insoluble. Canola concentrate is composed of 58.9 %DM of insoluble dietary fiber (IDF) and about 3.5 %DM of soluble dietary fiber

(SDF). It is characterized by such parameters as: neutral detergent fiber (36.0 %DM), acid detergent fiber (24.6 %DM), and acid detergent lignin (4.2 %DM). Taking into consideration the results presented in Table 3, Raptein™30 contains significantly more total dietary fiber than a similar ingredient that is permitted as a novel food ingredient in the European Union (EU), which is called partially defatted rapeseed powder (EFSA, 2020).

Table 3. Dietary fiber fraction content for Raptein™30 and rapeseed powder

Fiber fraction [%]	Raptein™30 *	Rapeseed powder**
Total dietary fiber (TDF)	59.5	40.0
Insoluble dietary fiber (IDF)	56.2	38.5
Soluble dietary fiber (SDF)	3.3	2.0

* Average values from five lots of Raptein™30; Insoluble dietary fiber determined as Insoluble high molecular weight dietary fiber (HMW-IDF); Soluble dietary fiber determined as soluble high molecular weight dietary fiber (HMW-SDF)
** Average values from seven lots of partially defatted rapeseed powder (EFSA, 2020)

2.1.2. Presence of undesirable anti-nutritional factors

Rapeseeds are known to contain a number of anti-nutritional factors, such as phenolic compounds, glucosinolates, erucic acid, phytic acid and protease inhibitors. Since the 1970's, low erucic acid and low glucosinolate rapeseed (canola) varieties have been developed by genetic engineering or breeding techniques (double low or “00” rapeseed varieties). The potential for adverse impacts from the intake of these compounds is addressed in section 6.7 of this document.

2.2. Production process

Figure 1 presents a block flow diagram depicting the process for production of the canola (rapeseed) concentrate product (Raptein™30). The starting material for the product is cold-pressed rapeseed cake.

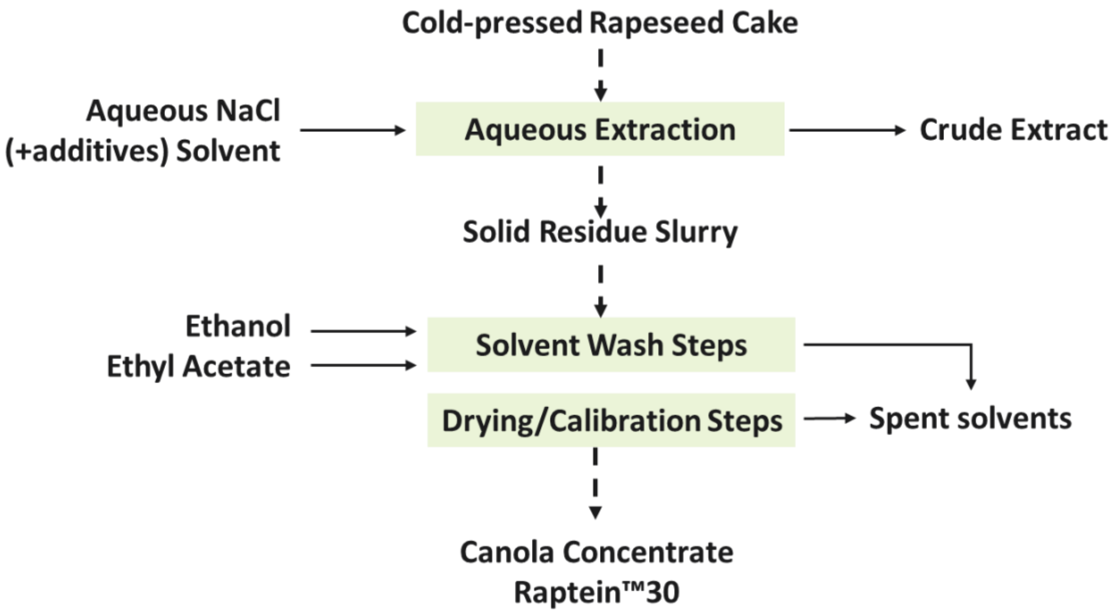

Figure 1. Block flow diagram of the process to produce canola (rapeseed) concentrate

The production process starts with an extraction step in which comminuted cold-pressed rapeseed cake, preferably devoid of hull fraction, is brought into contact with an aqueous solution (0-5% w/w NaCl, 0-1% w/w Na_2SO_3, 0-1% w/w ethanol, 0-1% w/w sodium benzoate). Cake-to-liquid ratio is kept between 1/3 to 1/20 (w/w), and the temperature is kept below 25°C. Liquid extraction medium may contain additives such as monovalent and divalent chlorides, that may improve protein extraction yield and additives that prevent oxidation and suppress microbial growth such as sulfites, benzoates and/or ethanol. Two outlet streams are generated in the extraction step: crude extract containing water-soluble components and a residual slurry containing non-soluble components such as fibers, lipids and non-soluble proteins.

Solid residue is processed to canola concentrate (Raptein™30).

Residual slurry after extraction is subjected to a solid-liquid separation step in a centrifuge or a filter press to remove the excess of the liquid phase from the solid cake.

Food-grade pH-adjusting titrants, such as hydrochloric acid, may be used prior to a solid-liquid separation step to enhance removal of anti-nutritional factors, such as phytates, from the solid residue.

The solid residue after solid/liquid separation step is washed at least twice with food grade solvents containing ethanol and ethyl acetate, or the mixture thereof. First wash step is with water-ethanol mixture containing 40-95% w/w ethanol and is aimed specifically to reduce the amount of anti-nutritional factors such as phenolic compounds and phytates. Subsequently, the solid phase is separated from the liquid solution by means of suitable techniques such as solid-liquid separation.

The wash step with ethyl acetate is then employed in order to remove impurities that are not soluble in ethanol, but which are soluble in ethyl acetate, such as fats and lipids. Subsequently, the solid phase is separated from the liquid solution by means of suitable techniques such as solid-liquid separation in a centrifuge or a filter press.

Organic solvents wash steps are carried out at temperatures between 0-50°C. Ratio solid-to-solvent in the wash steps is kept in the range 1:3 – 1:20 (w/w). Solid phase can be either dispersed in a stirred vessel or form a cake in a filter press.

Subsequently, the washed canola concentrate is subjected to drying in a dryer. Optionally after drying, the canola concentrate is conditioned in air or nitrogen at relative humidity between 30-99% and temperature not higher than 70°C.

Optionally, to remove residues of hull fraction from the final product, a suitable separation step can be employed involving elutriation, sieving, sifting, electrostatic separation and alike.

All processing aids used in the manufacturing of rapeseed (rapeseed) concentrate are appropriate and permitted for use in foods in the U.S. (see Table 4).

Table 4. List of processing aids with US Code of Federal Regulation (CFR) references

Name of processing aids	Code of Federal Regulations (CFR) - Title 21
ascorbic acid	§ 182.8013
citric acid	§ 184.1033
ethanol	§ 184.1293
ethyl acetate	§ 173.228
hydrochloric acid	§ 182.1057
sodium benzoate	§ 184.1733
sodium bisulfite	§ 182.3739

Name of processing aids	Code of Federal Regulations (CFR) - Title 21
sodium chloride	§ 182.1
sodium hydroxide	§ 184.1763
sodium phosphate	§ 182.1778
sodium sulfite	§ 182.3798

2.3. Specifications for food grade material

2.3.1. Product specification

Specifications for the product are presented in Table 5. Analytical results from five non-consecutive lots are provided in Appendix 1. A comparison of five non-consecutive lots of product and values from the specification below can be found in Table 6.

Table 5. Product specifications for canola concentrate (Raptein™30)

Parameter	Unit	Value	Method
Appearance	-	white to off-white dried powder	Visual
Composition**			
Total protein (N*6.25)	%	30 - 45	ISO 1871:2009
Carbohydrates	%	≤ 65	By difference*
Fat (direct)	%	≤ 2	PB/CH/16 version 3 from 09.11.2018
Ash	%	≤ 5	PN-A-79011-8:1998
Moisture	%	≤ 7	PN-A-79011-3:1998
Total dietary fiber (TDF)	%	40 - 70	AOAC 2011.25-M
Total glucosinolates	mmol/kg	≤ 0.3	ISO 9167-1:1992; AOCS Ak 1-92
Purity**			
Phytates	%	≤ 2	Analytical Biochemistry Vol. 77:536-539 (1977)
Lead	mg/kg	≤ 0.5	DIN EN 15763:2010 (2010-04), mod.
Arsenic	mg/kg	≤ 0.2	§64 LFGB L 00.00-19/3

Parameter	Unit	Value	Method
Cadmium	mg/kg	≤ 0.2	DIN EN 15763:2010 (2010-04), mod.
Mercury	mg/kg	≤ 0.1	DIN EN 15763:2010 (2010-04), mod.
Microbiological criteria			
Total plate count	cfu/g	$\leq 10^4$	PN-EN ISO 4833-1:2013-12
E. coli	-	absence in 10 g	PN-ISO 7251:2006
Salmonella spp.	-	absence in 25 g	PN-EN ISO 6579-1:2017-04
Yeast and Molds	cfu/g	≤ 100	PN-ISO 21527-2:2009
Total coliform count	cfu/g	≤ 10	PN-ISO 4831:2007
Enterobacteriaceae	cfu/g	< 10	PN- EN ISO 21528-2:2017-08

*Carbohydrates are calculated by difference as follows: 100 % - [protein (as is) % + moisture % + fat % + ash %] **On dry matter (except of moisture)
PN-A - polish standard; LFGB - Lebensmittel-und-Futtermittelgesetzbuch (German Food and Feed Act); DIN - Deutsches Institute für Normung (German institute for standardization); AOCS - American Oil Chemists’ Society; AOAC - Association of Official Agricultural Chemists

2.3.2. Batch data

The results of key attributes for 5 separate and representative batches of Raptein™30 are summarized in Table 6. Additional data about dietary fiber and fiber fractions are included in Table 7. The presented data show compliance with the specifications and consistency of the production process. The results of heavy metal content as well as of microbiological tests are considered within safe limits. Certificates of Analysis are provided in Appendix 1.

Table 6. Analyses of 5 production batches of canola concentrate (Raptein™30)

Parameter	**Specification**	**Lot No. R-03#35**	**Lot No. R-06#60**	**Lot No. R-09#41**	**Lot No. R-13#60**	**Lot No. R-15#66**
Appearance	white to off-white dried powder	white to off-white dried powder	white to off-white dried powder	white to off-white dried powder	white to off-white dried powder	white to off-white dried powder
Composition**						
Total protein (N*6.25) [%]	30-45	41.26	33.10	32.28	31.65	35.49
Moisture [%]	≤ 7	2.9	5.6	2.8	6.0	4.9
Fat (direct) [%]	≤ 2	0.89	0.86	0.51	0.67	0.83
Ash [%]	≤ 5	2.82	2.87	2.89	3.81	3.23
Total dietary fiber (TDF) [%]	40-70	51.2	66.4	63.6	65.9	64.7
Carbohydrates* [%]	≤ 65	55.04	63.17	64.31	63.87	60.45
Total glucosinolates [mmol/kg]	≤ 0.3	<LOQ (<0.05)	<LOQ (<0.05)	<LOQ (<0.05)	<LOQ (<0.05)	<LOQ (<0.05)
Purity**						
Phytates [%]	≤ 2	1.44	1.09	1.38	1.60	1.49
Lead [mg/kg]	≤ 0.5	0.154	0.233	0.257	0.117	0.089
Arsenic [mg/kg]	≤ 0.2	<LOQ (<0.1)	<LOQ (<0.1)	<LOQ (<0.1)	<LOQ (<0.1)	<LOQ (<0.1)
Cadmium [mg/kg]	≤ 0.2	0.026	0.018	0.020	0.030	0.026
Mercury [mg/kg]	≤ 0.1	<LOQ (<0.005)	<LOQ (<0.005)	<LOQ (<0.005)	<LOQ (<0.005)	<LOQ (<0.005)
Microbiological criteria						
Total plate count [cfu/g]	$\leq 10^4$	1 200	720	610	180	170
E. coli	absent in 10g	absent	absent	absent	absent	absent

