FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

Supporting Document 1

Risk assessment (at Approval) – Proposal P1031

Allergen Labelling Exemptions

Executive summary

Foods are mainly composed of proteins, carbohydrates and lipids. For allergenic foods, the risk to allergic consumers is almost always associated with proteins. The risk to allergic consumers is a function of the likelihood of an allergic reaction occurring at the dose of allergenic food consumed and the severity of reaction. The dose depends on the concentration of the residual protein in the product and amount consumed. Various products derived from allergenic foods contain little or no protein as a result of chemical and/ or physical processes which separate and remove the proteins. Although not all proteins in a food allergen are allergenic, minimising the total protein content in products derived from the allergenic sources would minimise the risk to allergic consumers.

The risk assessment in this report relates to four products derived from allergenic foods. The products are: soybean oil that has undergone a complete refining treatment, i.e. degummed, neutralised, bleached and deodorised (N/RBD) soybean oil; tocopherols and phytosterols derived from the deodoriser distillate of N/RBD soybean oil; glucose syrup from wheat starch; and alcohol distilled from wheat and whey.

FSANZ considered available evidence from published and unpublished oral challenges with each product in allergic patients. Also considered were analytical data on residual protein levels in samples from each of the products. Dietary exposure per meal was estimated based on the level of use in food and food consumption data from Australian national nutrition survey data. The assessment also considered information on the processing steps which account for reducing protein content in the final product. Where available, data on allergen thresholds were incorporated into the assessment. Thresholds are the highest amount of allergenic food that can be consumed in a single meal without causing an allergic reaction.

FSANZ undertook two consultations with the Food Allergy and Intolerance Scientific Advisory Group (FAISAG) to gain input from allergy clinicians in Australia and New Zealand. In particular, FSANZ sought input on suitable risk assessment terminology to describe the level of risk and to determine whether conclusions can be drawn from the available evidence.

The risk assessment concluded that N/RBD soybean oil presents negligible risk to soybean allergic consumers. The conclusion is based on the negative results of clinical studies of dose escalation oral challenges, the analytical data showing extremely low/ undetectable protein content in N/RBD soybean oil, and the limited dietary exposure to soybean oil in one meal. With regards to the effect of the processing steps on protein content, the data indicate that the bulk of soybean protein is removed during degumming, the first step in the oil production process. Residual impurities, including proteins, are reduced further by the neutralising step using alkali, and the bleaching step using activated clay or silica.

Finally, the soybean oil is deodorised to remove volatile substances such as odours and off-flavours. The full process results in N/RBD soybean oil virtually devoid of any protein. Analytical data of N/RBD soybean oils sourced from different countries indicate that soybean protein levels are consistently <1 mg/kg. Cold pressed soybean oil is not included in this assessment as the protein profile of cold-pressed soybean oil is very similar to that of soybean flour.

Phytosterols and tocopherols, highly processed products derived from the deodoriser distillate of N/RBD soybean oil were considered. The distillate is generated in the final step of N/RBD soybean oil production. Analytical data confirmed that protein was not detected in the distillate and tocopherols or phytosterols are also unlikely to contain detectable protein. This is not surprising since soybean protein is removed in the production of N/RBD soybean oil. It also follows then that, like N/RBD soybean oil, tocopherols and phytosterols present negligible risk to soybean protein allergic consumers.

The available clinical data suggests that acute dietary exposure (a single eating occasion) to no more than 1 mg of wheat protein is unlikely to provoke an IgE-mediated immunological response in the majority of wheat sensitive individuals. Consumption data indicates that the amount of food (confectionery or chocolate) consumed per day for high consumers was between 52–100 g for Australian 2–4 year olds; between 100-183 g/day for 5–14 year olds in Australia and between 100–232 g/day for 5-14 year olds in New Zealand. Confectionery products contain glucose syrup in various amounts between 1–70% of the final product, however not all the glucose syrup is derived from wheat, particularly in New Zealand where the sole manufacturer meets 90% of its glucose syrup requirements and this glucose syrup is corn based.

Analytical data from Australian produced glucose syrup shows that in samples taken from daily batch testing over 10 months, 90% of syrups contained less than 10 mg/kg gluten and the remaining 10% were below 20 mg/kg. Minimising gluten in all glucose syrup samples to as low as technically and practically achievable, would ensure that dietary exposure for most consumers does not exceed 1 mg of wheat protein in a single meal. The risk assessment concluded that based on the available evidence, consumption of wheat-derived glucose syrup that had been purified and prepared as described in Appendix 2 would present negligible risk to the majority of wheat allergic individuals; such syrups would also be suitable for those with coeliac disease.

Another category of products considered in this risk assessment were alcohol distillates from wheat and whey. There is general scientific agreement that, in a properly controlled distillation process, non-volatile substances such as lactose and proteins from wheat and whey are not found in the distillate. On this basis, distilled alcohol (and products made from distilled alcohol, such as vinegar) would not contain protein. The available analytical data confirm that protein is undetectable (i.e. <1 mg/kg) in distilled ethanol from whey and wheat. The risk assessment concluded that alcohol distilled from wheat and whey, including vinegar derived from distilled alcohol, present negligible risk to susceptible individuals.

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1 Soybean oil

Soybean (*Glycine max*) is the largest single source of edible oil in the world, accounting for roughly 50% of global production. Soybean oil is a rich source of essential fatty acids and vitamin E. Soybean oil is the main oil used by the food industry in a wide range of products as an ingredient, additive or processing aid, and for commercial food frying.

1.1 Soybean allergy

The soybean seed is approximately 37% protein on a dry weight basis. Like other food allergens, the protein component in soybean is responsible for allergic reactions. Eight soybean seed proteins have been identified as food allergens designated Gly m 1–7 (IUIS 2013). In addition, the soybean protein Gly m Bd 30K, also known as P34, is considered a major allergenic soybean protein. The characteristics and functions of allergenic soybean proteins were reviewed by L'Hocine and Boye (2007).

