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SCIENTIFIC OPINION

Scientific Opinion on the safety of "rapeseed protein isolate" as a Novel Food ingredient [1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) [2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of a "rapeseed protein isolate" (Isolexx™) as a novel food ingredient (NF) in the context of Regulation (EC) No 258/97. The NF is an aqueous extract with at least 90 % protein, isolated from rapeseed press cake originating from so-called canola varieties. The applicant intends to market the NF for the same food products, at similar concentrations and for corresponding purposes, as soy protein isolates. Total protein intake of "heavy" adult consumer may be estimated as the mean + 2 SD, i.e. 2.2 g/kg bw per day. The age group of 4 - 6 years is estimated to have the highest protein intake on a per kg bw basis with a mean and 95th percentile intake of up to 3 and up to 4.73 g/kg bw per day, respectively. A significant part of these estimated intakes could come from rapeseed protein. The Panel considers that the risk of sensitisation to rapeseed cannot be excluded and that it is likely that rapeseed trigger can allergic reactions in mustard allergic subjects. The biological value of rapeseed and soy protein, determined by the PDCAAS, appears to be similar. The Panel notes the source and nature of the novel food, the absence of a nutritional disadvantage at the proposed uses and use levels, the low concentrations of potentially adverse components in the NF, and the absence of toxicologically relevant effects in subchronic studies with rats conducted with rapeseed protein isolates with similar compositions. The Panel concludes that rapeseed protein isolate is safe under the proposed uses and use levels.

KEY WORDS

novel food, ingredient, rapeseed protein, plant protein

[1] On request from the European Commission, Question No EFSA-Q-2013-00231, adopted on 10 October 2013.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on the safety of a "rapeseed protein isolate". The novel food (NF) rapeseed protein isolate (Isolexx^{TM}) is an aqueous protein-rich extract from rapeseed press cake originating from the two *Brassicaceae* species, *Brassica napus* L. and *Brassica rapa* L., both so-called canola varieties. Canola varieties are characterised by their low content of erucic acid (≤ 2 % by mass in the oil) and glucosinolate content of below 30 μmol/g in the air-dried, oil-free meal. The albumin napin and the globulin cruciferin, are the two major storage proteins and represent the majority of proteins in rapeseed. The results from batch testing of twelve production batches showed compliance with the specifications as proposed by the applicant. The Panel considers that the information provided on the composition, specifications and stability and production process of the NF does not raise safety concerns.

The applicant intends to market the NF essentially for the same food products, at similar concentrations and for corresponding purposes, as soy protein isolates, namely: a) as a source of protein, for example, in meal replacements (formula diets), protein drinks (including "dairy analogues"), nutrition bars, soups and soup mixes, breakfast cereals, plant protein products (meat analogues), and b) for improving the texture of, for example, bakery products, chilled or frozen processed meat products (such as patties), pasta, desserts, and other foods and in food supplements. The NF is not intended for use in infant formulae and follow-on formulae. The applicant provided intake estimates based on the intended uses and on protein intake data provided in the Scientific Opinion on Dietary Reference Values for protein published by the EFSA NDA Panel in 2012. Total protein intake of "heavy" adult consumer may be estimated as the mean + 2 SD, i.e. 2.2 g/kg bw per day. The age group of 4 - 6 years is estimated to have the highest protein intake on a per kg bw basis with a mean and 95th percentile intake of up to 3 and up to 4.73 g/kg bw per day, respectively. A significant part of these intake estimates could come from rapeseed protein.

According to data provided by the applicant, the levels of compounds contained in the NF such as erucic acid, glucosinolates, AITC and polyphenols are either below detection limits or below levels which may raise concerns. In addition, the applicant provided two publications on two 13-week toxicity studies in rats which studied a cruciferin protein rich isolate and a napin protein rich isolate from canola quality rapeseed produced by another manufacturer. Both products contained erucic acid, total glucosinolates, AITC, total phytates, and phenolics at similar concentrations as the NF. The Panel notes that in the study with cruciferin protein isolate, no treatment-related effects were noted, whereas in the study with napin protein isolate lower feed intake associated with reduced body weight gain and a reduced feed efficiency was observed, which may be caused by a low palatability and in part by an antinutritional effect inducing discomfort and consequently a conditioned taste aversion.

Several Member States expressed concerns with regard to a potential risk of allergenicity of rapeseed proteins in general, and with regard to potential cross-allergenicity of rapeseed proteins with proteins of other *Brassicacae*, particularly of mustard. The applicant has not carried out any studies to determine the potential allergenicity of the rapeseed protein isolate to which the application relates. Food allergy to rapeseed (*Brassica rapa* L.) and oilseed rape (*Brassica napus* L.) has been reported to occur, as evidenced by studies in humans. In a study, 11 % (206/1887) of atopic Finnish children with suspected food allergies who were screened using skin prick tests showed sensitivity to seeds of *Brassica rapa* L. and/or *Brassica napus* L. A subsequent challenge test confirmed that 89 % of sensitised children were allergic. In another study by the same authors, a group of homologous proteins, 2S albumins or napins, were identified as new possible food allergens. The authors considered that even the smallest quantities of protein residues present in refined or cold-pressed rapeseed oils might be sufficient to produce sensitisation. There are also indications of cross reactivity between rapeseed and other foods. According to the authors, the cross-reactivity between mustard and rapeseed flours could be explained by the high amino acid sequence homology between the two proteins. Mustard allergy has been reported in France and has also been investigated also in Spain, including studies on cross-reactions within *Brassicaceae*. One study showed that seed storage proteins

of various members of *Brassicaceae*, including mustard, have highly homologous molecular level structures and present risks of allergic reactions and cross-reactions in sensitised individuals.

The Panel concludes that the risk of sensitisation to rapeseed, as well as the risk of cross-reactivity in subjects allergic to mustard, cannot be excluded.

The NF, a canola quality rapeseed protein isolate, shares many properties with soy protein isolates, which are isolated in a similar way from the press cake remaining from soy oil production. The macronutrient composition of the NF is similar to commercially available soy protein isolates. The biological value of rapeseed and soy protein, determined by the PDCAAS, appears to be similar.

The Panel notes that people may consume up to 2.2 g protein/kg bw per day, of which a significant part may come from rapeseed protein. The Panel also notes that some subgroups of the population, such as sportspeople, may consume even higher amounts of protein. Only in an extreme scenario, in which "high consumers", such as vegans would consume rapeseed protein isolates as their sole source of protein, can an antinutritional effect not be excluded. The Panel considers that such a worst case scenario is unrealistic, and it would imply the consumption of an unbalanced diet, which is generally not recommended.