Parameter	Specification	Lot No. R-03#35	Lot No. R-06#60	Lot No. R-09#41	Lot No. R-13#60	Lot No. R-15#66
Salmonella spp.	absent in 25 g	absent	absent	absent	absent	absent
Yeast and Molds [cfu/g]	≤ 100	<10	<10	<10	<10	<10
Total coliform count [cfu/g]	≤ 10	0	0	0	0	0
Enterobacteriaceae [cfu/g]	<10	<10	<10	<10	<10	<10

*Carbohydrates are calculated by difference as follows: 100 % - [protein (as is) % + moisture % + fat % + ash %]; **On dry matter (except of moisture); <LOQ – below the Limit of Quantification

Table 7. Analyses of 5 production batches of canola concentrate (Raptein™30) – additional data

Parameter*	Lot No. R-03#35	Lot No. R-06#60	Lot No. R-09#41	Lot No. R-13#60	Lot No. R-15#66
Insoluble high molecular weight dietary fiber (HMW-IDF) [%]	48.8	62.6	59.8	61.8	61.3
Soluble high molecular weight dietary fiber (HMW-SDF) [%]	2.4	3.8	3.8	4.0	3.4
Soluble low molecular weight dietary fiber (LMW-SDF) [%]	<0.6	<0.6	<0.6	<0.6	<0.6
Neutral detergent fiber (NDF) [%]	31.0	37.8	38.2	38.8	34.2
Acid detergent fiber (ADF) [%]	19.6	25.4	26.1	26.4	25.6
Acid detergent lignin (ADL) [%]	3.6	3.1	3.9	6.5	3.8

*On dry matter

2.3.3. Mycotoxin analysis

Mycotoxins are metabolites produced by microfungi that pose a threat to human health. Table 8 contains mycotoxin analyses for five lots of canola concentrate (for certificates of analyses, see Appendix 1). All analyzed compounds were found to be below LOQ, thus they are not considered as an object of concern. For example, the result for total aflatoxin content is <0.4 µg/kg, while according to US and EU regulations the maximum level should be 20 µg/kg (all foods except milk) and 4 µg/kg (all cereals, including maize and rice, intended for direct human consumption), respectively (FDA, 2000; Commision Regulation, 2010). Similarly, fumonisin levels in Raptein™30 were below 20 µg/kg, while Mycotoxin Regulations for Food in the US and EU recommend maximum limits in human food of 2000-4000 µg/kg (corn products; FDA, 2001) and 400-2000 µg/kg (maize products; Commission Regulation, 2006).

Table 8. Mycotoxins in canola concentrate (Raptein™30)

Mycotoxin	Unit	LOQ	Lot Number				
			R-03#35	R-06#60	R-09#41	R-13#60	R-15#66
3-Acetyldeoxynivalenol (3AcDON)	µg/kg	20	<20	<20	<20	<20	<20
BA-TM-03 15-Acetyldeoxynivalenol (15AcDON)	µg/kg	20	<20	<20	<20	<20	<20
Ochratoxin A	µg/kg	0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Deoxynivalenol (DON)	µg/kg	20	<20	<20	<20	<20	<20
Fusarenone X (Fus X)	µg/kg	20	<20	<20	<20	<20	<20
Nivalenol (NIV)	µg/kg	20	<20	<20	<20	<20	<20
T2 Toxin (T_2)	µg/kg	10	<10	<10	<10	<10	<10
HT2 Toxin (HT_2)	µg/kg	10	<10	<10	<10	<10	<10
Zearalenon (ZON)	µg/kg	10	<10	<10	<10	<10	<10
Aflatoxin B_1	µg/kg	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Aflatoxin B_2	µg/kg	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Aflatoxin G_1	µg/kg	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Aflatoxin G_2	µg/kg	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Total Aflatoxin	µg/kg	0.4	<0.4	<0.4	<0.4	<0.4	<0.4
Fumonisin B_1	µg/kg	20	<20	<20	<20	<20	<20
Fumonisin B_2	µg/kg	20	<20	<20	<20	<20	<20
Fumonisin B_3	µg/kg	20	<20	<20	<20	<20	<20

2.3.4. Pesticide analysis

Three lots of the canola concentrate (R-06#60, R-09#41, R-15#66) were screened for residues of about 600 pesticides using GC-MS and LC-MS methods. The content of 68 of the most significant compounds was assessed according to the EU Pesticides Database, as well as the Maximum Residue Limits (MRL) Database (Bryant Christie Inc.).

Glyphosate was detected in two lots of canola concentrate (Lot Number R-06#60, R-09#41). However, the concentrations were in the range of 0.012-0.020 mg/kg, while maximum permissible values are 10 and 20 mg/kg according to EU and US regulations, respectively. The determined content of phosphine in one lot (R-06#60) was 0.008 mg/kg, while the limit for processed food on the US market is 0.01 mg/kg. On the other hand, the maximum residue level in the EU is even higher, at 0.05 mg/kg. It is worth mentioning that most of all analyzed pesticides were below the LOQ. No other residues were found to be at a level of concern. Complete data is included in the Appendix 1.

2.3.5. Acrylamide formation

Acrylamide is a substance produced via chemical reaction between amino acids and reducing sugars while heating. Significant quantities of acrylamide can be formed in temperatures above 120ºC (Żyżelewicz et al., 2010). Since the production process of Raptein™30 as well as the seed pressing process does not exceed this temperature, it is unlikely that acrylamide will be formed.

Five lots of the canola concentrate were analyzed (Table 9). Each lot had an acrylamide concentration <30µg/kg, which is below the limit of quantification for the method (see Appendix 1). Obtained results are lower than benchmark levels for the presence of acrylamide in foodstuffs (40 – 4000 µg/kg) included in Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food (European Commission, 2017). The guidance for industry developed by FDA in 2016 did not specify any recommended maximum level for acrylamide (FDA, 2016e).

Table 9. Acrylamide in Raptein™30

Parameter	Unit	LOQ	Lot No. R-03#35	Lot No. R-06#60	Lot No. R-09#41	Lot No. R-13#60	Lot No. R-15#66
Acrylamide	µg/kg	30	<30	<30	<30	<30	<30

2.3.6. Residual solvents and processing aids

The process of canola concentrate production requires use of organic solvents, such as ethanol and ethyl acetate, to reduce the amount of anti-nutritional factors (phenolic compounds and phytates) and fats/lipids. Some other substances are also added during manufacturing (e.g., sodium benzoate and sulfites that prevent oxidation and suppress microbial growth). All processing aids are appropriate for use in foods. Residual solvents and additives were determined in five lots of canola concentrate and presented in Table 10.

Table 10. Residual of solvents and processing aids in Raptein™30

Lot number	Benzoic acid and its salts [mg/kg]	Sulphur dioxide [mg/kg]	Ethanol [mg/kg]	Ethyl acetate [mg/kg]
R-03#35	10	<10	17.5	71.0
R-06#60	<10	<10	117.0	53.4
R-09#41	<10	<10	12.3	<1.0
R-13#60	<10	<10	14.1	29.8
R-15#66	<10	<10	<1.0	14.9

The analytical results for the canola concentrate summarized in the above tables and included in Certificates of Analysis (COAs) in Appendix 1 confirm that the finished product meets the analytical specifications. Thus, it demonstrates that NapiFeryn's manufacturing process results in a consistently reproducible product, and confirms the lack of significant levels of impurities and/or contaminants (e.g., anti-nutrients, heavy metals, pesticides, mycotoxins, and microbiological contaminants).

2.3.7. Product stability

One batch of canola (rapeseed) concentrate (Lot No. R-03#35) was placed in a polyethylene (PE) package and was subjected to long-term and accelerated stability studies using two climatic chambers (25°C, 60% humidity and 40°C, 75% humidity). The protein and moisture content as well as microbiological tests (incl. aerobic bacteria count, yeast and molds, *Salmonella* spp.) were determined. Figures 2, 3 and 4 as well as Tables 11 and 12 show the stability profile over the period of 24 weeks. The full data is presented in Appendix 2.

According to the presented results of the stability studies of Raptein™30, no significant changes in the protein or moisture content were reported over the 24-weeks period of storage in 25 °C / 60 %RH. Moreover, the results of microbiological tests carried out at specific points in time were noted below any level of concern. Thus, this assessment confirms that canola concentrate is stable in the aforementioned conditions for at least 24 weeks.

Stability of the product was also assessed in an "accelerated protocol" that is based on a reasoned assumption that one week of shelf life at 40 °C is equivalent (within reasonable limits) to four weeks of shelf life at 20 °C. Adapting such a reasoning, key quality attributes for the samples of Raptein™30 stored in 40 °C/75 %RH should remain constant and material considered as safe during 80 weeks (more than 1.5 year) storage at 20 °C. After a 12-week storage of the product, an insignificant increase in the moisture content is measured, while the level of microbiological purity and protein content remain stable and unchanged. Thus, the results confirm that the product is safe for human consumption. The packaging used for commercial product will reflect the need to protect the product to maintain its quality during storage and transport, and ensure that it meets product specifications on moisture content.

It is worth mentioning that canola protein isolates - Puratein® and Supertein® - have been reported to be stable with protein content practically unchanged for a period of over two years (FDA, 2010). According to another safety study, the intended shelf-life of partially defatted rapeseed powder at ambient temperature and humidity is 6 months (EFSA, 2020). The significantly lower fat content in Raptein™30 (<2%) as compared to partially defatted rapeseed powder (18-21%) is expected to be beneficial to stability of Raptein™30.

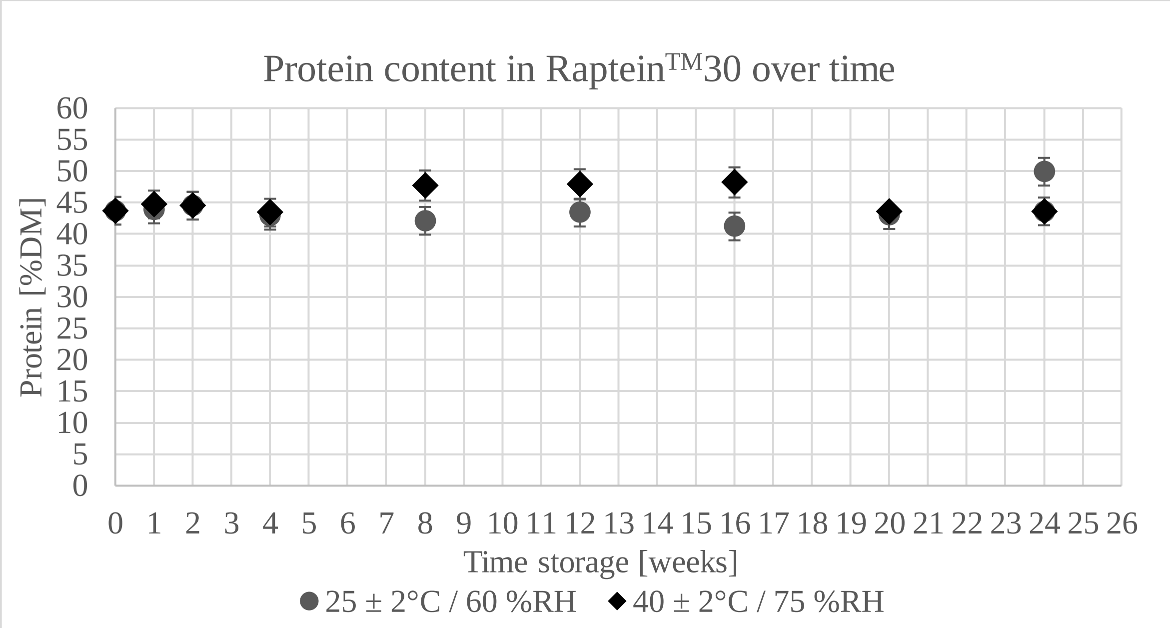

Figure 2. Protein content during stability studies of Raptein™30 (Lot No. R-03#35) in 25 °C / 60 %RH and 40 °C/75 %RH