Generally, soybean proteins show lower allergic reactivity when compared with other food allergens (Cordle, 2004). However, the amount of soybean protein reported to trigger an allergic reaction varies among soybean protein allergic individuals according to a European multi-centre study (Ballmer-Weber et al, 2007). In this study, 23 soybean protein allergic patients were challenged with soybean protein in a double blind placebo controlled food challenge (DBPCFC). The study reported that none of the patients reacted to 1 mg soybean protein following oral exposure. Subjective symptoms were triggered by cumulative soybean protein doses of 5.3 mg–26.5 g, and 240.6 mg–26.5 g for objective symptoms. (Ballmer-Weber et al, 2007).

1.2 Soybean oil terminology

It is recognised that terms used to refer to edible oils in general, including soybean oil, are not standardised making it difficult to interpret the literature Rigby et al (2011). For example, terms like 'fully refined', 'highly refined' or 'purified' oil are not well-defined in relation to the processing steps used in oil production. In the context of food allergy, this is particularly important because the processing steps contribute to the reduction of protein level in the oil (Appendix 1, Figure 1). To ensure clarity and consistency of terminology used in this report, the following terms are defined as follows:

Crude oil: is oil that has undergone solvent extraction or other steps to separate the oils from the seed solids.

Crude degummed oil: is crude oil that has undergone degumming, but no further processing.

Refined, Bleached and Deodorised (N/RBD or RBD) oil: is oil that has undergone a complete refining treatment, i.e. degummed, neutralised (alkali refined), bleached and deodorised.

1.3 Soybean oil – manufacturing process

The production process involves several steps and includes cleaning and drying, crushing and solvent extraction of soybeans. Crude soybean oil is degummed, neutralised, bleached and deodorised yielding N/RBD soybean oil. Soybean oil-based fats are interesterified and hydrogenated after bleaching and before deodorisation.

Protein is removed in this process but the final level of protein content depends on the quality and efficiency of the purification steps.

This assessment relates to soybean oil that has undergone a complete process, i.e. degummed and neutralised (refined), bleached and deodorised, referred to as RBD or N/RBD oil, for use in food. Cold pressed soybean oil is not included in this assessment.

1.3.1 Soybean oil extraction

Mechanical extraction is the traditional method of releasing oil by applying heavy pressure to the seeds. Due to friction, heat is produced during the process, averaging at about 40°C. Cold pressed oil is produced by mechanical extraction while maintaining the temperature below 49°C. Cold pressed oil is used for its flavour and is not refined. Martín-Hernández et al (2008) reported that the protein profile of cold-pressed soybean oil is very similar to that of soybean flour so it is not included in this assessment.

To overcome the low yield of oil recovery by mechanical extraction, modern soybean oil production is based on hot solvent extraction. The high percentage of oil recovered from solvent extraction made it the most popular method of oil extraction. In this process, the cleaned and dehulled soybean seeds are flaked and the oil is extracted using an organic solvent as described below.

A typical commercial solvent to extract the oil from the seed flakes would consist mainly of six-carbon alkanes, hence the name 'hexane' by which these solvents are commonly known. Higher temperature in the extractor enhances the solubility of oil which considerably increases the rate of extraction. The resulting solution of oil in solvent is distilled allowing the solvent to be recovered for reuse leaving behind the soybean oil. The physicochemical properties exploited in this process are: the high solubility of oil in hexane and poor solubility for non-oil components, and the low boiling point of hexane (67°C) to separate it from the oil.

The soybean oil obtained by extraction from the oilseeds is designated 'crude soybean oil'. Crude soybean oil contains more than 95% triglyceride mixtures, but also contains various substances from the soybean seed, including protein. The flavour, stability and functional properties of the oil are negatively affected by these substances, which are removed by further treatment.

1.3.2 Production of neutralised/refined, bleached and deodorised soybean oil

The complete treatment of crude soybean oil to produce neutralised/refined, bleached and deodorised (N/RBD) soybean oil involves degumming and neutralising (also known as refining), bleaching and deodorisation. Analytical evidence indicates that N/RBD soybean oil contains extremely low or undetectable levels of protein.

An outline of the complete process to produce N/RBD soybean oil, including a flow diagram (Figure 1), based on detailed description in the literature (Berk, 1992; Fereidoon, 2005; Inturissi 2007), is at Appendix 1.

1.4 Quantification of protein in oil

Protein levels in various edible oils have been reported in the literature (Crevel 2000; Nordlee et al 2002; Hidalgo and Zamora, 2006; Ramazzotti et al, 2008; Martín-Hernández et al 2008). However, in most studies, little or no information is provided on the process used in oil extraction and refining. In addition, without validated and suitable methods for the measurement of protein in oil matrices it is difficult to compare protein levels reported in various studies.

This situation has resulted in uncertainty over the true protein content of oils and led to difficulties in interpreting, from a safety perspective, studies reporting reactions to different edible oils.

More recently, Rigby et al (2011) reported a method for extracting and quantifying residual protein in fully and partially refined soybean oils. Initially three different extraction methods were assessed but, because of technical issues arising from the very low protein levels and in order to improve reproducibility, a further, low-volume extraction method (micro-borate method) was developed during the course of the study. Residual protein was extracted by the micro-borate method and quantified by the 3-(4-carboxybenzoyl) quinolone-2-carboxaldehyde (CBQCA) assay. In this assay, the non-fluorescent CBQCA generates a highly fluorescent derivative upon reacting with primary and accessible amines in the presence of cyanide or thiols. The CBQCA assay is significantly more sensitive than other techniques and resistant to lipid interference, making it particularly suitable for measuring protein in oil.

1.5 Effect of processing steps on protein content in soybean oil

The amount of protein remaining in refined oil is the component that is relevant to allergenicity. Therefore, it is important to determine the effect of the production process on protein content of soybean oil.

Rigby et al (2011) compared the protein content of crude non-degummed soybean oil and N/RBD oil using the micro borate CBQCA assay. The reduction of protein content was clearly demonstrated with crude non-degummed soybean oil containing about 340 times more protein than the corresponding N/RBD oil (average 87.250 mg/kg versus 0.256 mg/kg). Therefore, through the complete refining process, the protein content of N/RBD oil is reduced to levels < 1mg/kg. These results illustrate the effect of the process on the level of protein in crude soybean oil. While the bulk of the protein appears to be removed in the degumming step, protein removal depends on the quality and efficiency of all the processing steps. The final product, N/RBD soybean oil, is clear and bright in colour.