The Panel considers that the risk of sensitisation to rapeseed cannot be excluded and that it is likely that rapeseed trigger can allergic reactions in mustard allergic subjects.

The Panel notes the source and nature of the novel food, the absence of a nutritional disadvantage at the proposed uses and use levels, the low concentrations of potentially adverse components in the NF, the extended use of rapeseed press cake in farm animals, and the absence of toxicologically relevant effects in subchronic studies with rats of other rapeseed protein isolates with similar compositions. The Panel notes that based on the results of one of the rat studies, the possibility of an antinutritional effect caused by the novel food at high intakes, i.e. if rapeseed protein isolate was the main protein source in the diet, cannot be excluded.

The Panel concludes that rapeseed protein isolate is safe under the proposed uses and use levels.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

On 25 June 2012, Bioresco Ltd., on behalf of the company Helm AG, submitted a request under Article 4 of the Novel Food Regulation (EC) N° 258/97 to place on the market "rapeseed protein isolate" as a novel food ingredient.

On 17 September 2012, the competent authorities of Ireland forwarded to the Commission their initial assessment report, which came to the conclusion that rapeseed protein isolate may be placed on the market.

On 4 October 2012, the Commission forwarded the initial assessment report to the other Member States. Several of the Member States submitted comments or raised objections.

The concerns of a scientific nature raised by the Member States can be summarised as follows:

- The specification should be extended to include limit values for the undesirable compounds: erucic acid, allyl isothiocyanate and phenolic compounds such as tannin and sinapin. The respective analytical methods must be properly described and validated.
- The protein fraction should be analysed in more detail. The identity of the different (soluble) proteins present in the novel ingredient should be analysed, for example by HPLC analysis.
- The carbohydrate fraction should be clarified since the ultracentrifugation step is intended to remove carbohydrates. Direct analysis of the carbohydrates would give assurance that there are no low-molecular proteins present in this fraction.
- Considering the potential impact of phytates on micronutrient absorption, data from batch testing should be provided to demonstrate that phytate levels are consistently within the specified limits.
- The absence of butane (used for the oil extraction) in the final product should be demonstrated.
- Information on the content of lead, cadmium and aluminium is lacking.
- Information should be provided whether phytic acid is completely broken down to inositol by the addition of phytase during the manufacturing process.
- It is not appropriate to compare glucosinolates present in the novel food ingredient with those in sprouts since they show different characteristics.
- The application dossier does not contain data on the stability and shelf life of the product, or any information regarding process quality management.
- Accreditation of the test laboratories issuing test reports is not apparent. Accreditation should be according to an internationally-recognised system for analysing food.
- The production process is insufficiently described, including time between primary extraction by mechanical pressure and subsequent treatment (with respect to potential fungal contamination and mycotoxin production), level of detail of the ultracentrifugation step, excipients used for the atomisation.
- The figure given for the potential intake of the novel ingredient is only a very rough estimate based on a series of assumptions. A more refined intake estimate for the intended target populations, including children, should be provided on the basis of the maximum levels of enrichment and a comprehensive list of foods to which the novel ingredient may be added.

- The extent to which consumption of the novel protein preparation might cause allergic reactions in susceptible individuals has been insufficiently investigated. Allergenic properties of rapeseeds 2S albumins have been described in the literature (Monsalve et al., 1997). Moreover, OECD's revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola) reports the publication of studies investigating the potential for *Brassica rapa* L. and *Brassica napus* L. to be food allergens in children (OECD, 2011).

- Studies cited by the applicant and the high degree of homology between mustard proteins and rapeseed proteins point to cross-reactivity between rapeseed and mustard or allergens associated with seeds of other plants of the *Brassicaceae* family used in the production of mustard. Unlike mustard which is generally used in small amounts as a condiment, exposure to rapeseed protein is likely to be far more widespread as it is intended to be incorporated into a range of foods, and allergy is therefore of more concern.

- The applicant should provide evidence, based on clinical studies, demonstrating the safety and digestibility of the product to which the application relates.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Food Safety Authority is asked to carry out the additional assessment for "rapeseed protein isolate" as a novel food ingredient in the context of Regulation (EC) N° 258/97.

EFSA is asked to carry out the additional assessment and to consider the elements of a scientific nature in the comments raised by the other Member States.

ASSESSMENT

In accordance with Commission Recommendation 97/618/EC, "rapeseed protein isolate" (Isolexx^{TM}) has been allocated to Class 2.1, i.e. foods or food ingredients that are 'complex novel food from non-GMO sources. The source of the novel food has a history of food use in the Community'. The assessment of the safety of this novel food ingredient is based on data supplied in the original application, the initial assessment by the competent authority of Ireland, the concerns and objections of the other Member States, and the responses of the applicant. The data are required to comply with the information required for the novel foods of Class 2.1, i.e. structured schemes I, II, III, IX, XI, XII and XIII of the Commission Recommendation 97/618/EC. In the text, these structured schemes are listed in nine sections. This assessment only concerns risk that might be associated with consumption, and is not an assessment of the efficacy of rapeseed protein isolate (Isolexx^{TM}) with regard to any claimed benefit.

1. SPECIFICATION OF THE NOVEL FOOD (NF)

The novel food (NF) rapeseed protein isolate (Isolexx^{TM}) is an aqueous protein-rich extract from rapeseed press cake originating from the two *Brassicaceae* species, *Brassica napus* L. and *Brassica rapa* L., both so-called canola varieties. Canola varieties are characterised by their low content of erucic acid (≤ 2 % by mass in the oil) and glucosinolate content of below 30 µmol/g in the air-dried, oil-free meal (OECD, 2011). The specifications of the NF provided by the applicant are shown in Table 1. The NF will be referred to as "rapeseed protein isolate" in the following assessment.