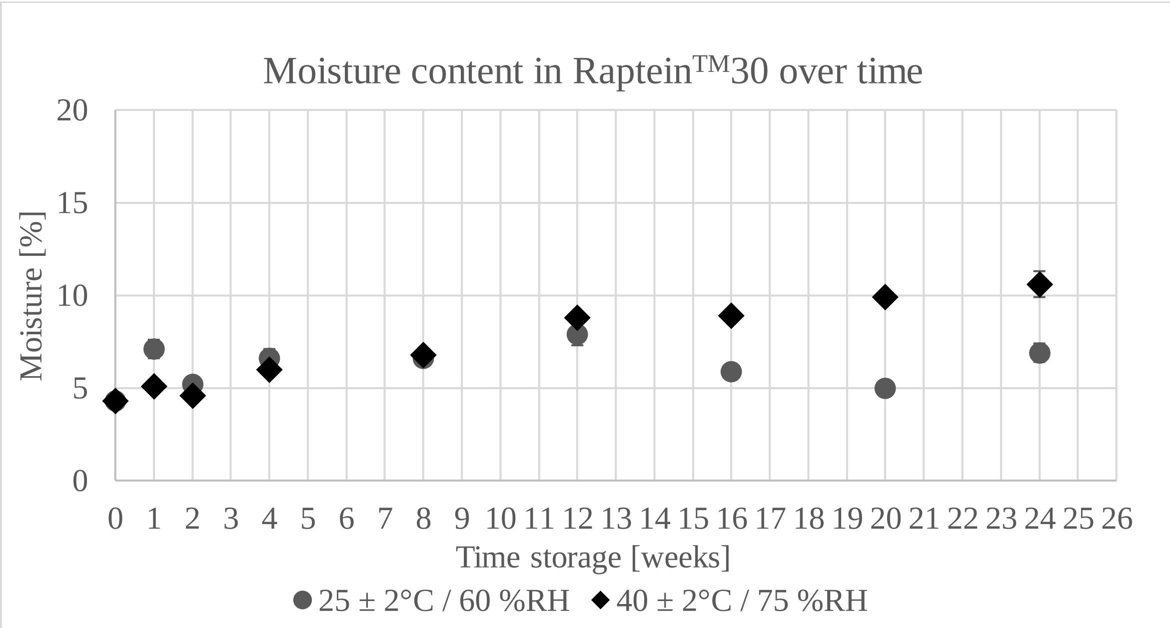

Figure 3. Moisture content during stability studies of Raptein™30 (Lot No. R-03#35) in 25 °C / 60 %RH and 40 °C/75 %RH

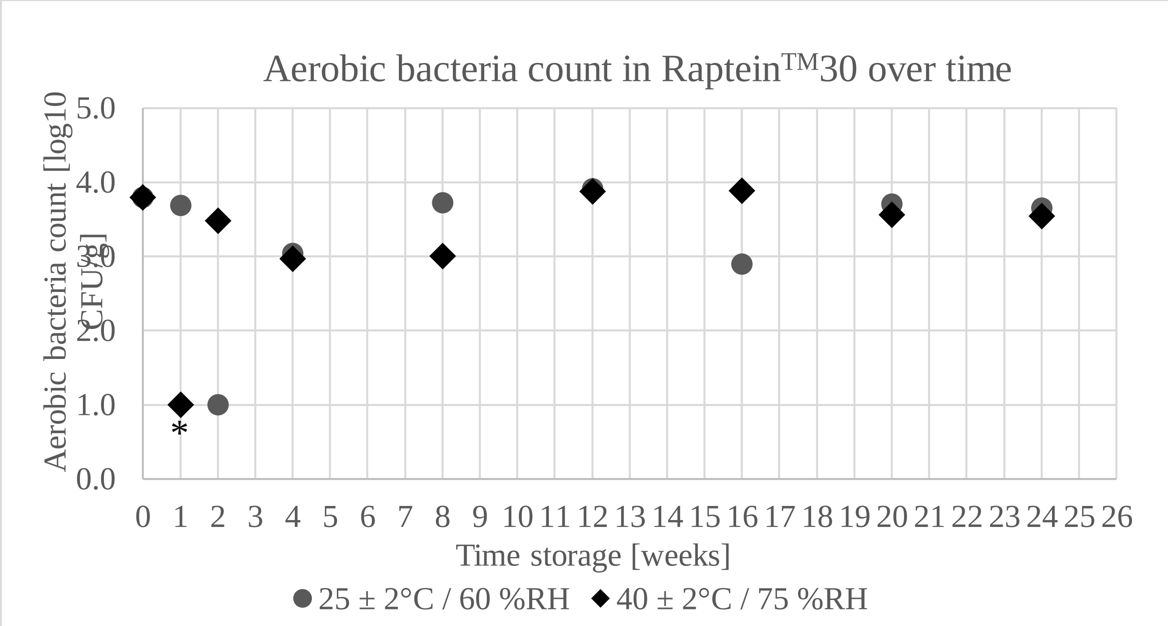

Figure 4. Aerobic bacteria count related to the stability studies of Raptein™30 (Lot No. R-03#35) in 25 °C/60 %RH and 40 °C/75 %RH

* Aerobic bacteria count: <10 CFU/g

Table 11. Microbiological results related to the stability studies of Raptein™30 (Lot No. R-03#35) in 25 °C/60 %RH

Weeks	Yeast and molds count [cfu/g]	Total coliform count [MPN/g]*	*Salmonella* [absence in 25g]
0	<10	0	absent
1	<10	0	absent
2	<10	0	absent
4	<10	0	absent
8	<10	0	absent
12	<10	0	absent
16	<10	0	absent
20	<10	0	absent
24	<10	0	absent

*MPN/g - the most probable number of coliforms

Table 12. Microbiological results related to the stability studies of Raptein™30 (Lot No. R-03#35) in 40 °C/75 %RH

Weeks	Yeast and molds count [cfu/g]	Total coliform count [MPN/g]*	*Salmonella* [absence in 25g]
0	<10	0	absent
1	<10	<10	absent
2	<10	0	absent
4	<10	0	absent
8	<10	0	absent
12	<10	0	absent
16	<10	0	absent
20	<10	0	absent
24	<10	0	absent

*MPN/g - the most probable number of coliforms

2.3.8. Raw materials

The raw material for production of canola concentrate (rapeseed concentrate) is press cake which is a by-product of oil production from *Brassica* species: *Brassica napus*, *Brassica juncea* and *Brassica rapa*.

Press cake is sourced from cGMP-qualified oilseed processing mills and is certified as suitable for use in food. See the cGMP+ certificate of one of the rapeseed press cake suppliers in Appendix 5. Table 13 summarizes the most important parameters of the raw materials used in production of canola concentrate batches described in this report.

Table 13. Physico-chemical composition of 3 lots of rapeseed press cake raw material

Parameter	Unit	Method	Lot No. A-00#58	Lot No. P-00#16	Lot No. A-00#59
Total protein [N*6.25]	%DM	ISO 1871:2009	33.65	32.40	43.49

Parameter	Unit	Method	Lot No. A-00#58	Lot No. P-00#16	Lot No. A-00#59
Fat (direct)	%DM	WE 152/2009 App. III	13.44	16.91	13.71
Ash	%DM	WE 152/2009 App. III	7.42	6.79	7.23
Moisture	%DM	WE 152/2009 App. III	6.56	8.94	8.16
Fiber	%DM	WE 152/2009 App. III	12.22	12.07	5.60
HCl-insoluble ash	%DM	PN-A-88022:1959	0.24	0.42	not analyzed

DM – dry matter

Risks of potential contaminants from the rapeseed cake are:

- Heavy metals

Arsenic, cadmium, lead and mercury were measured in rapeseed cakes used for canola concentrate production (Table 14). The obtained results were below any level of concern.

Table 14. Heavy metals content in three lots of the raw material rapeseed press cake

Rapeseed cake Lot no.	Arsenic (As) [mg/kg DM]	Cadmium (Cd) [mg/kg DM]	Lead (Pb) [mg/kg DM]	Mercury (Hg) [mg/kg DM]
Limit of quantification	<0.1 mg/kg	<0.005 mg/kg	<0.005 mg/kg	<0.005 mg/kg
A-00#58	<LOQ	0.077	0.009	<LOQ
P-00#16	<LOQ	0.063	0.040	<LOQ
A-00#59	0.22	0.120	0.015	<LOQ

- Pesticides

NapiFeryn BioTech obtained the raw materials from suppliers that follow cGMP+. Two lots of the rapeseed cake (no. A-00#58 and A-00#59) were tested. The material was screened for residues of about 600 pesticides using GC-MS and LC-MS methods. The content of 68 of the most significant compounds was assessed according to the EU Pesticides Database and the Maximum Residue Limits (MRL) Database (Bryant Christie Inc.).

The determined content of thiophanate-methyl in one of the rapeseed cake lot (no. A-00#59) was 0.15 mg/kg, while the maximum residual limits for this pesticide in canola seeds in the US and EU is 0.1 mg/kg. However, all other analyzed pesticides were below the LOQ. The results of isofetamid for all tested lots were below LOQ (<0.03 mg/kg), but the maximum residue level in US and EU is 0.015 mg/kg. No other residues were reported to be at a level of concern. Further, pesticide analyses of canola concentrate batches showed no pesticides at any level of concern. Complete data set is included in the Appendix 1.

- Microbiological contamination

Data for three independent and representative lots of rapeseed press cake are presented in Appendix 1. The results are considered within safe limits according to NapiFeryn BioTech’s and internal requirements for raw materials (see Table 15).

Table 15. Microbiology in three lots of the raw material rapeseed press cake

Parameter	Unit	Lot No. A-00#58	Lot No. P-00#16	Lot No. A-00#59
Total plate count	cfu/g	2 700	<10	28 000
E. coli	absent in 10g	not analyzed	absent	absent
Salmonella spp.	absent in 25 g	absent	absent	absent
Yeast and Molds	cfu/g	<10	<10	<10
Total coliform count	cfu/g	not analyzed	0	0
Sulphite reducing anaerobes	cfu/g	<10	<10	<10
Listeria monocytogenes	absent in 25 g	absent	absent	absent
Clostridium perfringens	cfu/g	<10	<10	<10

- Mycotoxins

The levels of mycotoxins in rapeseed press cake are monitored. As presented in Table 16, reported concentrations are below LOQ and hence are not of safety concern according US and EU regulations. See Appendix 1.