The identity of residual proteins was investigated using, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Matrix Assisted Laser Desorption/Ionisation–Time of Flight Mass Spectrometry (MALDI-ToF MS) (unpublished data). However, sufficient amount of protein could only be extracted from crude non-degummed soybean oil, but not from N/RBD oil. The analysis of crude non-degummed soybean oil identified the soybean protein Kunitz-type trypsin inhibitor A. There was no evidence that the major soybean allergens, Gly m Bd 30k or Gly m 4, were present in the crude non-degummed soybean oil. Therefore, it can be concluded that it is unlikely that these proteins would be present in N/RBD oil.

1.6 Use of soybean oil in food

Commercial uses of soybean oil range from use as a main ingredient in cooking oil and in salad dressing to its use as a minor ingredient e.g. emulsifier, or a processing aid. Examples of uses of soybean oil in food are:

- oil used in salad dressing, frying, cooking, and baking
- margarine, fat spreads, shortenings
- mayonnaise, salad dressing
- bakery products
- confectionery products
- snacks
- soups and sauces.

1.7 Soybean oil produced in Australia

Cressey et al (2011) reported analytical data on samples of soybean oil manufactured in Australia. Six unique 2 L batches of soybean oil were obtained from retail outlets in New Zealand over a period of five months. All samples were analysed for residual protein by ELISA and Bradford colourimetric method. The ELISA method used was a direct sandwich ELISA to the heat-stable soy trypsin inhibitor, validated for use with soybean oil as the test matrix. Soybean oil was spiked with non-fat soybean flour to a soybean protein content of 3.5 mg/kg and the soybean protein successfully recovered from the soybean oil. Soybean protein was not detected in any of the soybean oil samples analysed, above the limit of detection of 1 mg/kg. In addition, the researchers also applied the Bradford micro protein method using the low temperature acetone precipitation method described in the literature (Paschke et al 2001, and Rigby et al 2011). The method demonstrated 95% recovery of 3.5 mg/kg soybean protein spike (de-fatted soybean flour). These results show that soybean protein was not detected above the limit of detection (0.5 mg/kg) in any soybean oil samples.

The results reported by Cressey et al (2011) are consistent with protein content in N/RBD soybean oil samples sourced from a number of countries (0.082, 0.092, 0.698, 0.14, 0.047, 0.026, 0.33 mg/ kg) as reported by Rigby et al (2011).

1.8 Published clinical studies with soybean oil

The only published clinical study on the potential allergenicity of soybean oil was reported by Bush et al (1985). The authors reported that soybean oil did not elicit reactions in soybean allergic patients. Seven patients with well-documented allergy to soybean (systemic allergic reactions after ingesting soybean and positive skin prick test (SPT) to whole soybean extract) were enrolled in a double-blind crossover study. Sera from six of the patients showed elevated serum IgE to soybean extract (RAST assay binding values ranged from 2.3 to 28.1 times that of negative control). Before the oral challenges, all patients demonstrated negative SPT to three commercially available soybean oils and to olive oil (control). On four separate days, the patients were challenged with soybean oil and olive oil in random sequence. At 30-minute intervals doses of 2, 5 and 8 mL of one of the soybean oils or control contained in capsules were administered orally. No adverse reactions were observed with any of the soybean oils or control. The authors concluded that soybean oil ingestion does not appear to pose a risk to soybean-sensitive individuals.

However, a number of limitations were identified in the study. These include the small number of patients (seven); the use of capsules to administer the oil thus preventing direct allergen contact with the oral mucosa (recognised as an important route of allergen exposure); and lack of information on protein levels in the oils used in the study, two of which were refined, and one was cold-pressed.

1.9 Unpublished clinical studies with soybean oil

To address the safety of N/RBD soybean oil, three clinical studies were conducted by internationally recognised clinicians experienced in the performance and interpretation of food challenges. The studies are outlined below.

Study 1: Controlled challenge study in soybean allergic individuals

The study was designed as a controlled challenge study in 29 individuals with well-documented allergy to soybean, recruited through six clinical centres (3 in the USA, and one each in Canada and South Africa).

The test material was a pool of commercially representative N/RBD soybean oils (0.165±0.031 mg/kg protein content, as measured by PBS/total amino acid method (or 0.279±0.040 mg/kg, as later determined using the microborate extraction and ATTO-TAG assay). The control material in the clinical study was canola oil. The test, or control, material were given in successively increasing amounts i.e. 1, 5, and 10 g of oil, to a total of 16 g of oil with 30 minute interval between doses. The total of 16 g of oil was considered representative of the amount of soybean oil consumed in one sitting. Participants remained at the clinic for a further two hours for further observation. No objective reactions were observed and no subjective reactions were reported up to the cumulative dose of 16 g soybean oil.

Study 2: DBPCFC study in soybean allergic individuals

In this study, 32 individuals with demonstrated soybean allergy, confirmed by challenge, were recruited at each of three participating clinics (university clinics of Berlin, Utrecht and Zurich). All patients were challenged under the same conditions and using the same doses of a blend of six samples of N/RBD soybean oils. The predicted average protein content of the blend was 0.150 mg/kg, based on protein levels for individual samples using the bicarbonate/ATTO-TAG combination method. The challenge doses were 12, 24 and 48 mL of soybean or control oil (i.e. 84 mL of each in total of 800 g of mashed potato). The cumulative dose of 84 mL represents high dietary exposure to soybean oil. The control oil was canola oil. All subjects, except one who was too full to consume the full dose, completed the challenge. The majority of the volunteers reported no symptoms at all (90% after challenge with soybean oil; 70% control). In the remainder, all reported symptoms were mild, subjective and self-limiting. Based on these results, more reactions were reported with the control oil challenges. The occurrence of these reactions highlights the non-specific nature of the type of symptom reported. Since the oil matrix may delay absorption of the soybean proteins, participants in both studies were instructed to report any delayed symptoms that may occur after they left the clinic. No delayed reactions were reported by any of the participants.