Table 1: Specifications of the NF "rapeseed protein isolate", as proposed by the applicant

Parameters	Limit values [(a)]	Methods
Protein (N × 6.25)	≥ 90 %	AOCS Ba 4e-93
Soluble protein	≥ 85 %	Roe et al. (1990)
Moisture	≤ 7 %	AOCS Ba 2a-38
Carbohydrates	≤ 7 %	By difference [(b)]
Fat	< 2 %	AOCS Ba 3-38
Ash	≤ 4 %	AOCS Ba 5a-49
Fibre	≤ 0.5 %	AOCS Ba 6-84
Total glucosinolate	≤ 1 mmol/kg (450 mg/kg)	Method of the Canadian Grain Commission (Duan and MacGregor, 1981)
Total phytate	≤ 1.5 %	Colorimetric method (Gao et al., 2007)
Lead	≤ 0.5 mg/kg	Atomic absorption[(c)]
Aerobic bacteria count	≤ 10 000 cfu/g	MFHPB-18
E. coli	negative/10 g	MFHPB-34
Salmonella	negative/25 g	MFHPB-20
Total coliform count	≤ 10 cfu/g	MFHPB-34
Yeast and mold count	≤ 100 cfu/g	MFHPB-23

(a): Based on dry matter
(b): 100 % - [protein (as is) % + moisture % + fat % + ash % + fibre %]
(c): Alternatively, ICP-MS may be applied

The NF is a white to off-white, spray dried powder of which more than 90 % passes a US 80 mesh. It has a specified protein content of at least 90 %, with a minimum soluble protein content of at least 85 %. Upon request from EFSA, the applicant provided more detailed data on the protein composition of the NF. As determined by gel permeation chromatography, the product is composed of two major fractions: 60-65 % globulin (250 kDa) and 30-35 % albumin and other minor proteins (10-30 kDa). The albumin napin and the globulin cruciferin, are the two major storage proteins and represent the majority of proteins in rapeseed (Wu and Muir, 2008).

The applicant provided results of the composition of twelve production batches, analysed by an external laboratory (Appendix).

According to the applicant, the concentration of glucosinolates is at or below the detection limit (0.1 mmol/kg), which is supported by the results from batch testing. The concentration of allyl isothiocyanate (AITC) is limited by the specification limit given for glucosinolates.

According to the applicant, there is no need to add analytical data on erucic acid to the specification. The applicant justified the omission by the low content of erucic acid of canola quality rapeseed, the production process which reduces the oil fraction in the NF to a maximum of 2 %, and to the batch testing of the NF which showed that the level of erucic acid was below the limit of detection (0.1 % in the fat fraction). The Panel agrees with these arguments.

The Panel notes that the use of canola varieties limits the concentrations of both, thio-glucosinolates and thus of AITC, as well as of erucic acid (OECD, 2011).

Total phytates make up less than 1.5 % according to the batch testing.

The concentrations of "total phenolics" (expressed in gallic acid equivalents, extraction with water:methanol (50:50), analysis by the Folin-Ciocalteu, detection limit at 50 mg/kg) and of sinapin (by NMR) in the analysed batches were below 1.6 g/kg and 0.5 g/kg NF, respectively (Appendix).

A limit value for lead is set in the specification. Following comments from Member States, the applicant referred to analyses of four batches, which included lead, cadmium and aluminium analyses. The values ranged from < 0.01-0.02 ppm for Pb, 0.16-0.28 ppm for Cd and 7.5-12 ppm for Al. For Pb and Cd, the observed values are within the levels that typically apply to foods[4] (on an anhydrous basis). Upon request from Member States and EFSA regarding the high content of 75 ppm Al reported initially in one batch of the NF, the applicant clarified that this high value resulted from a transcription error from the laboratory report, and provided the original analytical report where an Al content of 7.5 ppm was reported. The applicant also provided analytical results of nine additional batches of the NF, where the Al content was found to be consistently below 10 ppm (range: 1.1-9.8 ppm). The Panel notes that maximum permitted levels of Al have not been established in EU food law. Assuming a high intake scenario of about 1.1 g NF/kg bw and day for adults (i.e. about 50 % of the high protein intake scenario, see Section 4) and a content of 10 ppm Al, the daily intake resulting from the consumption of the NF would amount to 0.77 mg Al per day (5.4 mg Al per week). EFSA has established a weekly tolerable intake level for aluminium from dietary intake at 1 mg/kg body weight (bw) per week (EFSA, 2008).

The applicant also set out the result of analyses on 307 different plant protection agents (pesticides), and on dioxins, PAHs and aflatoxins (B1, B2, G1 and G2). None was detectable above the limits of detection. The Panel notes that all foods, and hence also novel foods, have to comply with the existing food legislation.

With regard to the absence in the NF of n-butane used for the oil extraction, the applicant notes that n-butane may be used as a solvent in the production of any foodstuff in compliance with GMP, as per Directive 2009/32/EC[5]. The applicant referred to the analyses of six batches of the NF, which showed that this extraction solvent was not present at detectable levels (limit of detection: 10 ppm).

Regarding the stability and shelf-life of the NF, the applicant reported that after removal of the oil (typically at 60 °C), the filter cake (moisture content about 8 %) is cooled to ambient temperature. Further processing of the NF takes place not more than 8 hours later. The final product is packed in

[4] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in food stuffs, OJ L 364, 5-24.

[5] Directive 2009/32/EC of the European Parliament and of the Council on the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients. OJ L 141, 6 June 2009, pp. 3-9.

polylined multi paper bags or polylined cardboard boxes and should be stored below 25 °C and at 60 % relative humidity. The expiry date is 12 months from the data of manufacture. Given the nature of the NF, the Panel considers that the NF is generally stable and not susceptible to degradation if protected from humidity under the proposed storage conditions and shelf-life.

The Panel considers that the information provided on the composition, specifications and stability of the NF does not raise safety concerns.

2. EFFECT OF THE PRODUCTION PROCESS APPLIED TO THE NF

The applicant provided a summary and flow-chart of the production process, which employs conventional separation techniques.

The starting material for the production of the NF is the press cake that remains after cleaned, flaked and conditioned non-GM canola-quality rape seeds have been pressed to separate the oil. After milling to reduce particle size, the press cake may be extracted with n-butane in a stirred extractor to remove any remaining oil.

After completion of the extraction, water is added to displace the remaining solvent. The water contains about 300 ppm of a liquid preparation with phytase from *Aspergillus niger* to digest phytates naturally present in rapeseeds (Greiner and Konietzny, 2006; Kumar et al., 2010). Phytase from *A. niger* is included in the Codex Alimentarius Inventory of Processing Aids (CX/FA 09/41/8). In France, phytase from a specific strain of *A. niger* is authorised as a processing aid for use in bread dough. According to the applicant, phytase activity could not be detected in the NF.

The rapeseed meal/water slurry is then freed from any remaining solvent at elevated temperature under vacuum. The insoluble components (fibres, proteins) are removed by decantation. The obtained supernatant is pasteurised, centrifuged and concentrated by removing the bulk of water, mono- and oligosaccharides and other low-molecular weight solutes. Larger soluble polymeric carbohydrates such as xylans would remain with the protein fraction. The retentate is spray-dried to yield the rapeseed protein isolate.