Table 16. Mycotoxin concentration in three different and representative lots of rapeseed press cake

Mycotoxin	Unit	Lot Number		
		A-00#58	P-00#16	A-00#59
3-Acetyldeoxynivalenol (3AcDON)	µg/kg	<20	<20	<20
BA-TM-03 15-Acetyldeoxynivalenol (15AcDON)	µg/kg	<20	<20	<20
Ochratoxin A	µg/kg	<1	<1	<1
Deoxynivalenol (DON)	µg/kg	<20	<20	<20
Fusarenone X (Fus X)	µg/kg	<20	<20	<20
Nivalenol (NIV)	µg/kg	<20	<20	<20
T_2 Toxin (T_2)	µg/kg	<10	<10	<10
HT_2 Toxin (HT_2)	µg/kg	<10	<10	<10
Zearalenone (ZON)	µg/kg	<10	<10	<10
Aflatoxin B_1	µg/kg	<1	<1	<1
Aflatoxin B_2	µg/kg	<1	<1	<1
Aflatoxin G_1	µg/kg	<1	<1	<1
Aflatoxin G_2	µg/kg	<1	<1	<1
Total Aflatoxin	µg/kg	<4	<4	<4
Fumonisin B_1	µg/kg	<20	<20	<20
Fumonisin B_2	µg/kg	<20	<20	<20
Fumonisin B_3	µg/kg	<20	<20	<20

2.3.9. Information on the physical and technical effect

Proteins make up an integral part of many food products and are essential components of the daily diet of humans. Canola (rapeseed) proteins - cruciferin and napin - will be digested in standard metabolic processes like any other proteins, to yield break-down products in forms of amino acids and small peptides. The amino acids of canola (rapeseed) protein are relatively efficiently absorbed and utilized (Galibois et al., 1989; Bos et al., 2007; Fleddermann et al., 2013). According to a human feeding study, *Brassica napus* rapeseed protein isolate had an ileal digestibility value of 84%, which was lower than cereal-based proteins, but higher than legumes, such as lupin (Bos et al., 2007). The true nitrogen digestibility of canola protein isolate determined in rat assay showed 93.3%, which is only slightly lower than the 94.9% for soy protein isolate (Fleddermann et al., 2013). In the same research, increases of amino acids in human plasma was measured. Results showed that consumption of canola or soy protein led to significant increases of amino acids after 62.3 and 83.6 min, respectively (Fleddermann et al., 2013). This study showed that canola (rapeseed) protein has a high nutritional quality and can be considered as efficient as soy protein for a postprandial amino acid response (Fleddermann et al., 2013).

The US FDA recognizes FAO 1991 report and use of *in vivo* rat digestibility assay in determining protein quality (PDCAAS) for nutrient content claims and declaration of % DV of proteins on the Nutrition Facts Table on US food product labels. PDCAAS values for other canola protein isolates were estimated to be 86% (Fleddermann et al., 2013) and 87% (FDA, 2017a).

For completeness, the *in vitro* protein digestibility (IVPD) of raw vegetable proteins and protein isolates are as follows: lupin seeds 82.3-89.0%, Chinese bean seed (*Vigna unguiculata*) 83.1-86.1%, and pea protein isolate 79-87% (Pastor-Cavada et al., 2009; Tinus et al., 2012; Laguna et al., 2017). In comparison, IVPD was determined during digesting of raw and extruded canola flour under various conditions, and similar values were obtained, 79.5 and 78.9-81.3%, respectively (Zhang et al., 2017).

3. Dietary exposure

NapiFeryn intends to use canola concentrate (Raptein™30) in a variety of foods and beverages at use levels ranging from 5% to 100%. Canola concentrate (Raptein™30) can be incorporated into food products to provide nutritional value as a protein and/or fiber source and/or functional properties.

Dietary exposure was estimated on the basis of the application rate provided in Table 17 (and Table 1 in Appendix 3).

It is expected that Raptein™30 will be used in food and beverage products for consumption by adults and children 1 year of age and older. In summary, 79.66% of the total U.S. population 2+ years of age were identified as consumers from the selected food uses in the 2017-2018 survey (CDC 2022. NHANES 2017-2018 Dietary Data).

The canola concentrate (Raptein™30) will be added to food products as a protein or fiber substitute and therefore will not contribute to any additional exposure to protein and fiber for the consumers. Most of the protein intake in the population is, and will remain, in the form of unprocessed foods, including meat, poultry, fish and legumes. Dietary fiber is found in most fruits, vegetables, legumes and grains.

The Food and Drug Administration (FDA) has established a protein daily reference value (DRV) of 50g/day for adults and children four years of age or older (21 CFR § 101.9). The Institute of Medicine suggests a Recommended Dietary Allowance (RDA) for protein of 56 g/day for adult males and 46 g/day for adult females (IOM, 2006). However, the RDA does not represent an upper limit of consumption. It is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all healthy individuals in a group.

The Food and Drug Administration (FDA) has established a dietary fiber daily reference value (DRV) of 28 g/day for adults and children four years of age or older (21 CFR § 101.9). The Institute of Medicine recommends an Adequate Intake (AI) for total fiber of 38 g/day for adult males <50 years of age and 25 g/day for adult females <50 years of age. For adults >50 years of age, the AI of total fiber is 30 grams for men and 21 grams for women. The AI for fiber is based on the median fiber intake level observed to achieve the lowest risk of coronary heart disease (CHD) (IOM, 2006).

We do not realistically expect that the actual consumption of foods containing canola concentrate (Raptein™30) would result in a daily consumption of greater than the DRV or RDA for protein and DRV and AI for fiber. The canola concentrate is intended to be only an alternative source of protein and/or fiber for current uses in food.

Dietary and functional fibers are not regarded as essential nutrients. Therefore, inadequate intakes do not result in biochemical or clinical symptoms of a deficiency. As part of an overall healthy diet, high intake of dietary fiber will not cause adverse effects in healthy people (IOM, 2006).

Nevertheless, as the DGAC (Dietary Guidelines Advisory Committee, 2020) defines several nutrients are underconsumed, amongst them fiber. Underconsumption of fiber has been identified as a public health concern for the majority of the U.S. population. The AI for fiber is based on the association between higher intakes of fiber and reduced risk of coronary heart disease. Canola concentrate (Raptein™30) is composed of plant-based protein and fiber that can provide an alternative dietary protein source and fiber source. Consumption of food containing canola concentrate (Raptein™30) will allow to enrich the diet with both protein and fiber.

In addition, many protein products and fiber products are available in the marketplace. FDA has reviewed extensive published information and data as part of GRAS notifications for protein products and subsequently issued “no objection letters”. Among plant proteins, examples are: isolated wheat protein (FDA, 1999), canola protein isolates (FDA, 2010), canola protein isolate and hydrolyzed canola protein isolate (FDA, 2011), canola/rapeseed protein isolate (FDA, 2017), potato protein isolate (FDA, 2013), oat protein (FDA, 2015c), pea protein (FDA, 2016a; 2016c; 2018b; 2019; 2020a), and fava bean protein (FDA, 2020b).

FDA has also reviewed extensive data as part of GRAS notifications for fiber products and subsequently issued “no objection letters”. Among them, examples are: pecan shell fiber (FDA, 2016d), citrus fiber (FDA, 2016b), insoluble fiber from citrus peel (FDA, 2015b), pea fiber (FDA, 2014), rice hull fiber (FDA, 2015a), and corn fiber (FDA, 2012).

According to the Federal Meat Inspection Act and the Poultry Products Inspection Act, the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) is responsible for determining the efficacy and suitability of food ingredients intended for use in meat and poultry products.

The intended use of canola concentrate (Raptein™30) is as a flavoring agent or binder in various comminuted or whole muscle meat and poultry products at levels of up to 15%, where standards of identity permit. Suitability as a binder is determined by FSIS on a case-by-case basis by considering the proposed use of the substance and the specific meat or poultry product categories to which the ingredient is added. Appendix 6 includes a report of the evaluation of the quality characteristics of sausages containing 2% Raptein™30 as a partial replacement of soy protein concentrate in a sausage link product.

3.1. Estimated daily intake of canola concentrate

Canola concentrate (Raptein™30) can be used in various types of food formulations as a substitute for, and/or in conjunction with, other protein and/or fiber sources in conventional food products. Due to its functional properties, it is also intended for use as a protein source and/or fiber source, thickener or texturizer. The highest use level (g Raptein™30/100g of food as consumed) is for snack foods (e.g. popcorn, chips etc.). Raptein™30 can also constitute up to 100% (as is basis) of protein-fiber and nutritional powders.

Raptein™30 is intended to be used in a variety of categories of food and beverages including: bakery products, breakfast cereals, grain products, pastas, bars, protein-fiber and nutritional powders, beverages and beverage bases non-alcoholic, milk products and dairy analogs, coffee and tea, meat product analogs, fat, oils and salad dressings, egg substitutes, soup and soup mixes, sauces, gravies, condiments and dips, nut products, snack foods, fruit and water ices, confections, candies and frostings, sweet sauces, toppings, and syrups, jams, jellies, gelatins, puddings, fillings, processed fruits, vegetables, legumes, batter/breadings, spice and seasoning mixes, processed meat and poultry (Table 17; Table 1 in Appendix 3).

Table 17. Canola concentrate **(**Raptein™30) intended uses and use levels

Food Categories	Intended Food Uses	Max. use level (g Raptein™30 / 100g of food as consumed)
Bakery Products	Bakery products (e.g. breads, rolls, pies etc.) and baking mixes	20
Beverages	Beverages and beverage bases non-alcoholic (e.g. fruit and/or vegetable drinks, smoothies, energy drinks etc.)	7
	Coffee and tea (including instant coffee and tea)	7
Cereals and Other Grain Products	Breakfast cereals (e.g. oatmeal, grits, ready to eat breakfast cereal etc.)	35
	Grain products, pastas (e.g. noodles, macaroni etc.)	20
Dairy Products and Substitutes	Milk products and dairy analogs (e.g. cheeses, yoghurts, creams, frozen desserts etc.)	15
Egg and Egg Substitutes	Egg substitutes (e.g. powdered egg analogs etc.) and egg products analogs	7
Fats and Oils	Fat, oils and salad dressings (e.g. mayonnaise-type dressings, margarine-like spreads etc.)	10
Meat or Poultry and Substitutes	Meat product analogs (e.g. sausages, burgers, chops etc.)	20
	Processed meat and poultry	15
Fruits and Fruit Juices	Fruit and water ices	7
Miscellaneous	Batter/breadings	10
	Protein-fiber and nutritional powders	100*
	Spice and seasoning mixes**	5

Food Categories	Intended Food Uses	Max. use level (g Raptein™30 / 100g of food as consumed)
	Bars (e.g. nutritional, protein etc.)	20
Nuts and Seeds	Nut products (e.g. nut spreads, nut butters etc.)	30
Processed Fruits and Vegetables	Processed fruits, vegetables, legumes (e.g. vegetable spreads)	30
Sauces, Dips, Gravies and Condiments	Sauces, gravies, condiments and dips	10
Snacks	Snack foods (e.g. popcorn, chips etc.)	40
Soups	Soup and soup mixes	15
Sugars and Sweets	Confections, candies and frostings (e.g. baking chocolates, marshmallows, non-chocolate candies etc.)	20
	Jams, jellies, gelatins, puddings, fillings (including mixes)	30
	Sweet sauces, toppings, and syrups	10
* As is basis (content for dry powder), proposed serving – 30 g (one serving a day) ** No NHANES data exists; calculated independently		

The estimated daily intake of Raptein™30 is calculated on the basis of the exposure of individuals to Raptein™30 through consumption of the foods and beverages listed in Table 17 (and Table 1 in Appendix 3). Estimates for the intake of Raptein™30 were based on the food uses and maximum use level in Table 17 (and Table 1 in Appendix 3), in conjunction with food consumption data included in the National Center for Health Statistics' (NCHS) 2017-2018 National Health and Nutrition Examination Surveys (NHANES) (CDC, 2022; USDA, 2018). A total of 2582 food codes representative of each approved use were chosen from the Food and Nutrition Database for Dietary Studies (FNDDS) for the corresponding biennial NHANES survey. Calculations from NHANES for the mean and 90th percentile intakes were performed for representative food uses of Raptein™30.

The estimated "all-user" total intakes of Raptein™30 from 2582 proposed food uses listed in NHANES in the U.S. by population group is described in Table 18 (Table 2 in Appendix 3). In summary, 79.66 % of the total U.S. population 2+ years was identified as potential consumers of Raptein™30 from the selected food uses in the 2017-2018 survey.

The mean intakes of Raptein™30 from consumers ages 2+ from the selected food uses were estimated to be 19.29 g Raptein™30/person/day or 0.28 g Raptein™30/kg body weight/day. The heavy consumer (90^{th} percentile) intakes were estimated to be 40.80 g Raptein™30/person/day or 0.59 g/kg body weight/day.