Study 3: DBPCFC study in peanut allergic individuals

In this study, 30 individuals with a history of exquisite peanut food allergy, confirmed by DBPCFC, were recruited at each of two participating clinics (University clinics in Berlin and Utrecht).The study participants consumed increasing doses of soybean, or control, as outlined in Study 2. The protein content of 3 clinical blend batches used was the same as in Study 2. Nearly 75% of the participants reported no symptoms at all. In the remaining participants, all reported symptoms were mild, subjective and self-limiting and equally distributed between soybean oil and placebo challenges. No delayed reactions were reported.

1.10 Dietary exposure

The acute nature of Ig-E mediated reaction means that for estimates of dietary exposure the relevant dose is required to be ingested within a relatively short period of time, such as during a meal. The meal scenario considered by the European Food Safety Authority (EFSA), EFSA (2007a), assumed a maximum level of residual protein of 0.15 µg/g, and incorporated four food items made with soybean oil, i.e. margarine, salad dressing, mayonnaise and potato chips fried in soybean oil. Based on average serve size for each meal component, it was estimated that the meal scenario outlined below would provide a total intake of about 12.1 µg soybean protein in 80.5 g of soybean oil (Table 1).

Table 1: EU meal scenario (EFSA 2007a)

Meal item/ serve size	Soybean Oil content	Soybean Oil consumed	Soybean protein consumed
Margarine 10 g	80% N/RBD	8 g N/RBD	1.20 µg
Salad dressing 15 g	100% N/RBD	15 g N/RBD	2.25 µg
French fries 200 g	20% N/RBD	40 g N/RBD	6.00 µg
Mayonnaise 25 g	70% N/RBD	17.5 g N/RBD	2.63 µg
		Total 80.5 g	12.1 µg

FSANZ applied consumption data for 2–6 and 7–16 year olds to the same meal scenario. Consumption data were derived from the 2007 Australian National Children's Nutrition and Physical Activity Survey (ANCNPAS) using FSANZ's dietary exposure assessment program (DIAMOND). The 2007 ANCNPAS contains food consumption data for 4,487 respondents aged 12–16 years (mean body weight = 47 kg). The 2007 ANCNPAS has data for two 24-hour recalls. Further information on dietary modelling is available on the FSANZ website at http://www.foodstandards.gov.au/science/riskanalysis/exposure/documents/Principles%20_%20practices%20exposure%20assessment%202009.pdf

All food consumption data for consumers of each food presented in Table 2 below were for the average of these two days. The consumer mean, 90th percentile, 95th percentile and 97.5th percentile food consumption amounts for the selected food types are presented for 2–6 and 7–16 age groups.

Table 2: Consumption data for individuals aged 2-6 and 7-16 years from 2007 ANCNPAS

Food	Number of consumers	% consumers to respondents	Consumption of food for consumers[a] only (g/day)[b, c]			
			Mean	90th percentile	95th percentile	97.5th percentile
2-6 years						
French fries and chips	393	27	46	75	120	140
Salad dressing	37	3	6.4	15	20	n/a
Mayonnaise(commercial)	126	9	4.8	9.3	16	31
Margarine/ dairy blends	1058	72	7.4	14	19	23
7-16 years						
French fries and chips	967	32	72	130	150	190
Salad dressing	155	5	9.2	20	25	33
Mayonnaise (commercial)	577	19	7.2	19	23	28
Margarine and dairy blends	1845	61	8.3	17	20	24

[a]Consumers are those respondents from the nutrition survey who ate the food(s) of interest.
[b]Derived using an average of the Day 1 and Day 2 food consumption amounts and using the weighting factors.
[c]All food consumption amounts have been rounded to 2 significant figures.
n/a= there were too few consumers to allow for a sufficiently robust 97.5 percentile to be derived.

Table 3: Dietary exposure for soybean oil in a meal based on Australian consumption and food composition data

Food consumption (in grams)[1]	Food ID (NUTTAB 2010)	% oil in meal items	Soybean oil amount consumed (in grams)	Soybean protein consumed (in micrograms)
2-6 years				
Margarine (23 g)	04B20074	70%	16.1	2.4
Salad dressing (20 g)	10F20087	33.7% (Thousand island dressing)	6.7	1.0
French fries (140 g)	13A11631	17.2%	24.0	3.6
Mayonnaise (31 g)	10F20096	75.9%	23.5	3.5
Total			70.3	10.5 (based on protein concentration in oil of 0.15 mg/kg) or 35.5 (based on protein concentration in oil of 0.5 mg/kg)
7-16 years				
Margarine (24 g)	04B20074		16.8	2.5
Salad dressing (33 g)	10F20087		11.1	1.6
French fries (190 g) **Hot chips (190 g)**	13A11631		32.7	4.9
Mayonnaise (28 g)	10F20096		21.3	3.2
Total			82.0	12.3 (based on protein concentration in oil of 0.15 mg/kg) or 41.0 (based on protein concentration in oil of 0.5 mg/kg)

[1] 97.5th percentile of consumption for consumers of food per day (except for salad dressing for 2-6 year olds where the 90th percentile amount was used).

For the dietary exposure estimate, we applied food composition data from the Australian NUTTAB 2010 Database to the 97.5 percentile food consumption data for consumers for each of the selected foods for the 2–6 and 7–16 year age groups (90th percentile for salad dressing for 2–6 year olds). In this assessment the high percentile consumers amount was taken to represent an amount of each food that may be consumed in a single meal, which is likely an overestimate.

The meal included four items derived from or cooked with oil. The scenario assumed that all oil and fat contained in these foods was soybean oil; and that the soybean protein content was 0.15 µg/g or 0.5 µg/g (Table 3).

1.11 Summary

The product considered in this assessment is N/RBD soybean oil manufactured according to a well-defined process which includes hot solvent extraction, degumming, neutralising bleaching and deodorising. All the processing steps contribute to minimising protein levels. The final product, N/RBD soybean oil, is clear and bright in colour.

Analytical studies confirm that N/RBD soybean oils sourced from a number of countries contain extremely low levels of protein, <1 mg/kg. Similarly for N/RBD soybean oil manufactured in Australia and commercially available in Australia and New Zealand, residual protein content was below the level of detection (LOD) for commonly used protein detection methods, i.e. <1 mg/kg for the soybean protein-specific ELISA, and <0.5 mg/kg for the Bradford method.