The Panel considers that the production process is sufficiently described by the applicant and does not raise safety concerns.

3. HISTORY OF THE ORGANISM USED AS A SOURCE

Rapeseed is an important oilseed crop in many countries and is considered to be the second most abundant source of edible oil in the world. "Canola", a rapeseed variety with low contents of nutritionally undesirable compounds (primarily erucic acid and glucosinolates) was developed in Canada in the 1970s and has been widely used in the manufacturing of edible oil and animal feeds (OECD, 2011). The development of the "Canola" variety has aided in the development of technologies that can be used in the production of food grade items from the canola meal which is the by-product of oil extraction. Canola meal contains up to 50 % protein on a dry basis (Uppström, 1995).

The applicant intends to produce the NF from non-GM *Brassica napus* L. and *Brassica rapa* L. varieties of canola quality, which are characterised by a low content of erucic acid (≤ 2 % by mass in the oil) and glucosinolates (≤ 30 μmol/g) in the air-dried, oil-free meal (OECD, 2011).

4. ANTICIPATED INTAKE/EXTENT OF THE USE OF THE NF

The applicant intends to market the NF for addition to the same food products, at similar concentrations and for corresponding purposes, as soy protein isolates, namely: a) as a source of protein, for example, in meal replacements (formula diets), protein drinks (including "dairy analogues"), nutrition bars, soups and soup mixes, breakfast cereals, plant protein products (meat analogues), and b) for improving the texture of, for example, bakery products, chilled or frozen

processed meat products (such as patties), pasta, desserts, and other foods and in food supplements. The NF is not intended for use in infant formulae and follow-on formulae.

The applicant provided intake estimates based on protein intake data provided by the EFSA Scientific Opinion on Dietary Reference Values for protein (EFSA NDA Panel, 2012). In the EU, the average protein intakes of adults in absolute amounts range from approximately 67 to 114 g/day in men and from 59 to 102 g/day in women. Available data suggest an average intake of 0.8 to 1.25 g/kg bw per day for adults. According to the applicant, the total protein intake of adult "high" consumers may be estimated 2.2 g/kg bw per day.

Average protein intake varies in infants and young children from about 29 to 63 g/day. Average daily intakes increase with age to about 61-116 g/day in adolescents. In general, males have higher intakes than females. Only for a few Member States protein intake data per kg body weight are available. The estimated mean intakes vary from ≥ 3 g/kg bw per day in the youngest age groups to approximately 1.2-2.0 g/kg bw per day in children and adolescents aged 10-18 years. The age group of 4-6 years is estimated to have the highest protein intake on a per kg bw basis. In the EU, the highest mean and 95th percentile protein intake for children of this age group were observed in Italy with 3 and 4.73 g/kg bw per day, respectively (EFSA NDA Panel, 2012).

In the general population, about 75 % of protein intake stems from meat and meat products, grain and grain-based products, as well as milk and dairy products (EFSA NDA Panel, 2012). Another 7-8 % is ingested with seafood, eggs and egg products. The remaining 18 % of protein intake, corresponding to 0.15 g/kg bw per day, may in part represent protein isolates such as soy protein isolates which are added to processed foods. The latter may represent a higher proportion of the diet of vegans.

The Panel notes that adult "high" consumers may consume 2.2 g protein/kg bw per day, of which a significant part could come from rapeseed protein. However, the Panel also notes that some subgroups of the population, such as sports people, may consume even higher amounts of protein.

5. INFORMATION FROM PREVIOUS EXPOSURE TO THE NF OR ITS SOURCE

Varieties of rapeseed with low contents of erucic acid and glucosinolates have a significant history of safe food use as a source for oil worldwide. The applicant also referred to two already authorised rapeseed-derived oil based novel foods (EFSA, 2004a; EFSA, 2005). However, protein from the seed material that remains after the removal of the oil has only recently been examined as an alternative source of vegetable protein in food. According to the applicant, four distinct rapeseed protein-rich ingredients may be placed on the USA market following GRAS notices, including Isolexx^{TM}. However, according to the applicant rapeseed protein ingredients are currently not consumed to any significant degree in the USA.

6. NUTRITIONAL INFORMATION ON THE NF

In a study conducted in rats, the NF was provided as the sole dietary protein at levels of 7.5 %, 15 % and 30 % (Liebert, 2010, unpublished study report) and true fecal nitrogen (N) digestibility of 93.1 ± 1.9 %, 94.8 ± 1.2 % and 91.1 ± 1.4 % were observed, respectively. True fecal N-digestibility of 91.9 ± 1.3 % for the NF, 94.9 ± 0.4 % for a soy protein isolate (Dunasoy 90) and 97.4 ± 0.3 % for casein were found in a subsequent comparison in rats fed the respective proteins as the sole source of dietary protein at levels of ~ 15 %. Another study in rats fed a diet with 15 % canola protein isolate as the sole protein source reported true fecal N-digestibilities of 94.8 ± 1.2 % and 91.9 ± 1.3 % in two separate experiments (Fleddermann et al.,2013).

A study performed in humans equipped with an intestinal tube at the terminal ileum and receiving 30 g ^{15}N-labelled rapeseed protein isolate from other manufacturers reported a true ileal protein digestibility of 84.0 ± 8.8 % (Bos et al, 2007) and another study using the same approach reported true ileal protein digestibilities of 91 % and 87 % rapeseed protein in pigs and humans, respectively (Deglaire et al, 2009).

The Panel notes that rat fecal digestibility is not considered as a good model and usually overestimates protein digestibility for humans, that results obtained in humans are preferred.

The applicant provided information on the amino acid composition of four batches of the NF protein and compared it with the amino acid composition of four commercial soy protein preparations. The amount of lysine was about 15 % higher in soy protein, whereas the amount of sulphur containing amino acids (cysteine/cystine plus methionine) was about 65 % higher in the rapeseed protein. The values for other amino acids were comparable. According to the data provided by the applicant, the levels of lysine and all other essential amino acids in rapeseed protein meet the amino acid scoring pattern recommended by the US Institute of Medicines (IOM, 2006). In addition, the applicant referred to FAO (1991) reporting similar high protein digestibility (> 95 %) for rapeseed protein and soybean protein isolates. Protein-digestibility corrected amino acid scores[6] (PDCAAS) were 0.92 for soybean protein isolate vs. 0.83 for rapeseed protein isolate, not manufactured by the applicant (FAO, 1991).