Table 18. Estimated "All-user" Daily Intake (EDI) of NapiFeryn's Raptein™30 in Targeted Foods by Population Group (2017-2018 NHANES Data), excluding spices and seasoning

Population Group	N users	N popula-tion	% Users	Mean mass (kg)	Mean EDI (g)	90th % EDI (g)	Mean EDI (g/kg)	90th % EDI (g/kg)
ages 1-2	453	642	70.56	13.24	11.71	26.97	0.88	2.04
ages 2-5	365	529	69.00	21.08	13.46	30.00	0.64	1.42
ages 6-12	941	1258	74.80	41.89	16.37	36.6	0.39	0.87
ages 13-19	775	886	87.47	70.32	20.78	44.59	0.30	0.63
ages 20 and up	4632	5493	84.33	81.7	20.32	43.00	0.25	0.53
ages 2 and up	6713	8427	79.66	69.41	19.29	40.80	0.28	0.59

There are no food codes that can be used for stand-alone spice and seasoning mixes in NHANES. Accordingly, to calculate the estimated daily intake of Raptein™30 from its use in spice and seasoning mix, surrogate products were used to estimate its intake level.

The intakes of Raptein™30 for all food uses, including spices and seasonings at 1 g/day, are estimated to be the EDIs (calculated above) + 1 g, resulting in a mean EDI of 20.29 g/person/day or 0.29 g/kg body weight/day and the heavy consumer (90^{th} percentile) EDI of 41.8 g/person/day or 0.60 g/kg body weight/day.

More details can be found in the complete report: Raptein™30 Estimated Daily Intake Report attached as Appendix 3.

The Estimated Daily Intake (EDI) of Raptein™30 (excluding spices and seasoning mixes) for heavy consumer (90^{th} percentile) ranges from 2.04 g/kg body weight/day for population group: ‘1-2 ages’ to 0.53 g/kg body weight/day for consumers age 20 and up.

The exposure of Raptein™30 for heavy consumers is still much lower (approximately 7-to 22-fold lower) than the no-observed-adverse-effect level (NOAEL) values established for cruciferin-rich protein isolate: 11.24 g/kg body weight/day (male rats) and napin-rich protein isolate: 12.46 g/kg body weight/day (male rats) in 13-weeks toxicity studies (Mejia et al. 2009a, Mejia et al. 2009b). Therefore, these results support the product safety with the expected exposure.

The canola concentrate (Raptein™30) will be added to food products as a protein and/or fiber substitute and therefore will not contribute any additional exposure to protein and/or fiber for consumers. Most of the protein intake in the population is, and will remain, as unprocessed foods, including meat, poultry, fish and legumes. Dietary fiber is found in most fruits, vegetables, legumes and grains. Canola concentrate (Raptein™30) is only an alternative source of protein and/or fiber and will not replace all dietary protein and/or fiber sources, but will allow to enrich the diet with both protein and fiber.

4. Self-limiting levels of use

The use of canola protein as a food ingredient is self-limiting due to its sensory and food-technological properties. Therefore, it is not expected to exceed the amounts proposed in Table 17.

5. Experience based on common use in food before 1958

The statutory basis for the conclusion of GRAS use of canola concentrate (rapeseed concentrate) in this document is not based on common use in foods before 1958. The GRAS determination is based on scientific procedures. NapiFeryn BioTech is unaware of any use of rapeseed protein isolate prior to 1958. Before the appearance of low erucic acid varieties in the 1970's, rapeseed was mainly used for the production of animal feed (FDA, 2017).

6. Safety narrative

Literature review was conducted to assess available safety data on canola/rapeseed with particular reference to the topics of canola/rapeseed products and canola/rapeseed potential anti-nutritional factors, between 2001 and 2021. Apart from general internet searching, databases (e.g. ScienceDirect and PubMed) and reviews from organizations such as the U.S. Food and Drug Administration, USDA, U.S. Dept. of Health and Human Services, EFSA, European Commission and Food Standards Australia New Zealand, were considered. Because canola has been commonly consumed and studied for many decades, pre-2000 data sources have also been included. The most important and relevant data are summarized in this dossier.

6.1. Summary

This GRAS notification concerns canola concentrate (Raptein™30) obtained from the rapeseed (canola) press cake that is a by-product of rapeseed oil production. Rapeseed oil is produced by pressing the seeds of the rape plant from three varieties, *Brassica napus* and/or *Brassica juncea* and/or *Brassica rapa*. The rapeseed press cake is composed of crushed seeds. The canola concentrate is mainly composed of protein (30-45% dry matter) and dietary fiber (40-70% dry matter). The intended use of canola concentrate is a variety of foods and beverages. The use levels may be ranging from 5% to 100%. Canola concentrate can be incorporated into food products to provide nutritional value as a protein and/or fiber source and/or functional properties.

Nutrients in canola concentrate are expected to be absorbed, digested, metabolised and excreted identically to already authorised canola protein isolates and partially defatted rapeseed powder. Literature reveals that rapeseed (canola) contains specific anti-nutritional factors, such as phenolic compounds, glucosinolates, erucic acid, phytic acid and protease inhibitors. These anti-nutritional factors are minimized due to the canola concentrate manufacturing process (which is according to cGMP). Analytical testing for these compounds in the final product revealed that they are either below the level of detection of the analytical method or in negligible levels far below a concentration of concern. Potential risks of food allergy and celiac disease were evaluated using a bioinformatics approach. The search revealed that Raptein™30 proteins may elicit possible allergenic effects. The results also indicated that the proteins are unlikely to elicit celiac disease.

Based on the nature, source, and production process of canola concentrate, the product is not expected to be of toxicological concern and it is concluded to be safe for human consumption.

6.2. History of Use and Regulatory Approval

According to current knowledge, there is no formal approval for the use of canola concentrate in human nutrition in the U.S. However, canola protein isolate has been already recognized as GRAS by FDA for use as a food ingredient, protein source, thickener, water binder, emulsifier, gelling agent, foaming agent or texturizer. Table 19 contains a summary of previous effective GRAS notices for canola protein isolates. One of them (FDA, 2011) was also approved as novel food ingredient by EFSA (2013).

Furthermore, the partially defatted rapeseed powder has been authorized as novel food under Regulation (EU) 2015/2283 and recognized as safe for use as a food ingredient in the European Union (EFSA, 2020). The major constituents of this powder are dietary fiber (37-43%), protein (34-41%) and vegetable oil (18-21%). Because of high protein and high dietary fiber content, it is considered similar to canola concentrate described in this dossier. It is worth mentioning that another plant powder has received the FDA letter of no objection. The powder from duckweed contains 30-45% dietary fiber, 40-50% protein and max 10% fat (FDA, 2018a).

Table 19. GRAS Notices for canola protein isolates

Year	GRAS No.	Title	Intended use	FDA's Letter
2010	327	Cruciferin-rich canola/rapeseed protein isolate and napin-rich canola/rapeseed protein isolate	Ingredient in dairy products, grain products, fruit and vegetable juices and beverages, salad dressings, meal replacements, and nutritional bars	FDA has no questions
2011	386	Canola protein isolate and hydrolyzed canola protein isolate	Ingredients in foods such as bakery products, snack foods, beverages, soups, dairy products, dry instant milkshake mixes and protein drinks, instant powdered nutritional beverages, processed meat and poultry products, vegetarian food products/meat	FDA has no questions

Year	GRAS No.	Title	Intended use	FDA's Letter
			analogues, and meal replacement and nutritional bars	
2017	683	Canola protein isolate	Intended for use as a protein source, thickener, water binder, emulsifier, gelling agent, foaming agent or texturizer in prepared food (e.g. ready-to-eat meals, soups, pasta, snacks), meat analogues, bakery products (e.g. bread, rolls, doughnut, cookies, cakes, pies, batters, muffins, pasta, and cereal bars, cereals), protein enriched bakery products (e.g. bars, cookies), sports nutrition (e.g. protein drinks, energy bars), weight management (e.g. meal replacement, nutritional bars), beverages (e.g. fruit juices, soft drinks, juice blends), dairy products (e.g. desserts, ice cream, cheese, yogurt), medical nutrition (e.g. protein fortified drinks, ready-to-drink), elderly nutrition (e.g. foods specifically meant for the needs of elderly people) at use levels ranging from 5 to 30 percent.	FDA has no questions

6.3. Current safe use

Rapeseed, *Brassica napus* subspecies, is a large winter or spring annual oil crop in the *Brassicaceae* family and is also known as rape and oilseed rape, and for a specific group of cultivars, “canola”. As sources of common vegetables in the diet, *Brassica* species, such as mustard, cabbage, broccoli, cauliflower and turnip, have been consumed for centuries. Many of them are currently recognized as having desirable health benefits.

Species of the *Brassicaceae* family are important agricultural crops world-wide. The seeds of rapeseed are rich in oil and contain considerable amounts of protein (20 to 35% of seed dry weight; Wanasundara, 2011).

Rapeseed has three major protein fractions, namely cruciferin, napin and oleosin. Their primary, secondary and tertiary structures differ significantly, thus they have different properties and functionalities. The dominant storage proteins, cruciferin and napin, are located in protein storage vacuoles of embryonic tissues of the seed (Wanasundara et al., 2011). The ratio of cruciferin to napin is between 0.6 to 2.0 depending on erucic acid and glucosinolate levels (Wanasundara, 2011). Oleosin is a structural protein that is found in the membrane of the organelle called oil body which is the oil storage of the seed (Wanasundara et al., 2016).

Dietary fiber is defined as ‘the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine’. It includes polysaccharides, lignin, oligosaccharides and associated plant substances. It is well-known for its positive impact on human health. (AACC, 2001). Clinical studies have shown that the activity of fiber in the small intestine promotes the attenuation of blood cholesterol and glucose (mainly viscous/gel-forming fractions). Another crucial effect is the laxation as a result of insoluble and soluble fiber activity in the large intestine. As a consequence, dietary fiber helps to reduce the risk of cardiovascular diseases, facilitates weight loss in patients with metabolic problems and alleviates irritable bowel syndrome. Thus, a high-fiber diet is usually recommended. Dietary fiber can be commonly found in selected unprocessed foods. However, it is also obtained as a by-product from industrial processing (e.g. oat bran). Fiber supplements and fortified food products are widely consumed because of expected health benefits. (Lambeau and McRorie Jr., 2017; Elleuch et al., 2011).

Rapeseeds contain fiber fractions, which are mostly found in the hulls. Insoluble dietary fiber is a predominant fiber type in rapeseed seeds. This fraction is mostly composed of lignins, celluloses and usually some hemicelluloses. Soluble dietary fiber in rapeseed includes hemicelluloses, gums, pectins, mucilages and mixed β-glucans. Also, a wide range of indigestible constituents (such as glycoproteins) are associated with dietary fiber (Chmielewska et al., 2020; Joehnke et al., 2018).

The inhibitory effect of fiber on canola protein digestibility has been studied *in vitro* and *in vivo* (rats, chickens, pigs) (Bayley et. al 1975; Bjergegaard et. al, 1991; Joehnke et al., 2018). The reason for low availability of proteins is mostly related to the mentioned association of relatively big proportion of proteins with fiber fractions as well as to the resistance of dietary fiber to endogenous enzymes in the digestive tract (Bjergegaard et. al, 1991). However, recent studies have shown that fibers from dehulled rapeseed may be present in high quantities in the diet without any negative impact (Joehnke et al., 2018).

Rapeseed is a member of the family *Brassicaceae*. There are three main species namely *B. napus* (cultivated in Europe and Canada, having a black or reddish color), *B. rapa/campestris* (cultivated in Canada, having a black or yellow color), *B. juncea* (cultivated in Canada, having a yellow color). The main difference is in oil content (Daun et al., 2011; Wanasundara, 2011). There are two types of rapeseed: industrial and edible (also called canola).

Oilseed rape was first cultivated in India some 4,000 years ago and about 2,000 years later it was introduced to China. For the first time in Europe agro-scale production of rapeseed was recorded in the thirteenth century. Interest in breeding intensified in Canada soon after the crop was introduced from Europe in the 1940s. The first efforts were concentrated on improving the agronomic properties and the oil content. In the late 1970s, the name canola was adopted in North America to distinguish low erucic acid plants from other types of rapeseed (Snowdon, Lühs and Friedt, 2006).