Available clinical evidence demonstrates that N/RBD soybean oil does not trigger objective symptoms of allergic reactions in soybean allergic and peanut allergic consumers. No qualitative or quantitative differences were found between the responses to N/RBD soybean oil and control oils. None of the participants in the clinical challenges reported delayed or continuing reactions. The amount of N/RBD soybean oil used in the clinical challenges, considered to be at the upper limit of possible dietary exposure, provides a significant margin of safety.

Soybean oil is used as an ingredient in food or for frying, and is not likely to be consumed in large amounts. In a possible dietary exposure scenario it was estimated that the maximum amount of soybean oil likely to be consumed in one meal would contain ≤0.005 mg (i.e. ≤50 micrograms) of soybean protein. This amount of soybean protein is miniscule and is much lower than the reported cumulative soybean protein doses of 5.3 mg and 240.6 mg reported to trigger subjective and objective symptoms, respectively.

N/RBD soybean oil provides a safety benchmark which can be used to determine the equivalence of alternative processing methods which may be used to refine soybean oil.

1.12 Conclusions

Based on the available clinical and analytical evidence, N/RBD soybean oil presents negligible risk to soybean allergic consumers.

2 Phytosterols/ phytosterol esters and Tocopherols/ tocopherol esters

Phytosterols are a group of steroid alcohols that occur naturally in plants, including soybean. Tocopherols are a group of methylated phenols many of which have vitamin E activity. Natural source vitamin E products consist of natural mixed tocopherols, alpha-tocopherol, alpha-tocopherol acetate, and alpha-tocopherol succinate.

Phytosterols and tocopherols are a by-product of the processing of vegetable oils, including soybean oil. The final step in oil refining is deodorisation, i.e., treatment at high temperatures (steam stripped) and low pressure and recovering the volatiles in a vapour condenser. Deodorisation is mainly applied to remove volatile odoriferous substances which lead to deterioration of the quality of the oil. Deodorisation relies on the large volatility differences between the oil itself and the volatile compounds. The recovered distillate, generally referred to as vegetable oil deodoriser distillate (VOD), mainly contains free fatty acids, but also significant levels of phytosterols (8-20%) and tocopherols (5-15%) (Cantrill and Kawamura 2008).

2.1 Production process

The VOD, the starting material for the manufacturing of phytosterols and tocopherols is a complex mixture of free fatty acids, triglycerides, sterols and their esters, tocopherols, and breakdown products, waxes, and fatty acid esters. In a transesterification (methanolysis) step, the glycerides are converted into fatty acid methyl esters and glycerol, and the phytosterol-esters into free phytosterols and fatty acid methyl esters. Phytosterols and tocopherols are extracted by distillation. The phytosterols are separated from the tocopherols by crystallization and filtration. Phytosterols can be hydrogenated to obtain phytostanols (Cantrill and Kawamura 2008; Torres et al 2011).

The entire process, which includes distillation, filtration and crystallisation, produces phytosterols and tocopherols in good yield and high purity.

2.2 Use in food

Phytosterols are permitted to be added to specific foods (low fat milk, breakfast cereals, yoghurt, and edible oil spreads) in the Australia New Zealand Food Standards Code (the Code). Due to their structural similarity with cholesterol, phytosterols are added to foods for their properties to reduce absorption of cholesterol in the gut and thereby lower blood cholesterol levels.

Soybean-derived tocopherols are mainly used as a food antioxidant (INS 307) to prevent rancidity. The Code permits tocopherols to be added to edible oils and oil emulsions (GMP), infant formula products (10 mg/L), infant food (300 mg/kg of fat).

2.3 Protein content and allergenicity of phytosterols and tocopherols

Information including analytical studies, immunochemical studies and clinical studies, as reported in two EFSA Opinions (EFSA 2007b, EFSA 2007c), has been referred to in the preparation of this report and is outlined below. No data relating to the amount of soybean protein remaining in phytosterol and tocopherol preparations or their potential allergenicity were available to FSANZ.

2.3.1 Analytical studies

Two strategies were followed for the detection of residual soybean proteins in tocopherol and phytosterol samples, as follows:

(a) Extraction of hydrophilic proteins for ELISA analysis that detects hydrophilic proteins, and for analysis in immunoblotting that detects hydrophilic, lipophilic, and denatured proteins. The soybean specific ELISA allowed a detection of hydrophilic soybean proteins at or above 0.5–2 ng/ml in diluted extracts.

(b) Extraction of lipophilic and denatured proteins for analysis in immunoblotting. The immunoblotting method allowed the detection of 50 ng of internal reference soybean (IRS) extract and 100 ng of oleosin, respectively. Oleosin is a unique protein associated with the oil body surface. Oleosins have a high affinity for lipids. Each VOD and phytosterol sample extract was analysed for total protein, soybean protein and IgE-reactive soybean protein in a primary screening.

Protein detection – VOD samples

a) A VOD sample spiked with IRS protein extract prior to extraction was used to investigate IRS protein recovery. There was no protein (total) or soybean protein detected in any of the samples following SDS-PAGE/Sypro®Ruby (ruthenium based fluorescent) stain (limit of detection 10 mg/kg).There was no detectable hydrophilic protein in any of the nine VOD samples at or above 10 mg/kg.

b) A VOD sample was spiked with the oleosin fraction from soybean prior to extraction for investigation of lipophilic protein recovery. All protein extracts of VOD samples were analysed in SDS-PAGE/Sypro®Ruby. There was no detectable lipophilic protein in any of the nine VOD samples at or above 1 µg/g.

Protein detection – phytosterol samples

a) Selected phytosterol samples as well as the phytosterol blend used in the clinical study were analysed for residual soybean protein. Additionally, a phytosterol (blend) sample was spiked with IRS protein extract prior to extraction for investigation of IRS protein recovery. Using ELISA, soybean protein was not detectable at or above 10–20 µg/g in extracts of all investigated phytosterol samples including the blend sample.

b) The phytosterol blend was additionally spiked with the oleosin fraction from soybean prior to extraction for investigation of lipophilic protein recovery. In none of the investigated phytosterol samples including the phytosterol blend was soybean protein detectable at or above 10–20 µg/g.