The Panel notes that it is not satisfactory to compare profiles with other protein. The Panel notes that the PDCAAS should be calculated based on recent official reference patterns (WHO, 2007; EFSA, 2012). Using the most recent amino acid scoring pattern (EFSA NDA Panel, 2012) and a digestibility of 85 % from human studies, the PDCAAS of five batches of the NF has been calculated. The mean and the range of the PDCAAS of the five batches were 0.98 and 0.92-1.00, respectively, with mainly lysine (in four batches) or leucine (one batch) as potentially limiting amino acids. The Panel notes that this PDCAAS range is similar to the PDCAAS of soy protein with methionine+cysteine as limiting amino acids (EFSA NDA Panel, 2012). Fleddermann et al. (2013) applied the approach in compliance with the amino acid scoring pattern proposed by EFSA (EFSA NDA Panel, 2012) on one rapeseed protein isolate (called "CPI" in the article) with 4.78 g lysine per 100 g protein produced by the same producer of the NF, and the PDCAAS was 86 %.

In a double-blind cross-over study, Fleddermann et al. (2013) investigated the effect of rapeseed vs. soy proteins on plasma amino acid concentrations and N balance. After a three day run-in, 28 healthy male subjects consumed 30 g protein dissolved in tomato juice, as follows: Group A: canola protein isolate (n = 7), or soy protein isolate (SPI) (n = 7); Group B: canola protein hydrolysate (n = 7), or SPI (n = 7). Blood samples were collected at regular intervals up to 8 hours postprandial, and a urine sample was collected over 24 hours after ingestion. After a three week wash-out period, a second experiment was performed, where the protein sources were crossed within the four subgroups. Consumption of canola protein or soy protein led to significant increases in plasma amino acids after 62.3 and 83.6 minutes, respectively. Canola protein hydrolysate produced an earlier amino acid response compared to canola protein isolate and soy protein isolate, while the total amino acid response was comparable between all interventions. No statistical differences were found when comparing nitrogen balance of canola protein hydrolysate (0.73 ± 2.48 g N/day), canola protein isolate (2.16 ± 2.42 g N/day) and soy protein isolate (2.75 ± 3.41 g N/day (group A) and 1.61 ± 3.23 g N/day (Group B)). The Panel notes that the post-prandial kinetic of blood amino acid does not provide a suitable criterion to assess protein quality, and that nitrogen balance measured in acute conditions traduces the effect of the habitual diet of the subjects more than the quality of Canola protein.

The Panel notes that the quality of the NF rapeseed protein source measured by the PDCAAS is in the range of 92 - 100 %, depending on the batches of the NF protein, that lysine, which is particularly sensitive to food treatment, is the main limiting amino acid in the NF, and that the protein digestibility, biological value and PDCAAS seem to be highly dependent on the production process and batch-to-batch/product variation.

The Panel notes that the range of PDCAAS values calculated for five batches of the NF is not different from the value of around 95 % (with methionine + cysteine as limiting amino acids) estimated for soy protein. The Panel considers that intake of the NF is not nutritionally disadvantageous compared to

[6] PDCAAS = mg of amino acid in 1 g test protein : mg of amino acid in requirement pattern x true digestibility

soy protein isolate at the proposed conditions of use. However, the Panel also notes that due to the limiting amino acid lysine, this novel protein source cannot compensate lysine deficiency of cereal-based diets, in contrast to soy protein which is relatively high in lysine.

7. MICROBIOLOGICAL INFORMATION ON THE NF

The Panel considers that the data provided do not raise safety concerns with regard to the microbiological quality of the NF.

8. TOXICOLOGICAL INFORMATION ON THE NF

The applicant provided references and considerations on the type and amount of undesirable compounds contained in the NF.

With regard to erucic acid, the applicant referred to the Codex Standard 210-1999 for vegetable oils intended for human consumption (WHO, 1999), which sets the maximum concentration of erucic acid at 2 % of the oil. The applicant also referred to the provisional tolerable daily intake of 7.5 mg/kg bw erucic acid set in Australia/NZ (FSANZ, 2003). Considering the source, the production process, the specification of the NF (fat content < 2 %) and the batch testing (erucic acid not detected at a detection limit of 0.1 % of total fat), the Panel considers that the concentration of erucic acid in the NF is negligible.

According to the information provided by the applicant, the total level of glucosinolates in the NF is typically below the limit of detection (0.1 mmol/kg). According to the applicant, higher levels are only rarely found, and would not exceed 0.2 mmol/kg. However, considering the maximum level provided for glucosinolates in the specification of the NF (≤ 1 mmol/kg), and assuming a high daily intake scenario of e.g. 1.1 g NF/kg bw for adults (i.e. about 50 % of the high protein intake scenario, see Section 4), this would result in a maximum intake of ≤ 77 µmol (ca. 40 mg) glucosinolates. According to the information provided by the applicant, this is similar to the amount ingested with c. 7 g boiled brussels sprouts or 33 g cooked cauliflower. One Member State, as well as the applicant, indicated, however, that glucosinates are a heterogenous group of secondary plant metabolites. The major glucosinolates found in rapeseed are progoitrin, gluconapin, 4-hydroxyglucobrassicin and glucobrassicanapin (Millán et al., 2009). The Panel notes that the scenario above represents a conservative scenario. If, as claimed by the applicant and as supported by the batch testing, the level of glucosinolates is typically below 0.1 mmol/kg, and only rarely amount to up to 0.2 mmol/kg, then the actual intake of glucosinolates would be considerably lower.

According to the provided batch testing, AITC levels in the NF are below the limit of detection (1 mg/kg). EFSA has derived an acceptable daily intake (ADI) for allyl isothiocyanates of 20 µg/kg bw per day (EFSA, 2010a). A high daily intake by adults of 77 g of the NF with a 1 mg/kg AITC content would result in an intake of 77 µg or 1.1 µg/kg bw, far below the ADI established by EFSA. The Panel also notes the concentrations of AITC found in other foods such as horseradish (*Armoracia lapathifolia*) (1 500 – 9 000 mg/kg), Wasabi (*Wasabi japonica*, "Japanese horseradish") (9 600 mg/kg), cabbage (*Brassica oleracea*) (0.04-2.9 mg/kg) and cauliflower and broccoli (0.06 mg/kg) (TNO, 2009). Concentrations in mustard are also reported to vary considerably depending on the species of seed, ranging from 400-15 000 mg/kg (Velisek et al., 1995).