Low in erucic acid canola oil was recognized as GRAS in 1985 by the U.S. FDA (21 CFR §184.1555(c)), which is the edible oil obtained from *Brassica napus* or *Brassica campestris*. Later, this GRAS status was extended to canola oil from *Brassica juncea*.

The *Brassicaceae* family contain the same anti-nutritional factors as rape, namely, phytic acid and glucosinolates.

Therefore, there is long history of use as human a food ingredient source of rapeseeds, from which canola (rapeseed) oil and canola concentrate (Raptein™30) are obtained.

6.4. NapiFeryn BioTech's canola concentrate

Raptein™30 (canola/rapeseed concentrate) is produced according to cGMP by a multistep process starting from rapeseed cake, the by-product of rapeseed oil production. Rapeseed varieties used for the production of human edible vegetable oil are low in the anti-nutritional factors: erucic acid and glucosinolates.

The purified final product is a white to off-white powder, estimated to be stable at room temperature for more than 1 year (stability studies are being continued). See Sections 2.3 through 2.9 of the dossier.

Based on the manufacturing process information presented in Section 2.3, it is highly unlikely the process could adversely impact the safety of the product.

6.5. Contradictory information

NapiFeryn BioTech in consultation with Bioresco Ltd. critically evaluated the available literature (see Section 7) and summarized the findings in this GRAS dossier. Raw materials and canola concentrate (rapeseed concentrate) independent (non-consecutive) batches were tested for heavy metals, mycotoxins, pesticides as well as different anti-nutritional substances. All the information supports the safety of Raptein™30.

6.6. Toxicology

Extensive published scientific studies have been conducted to evaluate the toxicity of rapeseed products. Table 20 shows the results of various safety studies on rapeseed cake, rapeseed meals, rapeseed protein concentrates, rapeseed protein isolates and rapeseed powder.

Table 20. Review of published safety data on rapeseed products

Study	Protein product/Rapeseed product	Conclusions	Reference
Feeding of rats	Rapeseed cake	Acceptable protein source, toxic glucosides were removed	Matet et al., 1949
Feeding of rats	Mustard and rapeseed meals	Effect comparable to soy flour, toxic agent were removed	Goering et al., 1960
Toxicological test on hens	Rapeseed meals	Thyrotoxic effect, increase in the weight of thyroid gland	Jackson, 1970
Toxicological test on rats	Lipid-protein rapeseed concentrate	Effect comparable to control, higher frequency of renal calculi	Agren and Eklund, 1972
Reproduction in rats	Detoxified rapeseed protein concentrate	Litter size reduced, after detoxification toxic components were still present	Eklund, 1973

Study	Protein product/Rapeseed product	Conclusions	Reference
Reproduction in rats	Rapeseed protein concentrate	Loss of appetite, decrease in weight gain	Eklund, 1975
Feeding of rats and dogs	Rapeseed protein flours	No observed changes, no negative effect on thyroid gland	Loew et al., 1976
Reproduction in rats	Rapeseed protein concentrate, zinc supplementation	Zinc supplementation relieves side effect of rapeseed protein concentrate diet such as anorexia, zinc deficiency possibly caused by phytates present in rapeseed protein concentrate	Shah et al., 1979; Jones, 1979
Feeding of rats	Rapeseed meal	Slight enlargement of the liver and kidneys	Thompson et al., 1982
Thyroid gland stimulating tests in rats	Rapeseed meal, purified rapeseed protein isolate (from rapeseed flour), rapeseed extraction residue from rapeseed meal	Serious toxic effect of rapeseed meal on thyroid gland, slight abnormalities in the functioning of the thyroid gland in case of purified rapeseed protein isolate, no toxic effect of rapeseed extraction residue	Kroll and Przybilski, 1991
Feeding of rats	Rapeseed protein isolate and rapeseed extraction residue	Changes in kidneys and liver, no changes indicating a toxic effect	Plass et al., 1992
Toxicity studies on rats	Cruciferin-rich canola protein isolate	Slight increase of thyroid weight; NOEL was defined as 10%, NOAEL as 20% (the highest fed level, equivalent to 11,24 g/kg BW/day for males and 14,11 g/kg BW/day for females.)	Mejia et al., 2009a
Toxicity studies on rats	Napin-rich canola protein isolate	Reduction in food consumption and body weight gains linked to low palatability of tested product; NOEL was defined as 5%, NOAEL as 20% (the highest fed level; equivalent to 12,46 g/kg BW/day for males and 14,95 g/kg BW/day for females)	Mejia et al., 2009b

Study	Protein product/Rapeseed product	Conclusions	Reference
Assessment of the impact on the metabolic syndrome in rats and humans	Rapeseed protein	Insulin resistance prevention, probably due to high cysteine and arginine content of the rapeseed protein	Hermier et al., 2010
Genotoxicity	Rapeseed powder	No genotoxic potential was revealed	EFSA, 2020
Human study	Rapeseed powder	Human data did not point to rapeseed powder safety concerns	EFSA, 2020

Critical evaluation of these studies indicates that anti-nutritional substances present in rapeseed preparations may have possible toxic effects, especially glucosinolates and phytic acids or their derivatives. Eklund (1973, 1975) and Jones (1979) reported loss of appetite, apathy and decline in litter numbers in rat studies and linked it to the phytic acid presence in rapeseed protein meal and concentrate. Phytic acids can contribute to zinc chelation and zinc deficiency in animals leading to adverse effects. These adverse effects were not reported in pregnant rats supplemented with zinc (Shah et al. 1979). However, other causes of decreased consumption and weight loss are also possible and may be related to each other. For example, Mejia et al. (2009b) reported that in the case of napin-rich rapeseed isolates with 3.34% content of phytic acid, these effects may be due to low sensory characteristics.

Adverse effects of glucosinolates and their derivatives were reported after dietary feeding of rapeseed protein preparation in animals (Thompson et al., 1982). These compounds may contribute to animal weight loss and negative impact on thyroid gland functioning (Tripathi and Mishra, 2007). Linking such an impact with the presence of glucosinolates was confirmed by the researchers who reported the elimination of the negative effect on the thyroid gland after purification of rapeseed preparations from glucosinolates (Loew et al., 1976; Jones, 1979; Kroll and Przybilski, 1991).

The safety of canola/rapeseed protein isolates was examined in 13-week dietary feeding studies with rats. Two rapeseed preparations were tested: cruciferin-rich protein isolate - Puratein® (from ADM/Burcon) and napin-rich protein isolate - Supertein® (from

ADM/Burcon) described as low in anti-nutritional substances. In both studies, 20 rats per sex per group were treated with protein-free diet enriched with 5%, 10% or 20% rapeseed isolate. The control group diet was enriched with 20% casein without vitamins. The following indicators were reported: body weight, food consumption, clinical symptoms, physical abnormalities, eye abnormalities and mortality. Ten animals per sex per group were subjected to clinical pathology tests and functional observational battery and locomotor activity investigations. Necropsy was performed on all rats after test termination. It was reported that rapeseed protein enriched diet had no impact on functional observation tests, clinical chemistry, urinalysis, hematology and histopathology (Mejia et al. 2009a and 2009b).

No impact on body weight and food consumption up to 20% of Puratein® (cruciferin-rich protein isolate) was reported. No-observed-effect level (NOEL) was determined as 10% w/w. The no-observed-adverse-effect level (NOAEL) was reported as 20% w/w, which is approximately equivalent to 11.24 g/kg body weight/day (male) and 14.11g/kg body weight/day (females) (Mejia et al. 2009a and 2009b).

In the case of Supertein® (napin-rich protein isolate) supply, loss of body weight and food consumption were observed, but these effects were linked to a low palatability of rapeseed preparations. Since the lower body weight was associated with lower food intake, that is not considered as toxic effect. NOEL for Supertein® was determined as 5% w/w and NOAEL as 20% w/w, which corresponded to 12.46 g/kg body weight/day (males) and 14.95 g/kg body weight/day (females) (Mejia et al., 2009a; Mejia et al., 2009b).

These 13-week toxicity studies demonstrate that canola protein isolates are considered safe. These studies can support the safety of canola concentrate (Raptein™30), as this product has comparable amino acid profile with canola protein isolates (see Table 2). Further, NapiFeryn's product is characterized as low in anti-nutritional factors, similarly to ADM/Burcon products which is summarized in Table 22. Due to the potentially toxic nature of anti-nutritional compounds such as glucosinolates and phytic acid, low content of these substances allows to limit the toxic effects of canola preparations. The glucosinolates concentration of Raptein™30 is below that of previously notified canola protein isolates cruciferin-rich Puratein® and napin-rich Supertein®.

Absorption, distribution, metabolism and excretion (ADME) studies were performed for partially defatted rapeseed powder (Avena Nordic Grain Oy) as a part of EU Novel Food authorization. This product contains 33-43% protein, 14-22% lipids, 33-43% total fiber, 33-41% water-insoluble HMW fiber, 0.5-3% water-soluble HMW fiber, <0.3% water-soluble LMW fiber <13% lignin, <28% acid detergent fiber (as in the specification product). ADME analysis was focused on protein, fiber and other components, such as phytates or glucosinolates. The following models were used to study the fate of partially defatted rapeseed powder after human ingestion: upper intestinal model to determine protein and oil digestibility, ileal *in vitro* model to study protein digestibility and a colon fermentation model to study the fate of dietary fiber. The study concerning the upper intestinal model showed that proteins were digested by proteolytic enzymes, however the digestion process was approximately 30% less efficient in comparison to soy and whey proteins. *In vitro* studies showed that protein digestibility of the partially defatted rapeseed powder was similar to that of wheat bran. Fermentation of fiber from the partially defatted rapeseed powder was analyzed with the use of a colon model. Fatty acids released from mentioned fiber possessed a comparable profile to that of wheat bran (control). Moreover, insoluble rapeseed fiber absorption was considered negligible. In conclusion, most of partially defatted rapeseed powder compounds (such as protein or fat) will be absorbed in the digestive tract, but dietary fiber fraction will pass the small intestine and will be fermented in the large intestine (EFSA, 2020). It must be noted that a similar ADME pathway for Raptein™30 is expected because of the compositional similarity between these two products.

Genotoxicity studies were performed also for partially defatted rapeseed powder. The studies did not show genotoxic potential of partially defatted rapeseed powder and the EFSA Panel concluded that there were no mutagenicity concerns (EFSA, 2020).

Partially defatted rapeseed powder was also tested in human studies. Controlled, double-blind, 4-week randomized, parallel-group intervention, which was succeeded by a 2-week follow-up period, in 54 healthy subjects was performed. The aim was to evaluate the safety and tolerability of rapeseed powder with special focus on fiber. The subjects were consuming daily two test bars with a total of 20 g of partially defatted rapeseed powder or two control bars (containing 9.1% fiber). The main goal was to compare the frequency of symptoms associated with guts. As secondary outcomes following parameters were assessed: the number of reported adverse effects, clinically significant changes in blood safety tests, frequency of defecation, average stool consistency, BMI and test products' palatability. For the modified intention-to-treat population (N = 26 in the test group, N = 27 in the control group) the non-inferiority was shown, however for the per protocol population (N = 19 in the test group, N = 24 in the control group) was not shown. No clinically relevant findings were observed related to safety haematological parameters or clinical chemistry. The number, severity and causality of adverse effects were comparable between the two groups. The palatability of bar with partially defatted rapeseed powder was slightly lower, presumably because of drier consistency in comparison to the control bar. For intention-to-treat population, the significant differences in gastrointestinal symptoms between test and control group were not observed, in contrast to per protocol population. However, the panel noted that the increase of these symptoms in the test group may be related to higher content of fiber in the test product (14.2 g per day) than in the control product (7.3 g per day). These symptoms were also related to the consumption of other fiber-rich products and was not specific to partially defatted rapeseed powder. Further, the number and severity of other adverse effects were comparable between test and control groups (EFSA, 2020). In conclusion, the available human clinical data did not point to rapeseed powder safety concerns, and thus can support also the safety of Raptein™30.