Protein detection – tocopherol samples

a) Selected tocopherol samples as well as the tocopherol blend used in the clinical study were analysed for residual soybean protein. Additionally a tocopherol (blend) sample was spiked with IRS protein extract prior to extraction for investigation of IRS protein recovery. Using ELISA, hydrophilic soybean protein was not detectable at or above 10 µg/g in extracts of all investigated tocopherol samples including the blend sample.

b) The tocopherol blend was additionally spiked with the oleosin fraction from soybean prior to extraction for investigation of lipophilic protein recovery (oil bodies in seeds contain a unique protein known as oleosin associated with the oil body surface. Oleosins are unique in that they have a high affinity for lipids). In none of the investigated samples, including the tocopherol blend, was soybean protein detectable at or above 1 µg/g.

In addition, an ELISA inhibition assay was developed for soybean specific IgE. A range of tocopherols were analysed and none of the samples produced inhibition.

2.3.2 Immunochemical studies

Immunoblotting protocols using a polyclonal soybean protein-specific antiserum from rabbit (RBiopharm) had a LOD of 50 ng of IRS extract and 100 ng of oleosin. With the soybean protein allergic serum pool (using sera from nine soybean protein allergic patients), the immunoblotting protocol allowed the detection of 50 ng of IgE-reactive proteins from IRS extract. The oleosin fraction was shown to be a minor IgE-binding constituent of total soybean protein. All hydrophilic extracts of VOD samples were analysed by immunoblotting with soybean protein-specific antiserum, and by IgE-immunoblotting with a pooled human serum.

There was no protein or soybean protein detected in any of the samples following immunoblotting with soybean protein-specific antiserum from rabbit. There was no IgE binding with the VOD or the phytosterol samples using either the pooled human serum or the serum of one individual who had experienced mild oral allergy syndrome after a Double-Blind Placebo-Controlled Food Challenge (DBPCFC) with phytosterols. It was concluded that no IgE-binding proteins were present in VOD and phytosterol samples at or above 10 mg/kg.

2.3.3 Clinical studies

A European clinical study of 32 soybean allergic individuals was conducted in Berlin (Germany) and Zurich (Switzerland). The patients (17 males, age range 6 months to 65 years) with confirmed clinical food allergy to soybean protein participated in a clinical study to evaluate sensitisation and clinical reaction to phytosterols and tocopherols (Ballmer-Weber et al, 2007).

All participants were skin prick tested to commercial soybean extract, soybean isolate/soybean milk and the phytosterols blend. Twenty-two subjects had a positive skin reaction (minimum >3 mm wheal) to commercial soybean extract. None of the participants had a positive SPT to the phytosterols or tocopherols. All subjects participated in an open food challenge at the highest single exposure (3 g phytosterols or 500 mg natural mixed tocopherols).

Phytosterol challenge

The challenge material was a blend of equal amounts of phytosterols from a randomly selected batch of phytosterols from each of the three major manufacturers of phytosterols. The challenge was provided as a single dose mixed into commercially available rice and carob porridge or mashed potatoes.

Twenty-nine of the 32 subjects consumed the phytosterol blend under open food challenge. Three participants reported minor symptoms. These three participants subsequently underwent a double-blind placebo-controlled food challenge (DBPCFC). Two participants had no response, and one participant confirmed the symptoms (oral allergy syndrome, nasal itching) upon DBPCFC. *In vitro* diagnostic analysis of this patient's serum demonstrated no IgE binding to samples of phytosterols. The investigators attribute these symptoms to a non-IgE type reaction, although further evidence for this assumption is lacking. In a separate study, the same individual had reacted to a cumulative dose of 4 g soybean protein with oral allergy symptoms. On exposure to higher doses, the participant's oral symptoms persisted without developing a systemic reaction (Ballmer-Weber et al, 2007).

Tocopherol challenge

Thirty-one out of thirty-two subjects consumed tocopherols blend in an open food challenge without allergic reaction. One patient with a positive skin prick test to soybean protein and soybean protein-specific serum IgE reported mild oral allergy symptoms and nausea, which were confirmed in a DBPCFC. Immunoblotting analysis of this patient's serum failed to show IgE binding to samples of mixed tocopherols. As reported in the phytosterol study above, this patient had reacted with oral allergy symptoms to a cumulative dose of 4 g soybean protein without developing systemic symptoms after being challenged with higher doses (Ballmer-Weber et al, 2007).

Although the clinical studies were considered to be insufficient to predict the likelihood that the phytosterols and tocopherols from soybean oil may trigger an allergic reaction in susceptible individuals, the EFSA panel was satisfied that the available information regarding the residual protein was sufficient to indicate that it was unlikely that vegetable oils-derived phytosterols and tocopherols from soybean sources would trigger a severe allergic reaction in susceptible individuals.

2.4 Summary

Deodorisation is the last step of soybean/vegetable oil refining. The deodoriser distillate (VOD) is rich in phytosterols and tocopherols which can be extracted for use in food. The bulk of soybean protein is removed in the earlier steps of the refining process. Since VOD is a by-product of the final step of N/RBD soybean oil production, it follows that VOD would be expected to contain extremely low levels of protein. This was confirmed by analytical testing of VOD and the phytosterols and tocopherols. Although the clinical data on phytosterols and tocopherols were limited, a conclusion can be drawn based on lack of allergenicity in N/RBD soybean oil.

2.5 Conclusion

Tocopherols and phytosterols derived from deodoriser distillate in the manufacturing of N/RBD soybean oil present negligible risk to soybean protein allergic consumers.

3 Glucose syrup derived from wheat starch

Glucose syrup being over 90% glucose is a versatile food sweetener made from the hydrolysis of starch. In Australia, wheat starch is commonly used for the commercial manufacture of glucose syrup. Glucose syrup is used as a non-crystallising bulk sweetener to provide smooth texture in some food products including confectionery, centre-filled chocolates and some dairy desserts.

3.1 Immune-mediated reactions to wheat proteins

Wheat protein includes gluten as a major component therefore, wheat and its products, is one of the gluten-containing cereals subject to mandatory declaration requirements[1] in the Code. The requirements address the two types of immune-mediated reactions caused by dietary exposure to wheat protein, i.e. IgE-mediated wheat allergy and non-IgE-mediated disorders including coeliac disease (CD). Clinical manifestations and underlying mechanisms of wheat allergy and CD are well documented (Keet et al, 2009; Kaukinen et al, 2010).