The content of phytic acid of the NF ranges from 0.44-1.1 %. According to the data provided by the applicant, rapeseed and soybeans contain small and similar amounts of phytic acid. Phytic acid is known to reduce the bioavailability of minerals, if present in food or feed at a sufficient concentration (Kumar et al., 2010). Phytic acid is ingested with many plant-derived foods. Soy protein isolate is reported to contain 1.6-2.0 % phytic acid (Honig et al., 1984). Lower values (0.49-0.84 %) were reported more recently (Hurrell et al., 1992). In tofu, 1.46-2.90 % phytic acid was found (on a dry matter basis). Whole wheat bread contains 0.43-1.05 % phytic acid (on a dry matter basis) (Reddy et al., 2001). The Panel considers that the phytate concentration in the NF corresponds to that of commonly consumed foods.

The main phenolic substance present in rapeseed is 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid). Sinapic acid occurs in rapeseed (and mustard seed) in free acid form, as well as in esterified form (esterified with choline or glucose) (Thiyam et al., 2006; Khattab et al., 2010). Analysis of sinapin, the choline ester of sinapic acid, revealed concentrations of 100-500 mg/kg in the NF. This is rather lower than the levels of sinapic acid alone found in apple, pear, broccoli, potato flour and other common foods (Manach et al., 2004; Robbins et al., 2005).

Colorimetric analysis for total extractable phenolic compounds, which also include proanthocyanidins and extractable tannins, reveals contents of about 1-3 g/kg NF. These are levels that are commonly found in plant foods (on a dry matter basis) and which may result in daily intakes of 2.5 to 3 g/day for subjects consuming a Mediterranean diet (Saura-Calixto et al., 2007; Arranz et al., 2008). However, values of total phenolics vary depending upon the applied analytical method (Escarpa and González, 2001). The colorimetric method applied for the analysis of the NF, i.e. the Folin-Ciocalteu method, gives, for example, a 3.6 times higher polyphenol content in dried lentils than analysis by HPLC (Escarpa and González, 2001). The lower daily intake of polyphenols which has been reported for Finnish adults (0.86 g/day) may, therefore, not be the result of a difference of dietary habits only, but also of the analytical method applied (HPLC) (Ovaskainen et al., 2008). Phenolic substances are also present in soybeans (2.1-3.4 g/kg), and consequently in soy protein isolates (Tepavčević et al., 2010). In soy beans, sinapic acid, p-coumaric acid, ferulic acid and p-hydroxybenzoic acid are found among other phenolic acids (Schmidt and Pokorny, 2005; Kim et al., 2006; Seo and Morr, 1984). Taken together, the same phenolic acids occur as secondary aromatic plant metabolites in rapeseed and soy bean protein isolates. The applicant acknowledges that there are quantitative and qualitative differences, but considers that these differences are not of toxicological concern because other plant-derived foods contribute significant additional amounts to the total daily intake, and because some of these phenolic acids are also formed in and absorbed from the intestinal tract as microbial breakdown products of ingested flavonoids.

The applicant has not carried out any toxicological studies on the NF. Instead, the applicant provided a teratogenicity, a sub-acute and two sub-chronic rat studies on rapeseed protein concentrates or isolates manufactured by other producers.

No indication for teratogenicity was observed in a teratogenicity study with rats fed with a rapeseed protein concentrate which contained 0.2 mg glucosinolates/g protein concentrate (Sharpe et al., 1975).

In a 28 day sub-chronic feeding study, rats received diets with 0, 2.5, 5.0 and 10 % rapeseed protein isolates (Plass et al., 1992). Increased absolute liver weights were observed in the 5 and 10 % diet groups.

The Panel notes that the two studies do not provide sufficient information on the production and composition of the test preparations to allow conclusions to be drawn for the NF.

The applicant also provided two articles on two 90-day toxicity studies in rats (20 rats/sex/group) which studied two rapeseed (canola quality) protein isolates produced by a competitor (Mejia et al., 2009a, 2009b). One of the two protein isolates (Puratein^{TM}) consists mainly of cruciferin (the globulin storage protein of rapeseed; > 80 % of the protein fraction), the remainder being the albumin storage protein napin (Mejia et al., 2009a). The other protein isolate (Supertein^{TM}) consists mainly of napin (> 80 % of the protein fraction), the remainder being globulin (Mejia et al., 2009b). The protein composition of these two products differs from the NF with regard to both globulin and albumin fractions. Both products contain erucic acid, total glucosinolates, AITC, total phytates and phenolics at similar or higher concentrations than the NF. The two protein isolates were included in the test diets at levels of 0 (controls), 5, 10 and 20 %. A comparison group received a diet with 20 % casein. No test product-related effects on body weight, feed consumption, neurobehavioral and motor activity, or clinical chemical and hematological parameters were observed in rats fed diets with the cruciferin protein isolate (Mejia et al., 2009a). In contrast, rats fed diets with the napin protein isolate at 20 % consumed less feed than controls during most of the study, and the consumption was statistically

significantly lower during nine weeks in male rats and during three weeks in female rats. In the 10 % dose group, the feed intake was statistically lower during seven weeks for males and during two weeks for females, and in the 5 % dose group males had a statistically significant lower feed intake for three weeks (Mejia et al., 2009b).

The lower food intake was associated with significantly lower cumulative body weight gains in males and females of the 20 % dose group and males of the 10 % dose group (starting the first week of the study) and a significantly lower feed efficiency in the 20 % dose group (males during weeks 1 and weeks 4 and 5; females in the first week). The authors considered this effect to be due to a lower palatability, and they performed a four days separate palatability and preference study that confirmed a preference for the diet containing casein compared to the napin protein isolate.

However, the Panel notes that a low palatability effect is usually transient, and that a four day preference study performed and reported by Mejia et al. (2009b) does not allow exclusion of the possibility that the napin protein isolate produced discomfort which induced a conditioned taste aversion that durably reduced feed intake, as observed in the study. The observed effects on feed consumption and feed efficiency were present already from the very onset of the exposure in week 1. No pathological effects were observed by the analyses of haematology, coagulation and clinical chemistry parameters, and opthamological and histopathological examinations of organs and tissues did not reveal any treatment-related changes.

Organ weights were not affected by the treatment with the possible exception of the relative thyroid plus parathyroid weight, which was slightly but significantly increased in females, but not males, of the high dose napin protein isolate group (Mejia et al,. 2009b) and in both sexes of the high dose cruciferin protein isolate group (Mejia et al., 2009a). In the absence of histopathological changes of the thyroids, the Panel considered that the increases in thyroid weight were not of toxicological relevance.