6.7. Potential anti-nutritional factors

Rapeseeds/canola contain anti-nutritional factors, i. e. phenolic compounds, glucosinolates, erucic acid, phytic acid and protease inhibitors. Table 21 presents the levels of these compounds in five lots of canola concentrate (Raptein™30). Potential negative effects of their consumption are discussed below.

Table 21. Anti-nutritional factors in five lots of Raptein™30

Lot number	Erucic acid [%]	Total phenolics (expressed as sinapic acid) [%DM]	Phytic acid [%DM]	Glucosinolates [mol/kg DM]	Trypsin lnhibitor Activity [mg/g]
R-03#35	<0.1 (<LOQ)	0.011	1.44	<LOQ (<0.05 mmol/kg)	1.8
R-06#60	0.37%	0.004	1.09	<LOQ (<0.05 mmol/kg)	1.9
R-09#41	<0.1 (<LOQ)	0.007	1.38	<LOQ (<0.05 mmol/kg)	1.8
R-13#60	<0.1 (<LOQ)	0.017	1.60	<LOQ (<0.05 mmol/kg)	2.0

Lot number	Erucic acid [%]	Total phenolics (expressed as sinapic acid) [%DM]	Phytic acid [%DM]	Glucosinolates [mol/kg DM]	Trypsin lnhibitor Activity [mg/g]
R-15#66	<0.1 (<LOQ)	0.009	1.49	<LOQ (<0.05 mmol/kg)	2.8

6.7.1. Erucic acid

Erucic acid (docosenoic acid), a 22-carbon monounsaturated fatty acid, is present in small amounts in the lipid component of cruciferous plants, including rapeseed, rapeseed cake and canola. Studies conducted on laboratory animals in the early 1970s reported that erucic acid appeared to have toxic effects on the heart at high enough doses. However, more recent studies of erucic acid cast a doubt on the relevance of rat research to human health. Rats are unusual in their inability to process erucic acid. The symptoms in rats caused by a diet with high levels of erucic acid have not been reported in pigs, primates, or any other animals (Grice and Heggtveit, 1983; Food Standards Australia New Zealand, Australia NZ, 2003).

Similar myocardial lipidosis has also been observed in rats exposed to vegetable fatty acids suggesting that rats are less able to digest vegetable fats (containing erucic acid or not) than other animals (Neat, Thomassen and Osmundsen, 1981).

Food Standards Australia New Zealand (2003) set a provisional tolerable daily intake (PTDI) for an average adult of about 500 mg/day of erucic acid (7.5 mg erucic acid/kg bw/day), calculated based on 'the level that is associated with increased myocardial lipidosis in nursing pigs' (Food Standards Australia New Zealand, (Australia NZ, 2003)). 'There is a 120-fold safety margin between this level and the level that is associated with increased myocardial lipidosis in nursing pigs. The dietary exposure assessment concluded that the majority of exposure to erucic acid by the general population would come from the consumption of canola oil. The dietary intake of erucic acid by an individual consuming at the average level is well below the PTDI; therefore, there is no cause for concern in terms of public health and safety. However, the individual consuming at a high level has the potential to approach the PTDI. This would be particularly so if the level of erucic acid in colza oil were to exceed 2% of the total fatty acids' (Food Standards Australia New Zealand, (Australia NZ, 2003)).

In addition, most animal studies did not show any negative effect despite the high concentrations or unnatural exposure. In one case, neonate piglets (have a limited ability to absorb these fats) had their normal sow's milk replaced solely with rapeseed oil for one hundred percent of their caloric needs (Food Standards Australia New Zealand, (Australia NZ, 2003)).

Animal studies have shown that consumption of oils containing erucic acid over time can lead to a heart condition called myocardial lipidosis but this is temporary and reversible. Other effects reported in animals – including changes in the weight of the kidneys, liver, and skeletal muscle – occur at slightly higher doses (e.g. lowest observed adverse effect level of 0.02 g/kg bw per day was determined for liver toxicity in poultry). Based on this information, experts on EFSA's Panel on Contaminants in the Food Chain (CONTAM Panel) determined a tolerable daily intake of 7 mg/kg bw per day.

The average consumption erucic acid ranges from 0.3 to 4.4 mg/kg daily in different age groups. However, infants and other children may be exposed to up to 7.4 mg/kg per day. But experts noted that they likely overestimated this risk to account for the limitations of the available scientific knowledge. For most consumers, in particular for toddlers (1-2 years old) and other children (3-10 years old), the main exposure to erucic acid in the diet are pastries, cakes and biscuits. For infants (0-12 months), infant formulae are the main contributors (EFSA Panel on Contaminants in the Food Chain (CONTAM)).

Food-grade rapeseed oil (also known as canola oil, low erucic acid rapeseed oil, rapeseed 00 oil, and rapeseed canola-equivalent oil) is regulated to a maximum of 2% erucic acid by weight in the USA (U.S. Dept. of Health and Human Services, 2021) and with special regulations for infant food. Until 2018 EU limit was 5% erucic acid in oil for food uses (and significantly lower for infant foodstuffs), but this was set in 1976 (Commission of the European Communities, 1980) and is not consistent with other guidelines such as that in the Codex Alimentarius and the US FDA which both set a limit of 2% (21 CFR §184.1555). A new EU level of 2% was introduced in 2019 (Commission Regulation (EU) 2019/1870).

Rapeseed is, by design, low in erucic acid. FDA has defined a maximum level of 2% erucic acid for low erucic acid rapeseed oil to be used in food (21 CFR §184.1555). The fact that the Raptein™30 contains only a low amount of fat (<2%), means that any amount of erucic acid in these proteins would be negligible. Actual analyses of erucic acid in different production lots of the canola concentrates (Raptein™30) revealed undetectable (below limit of quantification: <0.1%) or low (0.37%) levels of this fatty acid. In summary, the erucic acid content in Raptein™30 is insignificant and does not represent any toxicological concern. As shown in Table 21, only low or traces of erucic acid are found in Raptein™30.

6.7.2. Total phenolics (expressed as sinapic acid)

Phenolic substances are common in plants and they are generally considered safe. Dietary exposure to them is substantial in human diet. Products rich in phenolic acids are: blueberry (1,881-2,112 mg/kg), cherry (290-1,280 mg/kg), pear (44-1,270 mg/kg), apple (2-258 mg/kg), orange (21-182 mg/kg, potato (100-190 mg/kg) and coffee (56 g/kg/dry weight) (FDA, 2010). Phenolics are in general considered as safe and have beneficial antioxidant effects.

Phenolic acids are present in rapeseed/canola seeds and products (Naczk et al., 1998; Wanasundara et al.. 2016; Khattab et al., 2010). The most prominent of these compounds are sinapic acid (3,5-Dimethoxy-4-hydroxycinnamic acid) and its derivative - sinapine (the choline ester of sinapic acid) (Naczk et al., 1998; Wanasundara et al., 2016; Khattab et al., 2010). Seeds are rich in phenolic compounds and most of them are not removed during the process of receiving oil (Khattab et al., 2010). Phenolic acids in various rapeseed products have been reported at levels from 1.1 to 1.8% (Naczk et al., 1998). Naturally occurring phenolics in rapeseed products do not cause toxicity, but have a negative impact on palatability. Phenolic acids are characterized by a bitter or astringent taste and also contribute to undesirable, dark color (Naczk et al., 1998; Wanasundara et al., 2016; Chmielewska et al., 2020).

Technology of production of canola concentrate (Raptein™30) developed by NapiFeryn BioTech is able to minimize the presence of these components and thus their unfavorable effect on the organoleptic properties of the products. Total content of phenolic compounds in canola concentrates (rapeseed concentrates) was determined using high performance liquid chromatography (HPLC). Obtained levels of total phenolics expressed as sinapic acid are presented in Table 21. Concentration is 0.004-0.017%DM.

This level is lower than the level of phenols in canola/rapeseed protein isolates presented in GRAS Notifications reviewed by FDA (2010, 2011 and 2017). In GRAS Notice No. 327 (FDA, 2010) total content of phenolic acids in Puratein® (cruciferin-rich protein isolate) and Supertein® (napin-rich protein isolate) was reported at 0.40% and 0.26%, respectively. Isolexx™ (canola protein isolate) contained 0.14% and Vitalexx™ (hydrolyzed canola protein isolate) possessed 0.39-0.48% of phenolics (FDA, 2011). In other GRAS Notice (FDA, 2016) concentration of total phenolics in RPI (rapeseed protein isolate) was reported with level 600-900 ppm (0.06-0.09%). Phenolic compounds were also analyzed in rapeseed powder, which was authorized to be placed on the EU market in 2021. The results were as follows: sinapine 0.14-0.19%, free ferulic acid 86-134 mg/kg, *p*-coumaric acid < 10 mg/kg and proanthocyanidins 214-270 mg/100g (EFSA, 2020; European Commission, 2021). Comparison in Table 22.

6.7.3. Phytic acid

Phytic acid (or phytate when in the form of a salt) is a major storage form of phosphorus in many plant tissues, especially seeds and bran. For example, it accounts for 60 – 80% of total phosphorus in mature cereal grains (NRC, 1973). It has strong chelating properties for essential minerals, such as iron, zinc and calcium, and therefore it can contribute to mineral deficiencies, by decreasing their bioavailability. It can also chelate vitamin B_3 (niacin), which could lead to vitamin B_3 deficiency (Reddy and Sathe, 2002) and create complexes with protein, which may interfere with protein utilization (Hídvégi and Lásztity, 2002). Some beneficial features of phytic acid including antioxidative, anticalcification, and potentially anticancerogenic properties have been reported (Schlemmer et al., 2009).

Phytic acid/phytate is present in many commonly consumed plant-based foods. It is present in such cereal products as maize (0.72 – 2.22 %DM), wheat (0.39 – 1.35 %DM), wheat bran (2.1 – 7.3 %DM), rice (0.06 – 1.08 %DM), barley (0.38 – 1.16 %DM), sorghum (0.57 – 3.35 %DM), oat (0.42 – 1.16 %DM), rye (0.54 – 1.46 %DM), millet (0.18 – 1.67 %DM), triticale (0.50 – 1.89 %DM), and wild rice (2.20 %DM). It has also been found in legumes, such as kidney beans (0.61 – 2.38 %DM), broad beans (0.51 – 1.77 %DM), peas (0.22 – 1.22 %DM), dry cowpeas (0.37 – 2.90 %DM), chickpeas (0.28

– 1.60%), and lentils (0.27 – 1.51 %DM). The content of phytic acid/phytate in different types of nuts is reported to be 0.20 – 6.69 %DM in walnuts, 0.23 – 0.92 %DM in hazelnuts, 0.29 – 2.83 %DM in pistachios, 0.35 – 0.42 %DM and in almonds (Schlemme, 2009). As for oilseeds, it has been presented that whole rapeseed contains 2.0 – 4.0% of phytic acid, defatted meal – 2.0 – 5.0%, protein concentrates – 5.0 – 7.5% and protein isolates – <1.0 – 9.8%, depending on the isolation method (Thompson, 1990). The content of phytic acid in soy protein isolate is reported to be 1.6 – 2.0% (Honig, Wolf and Rackis, 1984), but more recent studies indicate lower values of 0.49 – 0.84% (Hurrell et al., 1992). Phytates content in rapeseed powder which was authorized to be placed on the EU market ranged from 0.4 to 1 g/100 g (EFSA, 2020).

The level of phytic acid in canola concentrate is specified to be ≤2 %DM, which is consistent with specification of canola (rapeseed) protein presented in Commission Implementing Decision of 1 July 2014 authorizing the placing on the market of rapeseed protein as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (European Commission, 2014) and with specification of rapeseed powder presented in Commission Implementing Regulation (EU) 2021/120 of 2 February 2021 authorising the placing on the market of partially defatted rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470 (European Commission, 2021). The specific levels of phytic acid in different production batches of Raptein™30 is found to be 1.09-1.60 %DM (Table 21).