IgE-mediated allergy to wheat protein is most commonly observed in infants and usually resolves within the first few years of life (ASCIA, 2010; Inomata, 2009; Keet et al, 2009). Wheat allergy symptoms occur within minutes to a few hours and include urticaria and worsening eczema, angioedema, bronchial obstruction, nausea, abdominal pain and in severe cases, anaphylaxis. Another form of IgE-mediated wheat allergy is wheat-dependent exercise-induced anaphylaxis (WDEIA). This type of wheat allergy is triggered when wheat consumption is followed by physical exercise. Clinical symptoms range from local or generalised urticaria to anaphylaxis (Inomata 2009). Reports of cross reactivity to other gluten containing cereals in wheat allergic patients are infrequent in the medical literature.

Wheat also triggers CD, an immune-mediated enteropathy, in susceptible individuals. In relation to CD, gluten from wheat and other gluten-containing cereals is the protein responsible for triggering a non-IgE immune-mediated response in the gastrointestinal mucosa. The resulting inflammation and tissue damage cause abdominal symptoms and lead to poor nutrient absorption from food (Sapone et al, 2012).

[1] Standard 1.2.3 – Information requirements – warning statements, advisory statements and declarations http://www.foodstandards.gov.au/code/Pages/Food-Standards-Code-from-1-March-2016.aspx

Gluten is involved in wheat allergy and in CD. The wheat gluten protein, ω-gliadin, is recognised as a major allergen in WDEIA; and also in children with immediate food allergy and anaphylaxis to wheat (Palosuo et al, 2001; Matsuo et al, 2004, Sapone et al, 2012). In addition, it has been shown that IgE antibodies from a majority (>80%) of wheat allergic children with atopic dermatitis bind to non-gluten proteins (albumin and globulins) (Battais et al, 2005; Rodríguez del Río et al, 2014). In adults with anaphylaxis or urticaria the major allergen was shown to be ω-gliadin (Battais et al, 2005).

3.2 Gluten detection

Studies of the USDA World Wheat Collection indicated an approximate 3-fold variation in total protein content (from 7–22%), with only about one-third of this variability being under genetic control (Vogel et al, 1978). Wheat proteins are often classified on the basis of their aqueous solubility following a sequential Osborne extraction procedure; a water/salt-soluble fraction which includes albumins and globulins; and a water/salt insoluble fraction which includes gliadins and glutenins. Albumins and globulins of wheat that are mainly enzymes and proteins involved in cell function represent 20% to 25% of total grain protein (Merlino et al, 2009). Wheat gluten is a complex of monomeric α/β-, γ-, and ω-gliadins (classified according to their electrophoretic mobility) and high and low molecular weight glutenins that form polymers in vivo. Wheat gluten, with a gliadin:glutenin ratio of about 1:1, constitutes about 75–85% of the total grain protein.

The monoclonal antibody R5 used in several commercial available sandwich ELISA test kits recognises the sequence Glutamine-Glutamine-Proline-Phenylalanine-Proline (QQPFP) and 5 other related (QQQFP, LQPFP, and QLPFP) epitopes. These five amino acid sequences occur repeatedly in α/β-, γ-, and ω-gliadin fractions, and also occur in barley (hordeins) and rye (secalins) (Osman et al, 2001; Valdés et al, 2003). The linear or sequential amino acid sequence of the epitope means that antibody binding is unlikely to be affected by heat that frequently interferes with conformation-dependent epitopes which require native secondary and tertiary protein structures. Peptide sequences rich in proline are typically either resistant or only slowly hydrolysed by proteases. These two features allow for intact gluten or gluten derived peptide quantification in heat-treated and partially hydrolysed foods. However, since mAb R5 was raised against gliadin this ELISA method is specific for gliadin or gliadin peptides with at least two epitopes.

Consequently, it will be unable to detect small gliadin peptide fragments or any of the glutenins in gluten. This latter problem is partially overcome by assuming that the gliadin:glutenin ratio is always 1:1 and doubling the gliadin concentration to estimate the gluten concentration. A difficulty with small peptide fragments has now been overcome with the development of a competitive ELISA method with mAb R5 (Haas-Lauterbach et al, 2012). The mAb R5 sandwich ELISA method has been validated in a collaborative trial conducted by the Prolamin Working Group (Mendez et al, 2005). It was also declared a type I method (highest level, defining method) at the 27th session of the Codex Committee on Methods of Analysis and Sampling (CCMAS, 2006). A second developmental phase of anti-gluten reagents involving antibodies raised against biologically relevant peptides such as the G12 monoclonal antibody (Shan et al, 2002) directed against the toxic 33mer of α-gliadin has commenced.

The Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (118-1979; revised 2008) specifies criteria that methods for gluten quantitation need to fulfil. An important criterion is that the method must either an immunochemical method or a non-immunochemical method but it must have equal specificity and sensitivity, and that (2) the limit of detection is 10 mg/kg or below. Any immunochemical method that meets these requirements would comply with the Codex Standard.

3.3 Glucose syrup from wheat

Wheat flour can be processed to separate out protein from starch. However, this process is incomplete and will always result in residual wheat protein, including gluten, being present in the starch fraction. Starch granules from wheat contain intrinsic proteins embedded in the starch matrix; mainly enzymes involved in starch synthesis (Rahman et al., 1995). Many different proteins have been detected in wheat starch, including gluten (glutenins and gliadins) and non-gluten proteins (albumins and globulins) (Kasarda et al., 2008). Wheat protein reported in four commercial wheat starch preparations, of which only two were food grade, ranged between 0.11% and 0.198% (1100–1980 mg/kg by the Kjeldahl nitrogen method). The gluten concentration, as determined by mAb R5 ELISA, ranged between 5 mg/kg–363 mg/kg with the two highest values (75 and 363 mg/kg) being associated with the food grade starches. The authors reported that the starch which contained the lowest gluten concentration (5 mg/kg) was intended to be used in making carbonless paper and had been fractionated by an undisclosed proprietary method (Kasarda et al., 2008).