The Panel considers that in the study with cruciferin protein isolate, no treatment-related effects were noted, whereas in the study with napin protein isolate lower feed intake associated with reduced body weight gain and a reduced feed efficiency was observed, which may be caused by a low palatability and in part by an antinutritional effect inducing discomfort and consequently a conditioned taste aversion.

The Panel notes that, although both products studied by Mejia et al. (2009a, b) are not identical to the NF, the composition of cruciferin protein isolate, which did not show any adverse effect (Mejia et al., 2009a), was more similar to the NF than the napin protein isolate (Mejia et al., 2009b).

9. ALLERGENICITY

Several Member States expressed concerns with regard to a potential risk of allergenicity of rapeseed proteins in general, and with regard to potential cross-allergenicity of rapeseed proteins with proteins of other *Brassicacae*, particularly of mustard.

To date, rapeseed has been used as a food in the EU only in the form of rapeseed oil, which normally contains only traces of rapeseed protein. Foods with a higher content of rapeseed protein have not been consumed in significant quantities. The applicant has not carried out any studies to determine the potential allergenicity of the NF.

Food allergy to rapeseed (*Brassica rapa* L.) and oilseed rape (*Brassica napus* L.) has been reported to occur, as evidenced by studies by Puumalainen et al. (2006), Poikonen et al. (2006) and Poikonen et al. (2008). In the study by Poikonen et al. (2006), 11 % (206/1 887) of atopic Finnish children with suspected food allergies who were screened using skin prick tests showed sensitivity to seeds of *Brassica rapa* L. and/or *Brassica napus* L. In a subsequent challenge test 89 % of these sensitised children reacted to rapeseed. In parallel, a group of homologous proteins, 2S albumins or napins, were identified as new possible food allergens (Puumalainen et al., 2006). The authors considered that even

the smallest quantities of protein residues present in refined or cold-pressed rapeseed oils might be sufficient to produce sensitisation. Monsalve et al. (1997) identified 2S storage proteins ('napins') in *Brassica napus* L. seeds which may cause allergic reactions by aerogen exposure. BnIII napin, which accounts for 30 % of all napins occurring in *Brassica napus* L., was identified as the major allergen. Napins consist of a small and large chain linked by disulfide bonds (Lönnerdahl and Janson, 1972) and are extremely resistant to pepsin digestion and denaturation caused by heat and low pH (Murtagh et al., 2003). Napins represent approximately 20 % of the total protein of the seeds, and according to the applicant about one third of the NF.

There are also indications of cross reactivity between rapeseed and other foods. Several authors have previously reported on cross reactivity between the proteins of rape and mustard seeds (Meding 1985, Widstrom and Johansson 1986, Monreal et al., 1992). Monsalve et al. (1997) demonstrated IgE and IgG cross-reactivity between BnIII napin and Sin a1, the major allergen in seeds of the *Brassica alba* L. plant used in the production of yellow mustard, by inhibition ELISA. Mustard allergy has been reported in France, and has been investigated also in Spain, including studies on cross-reactions within *Brassicaceae* (Rance, 2003, Figueroa et al, 2005). Recombinant rapeseed 2S pronapin precursor protein was found to bind IgE in sera from mustard (Sin a 1) allergic patients as well as IgE in serum from a rapeseed allergic patient (Palomares et al., 2002). Seed storage proteins of various members of *Brassicaceae*, including mustard, have highly homologous molecular level structures with up to 94 % sequence similarity, and present risks of allergic reactions and cross-reactions in sensitised individuals (Monsalve et al., 2001; Poikonen et al., 2009).

The applicant proposed to inform individuals with mustard allergy about the potential unsuitability of foods formulated with canola protein isolate for their consumption.

The Panel considers that the risk of sensitisation to rapeseed cannot be excluded and that it is likely that rapeseed trigger can allergic reactions in mustard allergic subjects.

DISCUSSION

While canola oil has a pre-1997 history of safe food use, canola protein isolates have not been consumed in the EU in significant quantities. The presence of antinutritive substances in rapeseed (i.e. erucic acid, phytic acid and glucosinolates) previously limited the potential of rapeseed as a source of protein for food and feed. With the introduction of rapeseed cultivars with a naturally low content of erucic acid and glucosinolates (canola quality), this protein source has become potentially fit for human consumption. The rapeseed proteins can now be isolated with conventional separation techniques from the press cake (rapeseed meal), and purified up to the level of protein isolates (> 90 % protein). According to data provided by the applicant, the levels of undesirable compounds contained in the NF, such as erucic acid, glucosinolates and phytates, are either below detection limits or below levels which raise concerns.

The Panel considers that the information provided on the manufacturing process, as well as on the composition, specification and nutritional value of the NF, is sufficient and does not raise safety concerns.

The NF, a canola quality rapeseed protein isolate, shares many properties with soy protein isolates, which are isolated in a similar way from the press cake remaining from soy oil production. The macronutrient composition of the NF is similar to commercially available soy protein isolates. The biological value of rapeseed and soy protein, determined by the PDCAAS, appears to be similar.

The Panel notes that in one 13-week rat study with cruciferin protein isolate, no treatment-related effects were noted, whereas in the study with napin protein isolate lower feed intake associated with reduced body weight gain and a reduced feed efficiency was observed, which may have been caused by a low palatability and in part by an antinutritional effect inducing discomfort.

The Panel notes that people may consume up to 2.2 g protein/kg bw per day, of which a significant part may come from rapeseed protein. The Panel also notes that some subgroups of the population, such as sportspeople, may consume even higher amounts of protein. Only in an extreme scenario, in which "high consumers", such as vegans would consume rapeseed protein isolates as their sole source of protein, can an antinutritional effect not be excluded. The Panel considers that such a worst case scenario is unrealistic, and it would imply the consumption of an unbalanced diet, which is generally not recommended.

The Panel considers that the risk of sensitisation to rapeseed cannot be excluded and that it is likely that rapeseed trigger can allergic reactions in mustard allergic subjects.

The Panel notes the source and nature of the novel food, the absence of a nutritional disadvantage at the proposed uses and use levels, the low concentrations of potentially adverse components in the NF, the extended use of rapeseed press cake in farm animals, and the absence of toxicologically relevant effects in subchronic studies with rats of other rapeseed protein isolates with similar compositions. The Panel notes that based on the results of one of the rat studies, the possibility of an antinutritional effect caused by the novel food at high intakes, i.e. if rapeseed protein isolate was the main protein source in the diet, cannot be excluded.