Several GRAS Notices regarding canola/rapeseed products with reported phytic acid/phytate content have been previously submitted and obtained “no further questions letter” from the FDA. Puratein® (cruciferin–rich canola protein isolate) and Supertein® (napin–rich canola protein isolate) have an average content of phytic acid of 0.32% and 3.34%, respectively (FDA, 2010). In GRAS Notice No. 386 (FDA, 2011), the level of phytic acid was specified to be <1.25 %DM for Isolexx™ (canola protein isolate) and <1.0 %DM for Vitalexx™ (hydrolyzed canola protein isolate), with the average levels reported to be 0.85% for Isolexx™ and 0.5% for Vitalexx™. In GRAS Notice No. 683 (FDA, 2017) the content of phytate in canola protein isolates was reported to be <0.14%.

Specified level (≤2%DM) of phytic acid content is far below the 5.25% phytate content which caused adverse effects in rats fed rapeseed flours from early rapeseed cultivars

(Anderson et al., 1976). Also, Mejia et al. (2009b) performed 13-week toxicity studies with napin-rich protein isolate (Supertein®) with phytate content of 3.34% and showed that fed orally to rats at up to 20% of their diet did not affect the plasma concentration of the trace mineral zinc. Accordingly, phytic acid content in Raptein™30 is not likely to be of nutritional or toxicological concern.

6.7.4. Glucosinolates

Glucosinolates (GSL) are sulphur- and nitrogen-containing molecules derived from amino acids. (Nugon-Baudon and Rabot, 1994; Liu et al., 2019). GSL occurs commonly in the Brassicales (Cruciales), order of flowering plants, which include rapeseed and other edible plants species like mustard, cabbage, capers, cauliflower or broccoli (Nugon-Baudon and Rabot, 1994; Liu et al., 2019).

Glucosinolates concentrations have been reported for cabbage (39-70 mg GSL/100g), broccoli (47-121 mg GSL/100g), turnip (99-230 mg GSL/100g), cauliflower (14-208 mg GSL/100g) and radish (44- -252 mg GSL/100g) (FDA, 2010).

GSLs are innocuous. However, the enzyme myrosinase (thioglucoside glucohydrolase) present in plants, hydrolyzes the glucosinolates in case of injury of plant tissues during processing. Created unstable molecules are spontaneously transformed into potential toxic derivatives including isothiocyanates (e.g. allyl isothiocyanates), thiocyanates, nitriles, 5-vinyloxazolidine-2-thione, epithioalkanes, and oxazolidinethions (Nugon-Baudon and Rabot, 1994; Tripathi and Mishra, 2007; Von Der Haar et al., 2014; Kaiser et al., 2021). Similarly, a wide range of GLSs breakdown products can be produced by intestinal microflora, which also produces myrosinase (Nugon-Baudon and Rabot 1994; Tripathi and Mishra, 2007).

Glucosinolates and products of their degradation were reported to reduce palatability of products (Tripathi and Mishra, 2007; Kaiser et. al., 2021). Deleterious effects of intake GSL and derivatives in animals include reduced feed ingestion, growth inhibition and also impaired thyroid function and damage of liver, kidneys and thyroid (Tripathi and Mishra, 2007; Nugon-Baudon and Rabot, 1994; Kaiser et. al., 2021).

The raw material used for the production of Raptein™30 is low in glucosinolates. In addition, production of canola concentrate developed by NapiFeryn BioTech minimizes the presence of these anti-nutritional components in the final product. Total content of glucosinolates in canola concentrate (Raptein™30) is presented in Table 21 and is at levels less than 0.05 mmol/kg (below detection limit). This level complies with specification presented in EU Commission Implementing Decision of 1 July 2014 authorizing the placing on the market of rapeseed protein as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council (not more than 1 mmol/kg) and specification presented in Commission Implementing Regulation (EU) 2021/120 of 2 February 2021 authorizing the placing on the market of partially defatted rapeseed powder from *Brassica rapa* L. and *Brassica napus* L. as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470 (< 0.3 mmol/kg) (EFSA, 2020).

The level of glucosinolates in canola/rapeseed protein isolates presented in GRAS Notifications reviewed by FDA (2010, 2011 and 2017) is similar or higher than the content in the product of NapiFeryn BioTech. Rapeseed protein isolates (RPI) presented in GRAS Notice No. 683 (FDA, 2017) possessed <0.1 µmol/g glucosinolates. In GRAS Notice No. 386 (FDA, 2011) levels of glucosinolates was reported from 0.05 to 0.15 µmol/g for Isolexx™ (canola protein isolate) and from 0.14 to 0.23 µmol/g for Vitalexx™ (hydrolyzed canola protein isolate) respectively. Total content of glucosinolates in Puratein® (cruciferin-rich protein isolate) and Supertein® (napin-rich protein isolate) was reported at level 1.09-2.53 µmol/g and 0.39-1.02 µmol/g respectively (FDA, 2010). Residual glucosinolates were also analyzed in rapeseed powder authorized to be placed on the EU market. The content of progoitrin ranged from <0.05 to 0.19 mmol/kg, gluconapin from <0.05 to 0.08 mmol/kg, whereas total concentration was determined to be from <LOQ to 0.27 mmol/kg (EFSA, 2020).

6.7.5. Protease inhibitors

Protease inhibitors are present in many plants. Soybean based products have been the primary concern as a source of protease inhibitors (Rackis and Gumbmann, 1981), but other legumes also contain these compounds (Kadam and Smithard, 1987; Carvalho, 1997). Presence of protease inhibitors in wheat and potatoes was reported by Habib and Fazili (2007). Protease inhibitors at high concentrations can limit nutritional value of a protein (Rackis and Gumbmann, 1981). Heating proteins is known to inactivate the protein digestion inhibitors (Osborne and Mendel, 1917).

The *Brassica* genus of plants contains protease inhibitors (Ceciliani et al., 1994), therefore Raptein™30 may contain a protease inhibitor. Analysis of five samples collected during product development revealed an average protease inhibitor concentration of 2.1 mg/g. See Table 21.

Many of the products proposed to contain Raptein™30 are heat treated, which will lead to some level of degradation of the protease inhibitor. Therefore, the potential for an excessive intake of the protease inhibitor is very low. But even if no degradation were to occur, if the highest use level, 40 g of Raptein™30/100 g of food as consumed for snack foods and 100 g of Raptein™30 for protein and nutritional powders (as is basis), is used as a model for exposure to the protease inhibitor, the average concentration would be 2.1 mg/g of of Raptein™30. Converted to the protein content of the Raptein™30, the average concentration is 6.3 mg/g of protein. This concentration is lower than in other consumer goods such as soy milk - approximately 12,6 mg/g protein (Xiao et al., 2012), or tofu – 9.2 mg/g protein and fresh cabbage – 10.5 mg/g protein (Doell et al., 1981).

6.7.6. Comparison of anti-nutritional compounds in Raptein™30 to other rapeseed products

The levels of anti-nutritional compounds in Raptein™30 can be compared to canola protein ingedients that were previously notified to the FDA and obtained "no questions letter" and therefore GRAS status, as well as partially defatted rapeseed powder which was authorized to be placed on the EU market (EFSA, 2020). It should be emphasized that the total phenolics content in Raptein™30 is estimated to be from 7 to even 45 times lower than the content in other canola products. Phytic acid concentration might be about 2 times higher in the canola concentrate than in the rapeseed powder, but at the same time about 2.5 times lower than in Supertein®. Total glucosinolates were found to be below LOQ in most of Raptein™30 samples or were found to be as low as in Isolexx™ and Vitalexx™. According to results from the external laboratory, erucic acid content in most of the canola concentrate lots was below LOQ, thus it is difficult to compare results. Protease inhibitors were measured only in RPI90 CanolaPro and reached about 10 times higher values than in Raptein™30. The summary of anti-nutritional factors is presented in Table 22.

Table 22. Anti-nutritional compounds in Raptein™30 and other products

Anti-nutritional compound	Raptein ™30	Rapeseed powder (EFSA, 2020)	RPI90 CanolaPro (FDA, 2017)	Isolexx ™ (FDA, 2011)	Vitalexx ™ (FDA, 2011)	Puratein ® (FDA, 2010)	Supertein ® (FDA, 2010)
Erucic acid [%]	< 0.1 - 0.37	< 0.1 of total fatty acid	< 0.005	0.002	0.003	< 0.0025	< 0.0025
Total phenolics [%]	0.010	0.17 – 0.23*	0.07	0.14	0.44	0.40	0.26
Phytic acid [%]	1.34	0.4 – 1	< 0.14	0.85	0.5	0.32	3.34
Total glucosinolates [µmol/g]	<0.05	< LOQ (< 0.27)	< 0.1	0.09	0.19	1.22	0.80
Trypsin lnhibitor Activity [mg/g]	2.1	not analyzed	21.1	not analyzed	not analyzed	not analyzed	not analyzed

* See Section 6.6.2

6.7.7. Additional considerations

Canola concentrate batches (Lots Number R-09#41, R-06#60 and R-03#35) were sent for additional safety evaluation (potential risks of food allergy and celiac disease) to VTT Technical Research Centre of Finland. Bioinformatics searches were performed using AllergenOnline database and Celiac Database (CD). The sequence searches (using FARRP allergen protein database) indicated the possible allergenic effects of the Raptein™30 proteins. There was no match using the CD peptide dataset, thus these proteins are unlikely to elicit celiac disease. The complete description of results is provided in Appendix 4.

6.8. GRAS Panel Evaluation

The GRAS panel convened at the request of NapiFeryn BioTech, independently and critically evaluated all data and information presented herein, and concluded that Raptein™30 canola concentrate (rapeseed concentrate) (containing ≥30% protein) is GRAS for use as an ingredient in human food, based on scientific procedures. Appendix VIII includes the Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Uses of NapiFeryn's Canola Concentrate (Rapeseed Concentrate) (RapteinTM30).

6.9. Environmental safety

Proteins are built with amino acids. Amino acids are used by all forms of living organisms to build the proteins to their needs. Humans, animals and most microorganisms are capable of breaking down proteins into amino acids. Raptein™30 is composed of the same amino acids that are present in all plant, animal and human proteins.

Dietary fiber is defined as 'the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine'. It includes polysaccharides, lignin, oligosaccharides and associated plant substances and has a positive impact on human health (AACC, 2001). Raptein™30 contains 40-70 %DM of total dietary fiber, most of which is insoluble.

Rapeseed press cake has been used as an ingredient in animal food for over several decades in Europe and America or disposed of in landfills without adverse environmental consequences. Therefore, the accidental release of canola concentrate (rapeseed concentrate) into the environment is not expected to adversely affect the air, soil or water.

NapiFeryn canola concentrate (rapeseed concentrate), Raptein™30 is a GRAS substance and per 21 CFR §25.32, foods, food additives and color additives, including GRAS substances, are categorically excluded from the requirement to submit an environmental impact statement or an environmental assessment.

7. Supporting data and information

7.1. References

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7.2. List of appendices

Appendix 1 - Reports of analysis for products and raw materials

Appendix 2 - Reports of analysis from stability studies

Appendix 3 - Raptein™30 Estimated Daily Intake Report

Appendix 4 - Allergenicity analysis of rapeseed concentrate Raptein™30 for food safety evaluation

Appendix 5 - GMP+ certificate of the rapeseed press cake supplier

Appendix 6 - Use of canola concentrate as a functional ingredient as extender in sausage meat product

Appendix 7 - Protein separation by SDS PAGE

Appendix 8 - Consensus Statement Concerning the Generally Recognized as Safe (GRAS) Status of the Proposed Uses of NapiFeryn's Canola Concentrate (Rapeseed Concentrate) (Raptein™30)