CONCLUSIONS

The Panel concludes that rapeseed protein isolate is safe under the proposed uses and use levels.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier 'Rapeseed Protein (Isolexx^{TM})' under Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning Novel Foods and Novel Food Ingredients" received on 14/02/2013. Submitted by Bioresco Ltd. on behalf of Helm AG. Additional data were provided by the applicant on 04/04/2013, 17/06/2013 and 02/09/2013.
2. Letter from the European Commission to the European Food Safety Authority with the request for an opinion on the safety of 'Rapeseed Protein (Isolexx^{TM})', received on 14/02/2013; Ref. Ares (2013)191734 - 14/02/2013.
3. Initial assessment report carried out by the Food Safety Authority of Ireland: 'Safety Assessment of Rapeseed Protein (Isolexx^{TM})'.
4. Member States' comments and objections.
5. Response by the applicant to the initial assessment report and the Member States' comments and objections.

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APPENDIX

Analytical results from testing of twelve batches

Component (unit)	Specifications	Limit of detection	Typical contents [a]	BBI10004 [b]	BBI10007 [b]	BBI10008 [b]	BBI10009 [b]	BIOEXX 20120109 [a]	BIOEXX 20120110 [a]	BIOEXX 20120213 [a]	BIOEXX 20120214 [a]	BIOEXX 20120215 [a]	BIOEXX 20120221 [a]	BIOEXX 20120227 [a]	BIOEXX 20120313 [a]
Protein (%) [c]	≥ 90 %	n. s.	92.7	95.3	91.7	90.7	93.3	92.2	91.9	90.7	92.7	96.6	89.7	91.6	91.2
Solubility (%)	n. s.	n. s.	n.s.	99	97	97	96	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Pass through US 80 mesh (%)	> 90 %	n. a.	n.s.	95	95	95	95	95	95	94	94	94	90	89	74
Moisture (%)	≤ 7 %	n. s.	3.7	3.3	3.7	3.2	1.5	5.04	5.4	4.95	4.75	5.08	4.65	4.47	5.88
Fat (%) direct [d]	n. s.	n. s.	≤ 0.5	0.26	0.23	0.22	0.13	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Fat (%) total [e]	< 2 %	n. s.	1.8	n. a.	1.7	n. a.	n. a.	1.28	0.72	0.79	0.71	0.34	1.74	1.09	0.6
Fibre (%)	≤ 0.5 %	n. s.	< 0.1	0.0	< 0.02	0.06	0.05	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Ash (%)	≤ 4 %	n. s.	2.3	2.2	2.0	2.3	2.3	2.4	2.68	2.4	2.7	1.3	1.4	1.48	1.20
Carbohydrates (%) [f]	≤ 7 %	n. s.	4.1	2.2	6.1	6.7	4.2	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Na (ppm)	n. s.	n. s.	660	550	246	200	170	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
K (ppm)	n. s.	n. s.	430	420	291	530	280	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Ca (ppm)	n. s.	n. s.	3 810	4 100	4 160	3 850	4 070	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
P (ppm)	n. s.	n. s.	4300	3600	4500	4500	4 700	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Fe (ppm)	n. s.	n. s.	85	84	83	75	80	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Mg (ppm)	n. s.	n. s.	1 500	1 600	1 780	1 870	1 860	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Zn (ppm)	n. s.	n. s.	52	49	49	50	57	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Pb (ppm)	≤ 0.5 mg/kg	n. s.	< 0.01	0.02	0.02	0.02	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
As (ppm)	n. s	≤ 0.05	n. s	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Cd (ppm)	n. s.	n. s.	n. s.	0.28	0.19	0.25	0.16	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Al (ppm)	n. s.	n. s.	n. s.	7.6	12	9.2	7.5[l]	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.

Erucic acid (% of total fat)	n. s.	0.1[a, g]	n. d. [h]	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Total glucosinolates (mmol/kg)	≤ 1	0.1[g]	≤ 0.1	n. a.	n. d.	n. d.	n. d.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Allyl isothiocyanate (mg/kg)	n. s.	1[g, i]	n. d.	n. d.	n. d.	n. d.	n. d.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Total phytates (g/100 g)	≤ 1.5	0.4[g]	≤ 1	0.44	0.83	1.14	1.03	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Phenolics (total in gallic acid equivalents) (g/kg)	n. s.	0.05[j]	< 2	n. a.	1.53[k]	1.52[k]	1.12[k]	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Sinapin (mg/kg)	n. s.	50[a]	≤ 500	200[j]	180[j]	80[j]	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.	n. a.
Aerobic bacteria count	≤ 10 000 cfu/g	< 5	n. s.	410	80	6 700	9 800	460	9 000	3 300	630	300	1 800	300	1 800
E. coli (cfu)	neg./10 g	< 5	neg.	neg.	< 5	< 5	< 5	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Salmonella (cfu)	neg./25 g	< 5	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Yeast and mold counts (cfu /g)	100	< 5	n. s.	120	< 5	< 5	280	10	10	10	10	10	10	10	10

Abbreviations: cfu = colony forming units, n. a. = not available, n. d. = not detected, n. s. = not specified, neg. = negative
(a): Provided by BioExx Proteins of Saskatoon Inc., Canada
(b): Analysed by POS Pilot Plants Saskatoon SK, Canada
(c): Based on dry matter
(d): Free fat measured by direct extraction with ether
(e): Total fat measured by HCl hydrolysis followed by ether extraction
(f) : Calculated by difference. Values are overestimates since for fat only values for extractable rather than total fat are available.
(g): Provided by Eurofins
(h): Analyses by Eurofins of Isolexx (dated 13/03/09 and 03/06/10)
(i): GC-MS analyses by Eurofins of Isolexx (dated 02/08/2011)
(j): Analysis by University of Nebraska Lincoln (dated 04/06/2011)
(k): Analyses by University of Saskatoon
(l): Misreported as 75 in initial application dossier

ABBREVIATIONS

ADI	Acceptable daily intake
AITC	Allyl isothiocyanates
Bw	Body weight
Cfu	Colony forming unit
FTU	Phytase unit
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GM(O)	Genetic Modification/Modified (organism)
GRAS	Generally recognized as safe
IOM	Institute of Medicine
kDa	kiloDaltons
NF(I)	Novel Food (Ingredient)
NMR	Nuclear Magnetic Resonance
NOAEL	No Observed-Adverse-Effect Level
PDCAAS	Protein-digestibility corrected amino acid
SCF	Scientific Committee on Food