EFSA reported that in Europe, starches from wheat prior to 2005 were found to contain up to 279.3 mg/kg of gluten but that subsequently no detected gluten at levels higher than 25.3 mg/kg (LOD 3.1 mg/kg) in glucose syrups and dextrose samples (starch hydrolysate) for 2005 and 2006 (3–43 samples per year) were found (EFSA 2007d). One glucose syrup sample had a gluten content of 39.6 mg/kg but this was assumed to be through accidental contamination. The gluten detection method used was the sandwich mAb R5 enzyme-linked immunosorbent assay (ELISA) which has with a limit of detection (LOD) of 3 mg/kg (ie. 2x1.5 mg/kg gliadin).

In another survey EFSA reported that of twenty-one European samples (14 wheat glucose syrups, 3 crystalline dextrose, 4 glucose syrups) which had undergone the comprehensive purification scheme as outlined in Appendix 2, the total protein concentration measured by high-pressure liquid chromatography ranged from only 0.3–1.4 mg/kg (EFSA 2007b). Subsequent to EFSA's evaluation, analytical data on wheat starch-based syrups produced in Europe using sandwich and competitive ELISA kits (R5 and another two mAbs) were published (Dostalek et al., 2009). The results showed residual gluten content to be <3 mg/kg in all syrup samples tested (n=9).

3.4 Analytical data on glucose syrup manufactured in Australia

Cressey et al (2010) published analytical data on gluten and total wheat protein levels in glucose syrup samples submitted by an Australian manufacturer. Six samples from different production runs of wheat glucose syrup, collected during 2009, were analysed using a gliadin-specific (R5 mAb) ELISA method and the Bradford protein assay.

Gluten content was determined using Neogen Biokits (Neogen Corporation, Auchincruive, Scotland). The method is a sandwich R5 mAb ELISA, based on the presence of two separate epitopes on the ω-gliadins. Applicability of the ELISA method to glucose syrup substrates was confirmed by adding the gluten control material provided with the kit to glucose syrup samples. The LOD was 3 mg/kg, and results were within the acceptable range for the gluten control.

For validation of the Bradford micro protein method (Coomassie brilliant blue G250 binding), all samples were spiked with bovine serum albumin (BSA) at a spike concentration of 6 mg/L. Spike recoveries were in the range 75.4–118.1% (mean = 92.8%). All samples were analysed in quadruplicate, all within batch coefficients of variation less than 3%. The method's LOD was in the range 0.6–0.7 mg/L, based on the standard deviation of blank determinations.

Due to the viscosity of some samples, analysis was carried out at a ten-fold dilution, meaning that the detection limit equates to 6-7 mg/L in the product as received. Gluten levels were below the LOD, i.e. <3 mg/kg, in three out of six glucose syrup samples; and were 8, 15 and 22 mg/kg in the remaining three samples. Total protein levels detected ranged from 8–16 mg/kg.

Following the instalment of a new microfiltration system at the commercial production plant, new analytical data were available on samples of glucose syrup produced over a 30-day period during June–July 2012. Sixty samples were collected in duplicate for protein content and gluten measurement by the Bradford method and gliadin sandwich R5 mAb ELISA method respectively.

In the Bradford method, protein content of the samples was measured relative to standard protein solutions at 0, 2.7, 4.1, 6.9 and 9.6 mg/L, and the results were reported in bandwidths between standards. All samples were <6.9 mg/L and 51/60 samples were <2.7 mg/L.

Results of ELISA testing of the same sixty glucose samples were also provided. Fifty seven out of sixty samples (95%) were below LOD (<3 mg/kg), and one sample was just above detection limit and the remaining two samples were >20 mg/kg. The higher gluten concentration in two samples was attributed to sample cross-contamination with gluten or wheat flour in the laboratory. While this cannot be confirmed, it is a plausible explanation based on lack of correlation with results for the same samples using the Bradford method.

In the Australian glucose syrup production plant, in addition to routine protein testing, the syrup batches are tested for viscosity, colour, etc. As part of quality control, all testing results are evaluated and batches are withheld, or blended, to ensure the final product meets the specifications.

In 2012 the Australian glucose syrup manufacturer advised that the glucose manufacturing system currently in place wassdesigned to provide <5 mg gluten/kg in 90% of samples and <10 mg gluten/kg in 100% of samples. This is consistent with published analytical data on glucose syrup from Europe which showed that the residual gluten content were less than the limit of quantification (<3 mg/kg) in nine glucose syrup samples tested, and in four food products (various flavoured confectionery bars) containing glucose syrup (Dostálek et al., 2009). According to the Australian glucose syrup manufacturer, the processing system recently put in place has been supplied and tested by its European supplier to achieve the same performance characteristics as the commercial equipment in Europe. In addition, the Australian manufacturer has developed a Code of Practice to ensure that all necessary control measures are implemented in the processing environment to avoid cross-contamination with wheat flour and gluten, and to ensure the product, wheat-based glucose syrup, consistently meets the minimal gluten content of wheat-derived glucose syrup that is available in Europe.

In 2016 the Australian glucose syrup manufacturer advised that the ‘RIDAQUIK’ testing kit had shown that, over a course of ten months, only 90% of samples had <10 mg gluten/kg and 100% were <20 mg gluten/kg.

3.5 Clinical data

3.5.1 Coeliac disease

CD is an immune-mediated gastrointestinal disease triggered by the ingestion of gluten in genetically susceptible persons. The abnormal immune response is characterised by an inflammatory reaction in the small intestine leading to flattening of the mucosa. As a result, affected individuals absorb food and nutrients poorly. This can result in bowel symptoms and deficiencies of vitamins, minerals and other nutrients.

CD has several autoimmune features, including the production of highly disease-specific IgA and IgG autoantibodies when patients are on a gluten containing diet (Kaukinen et al., 2010).

The gliadin fraction of cereal protein has been demonstrated to trigger CD with symptoms including mucosal flattening. Although all gliadins are toxic to coeliac patients, the most severe effects are caused by α-gliadins (Hischenhuber et al., 2006). CD is now recognised as a significant health issue worldwide. Currently, the only effective treatment for CD is a life-long strict avoidance of dietary gluten (Kaukinen et al., 2010).

In their review article Hischenhuber et al (2006) noted that available gluten challenge studies in patients with CD indicated that daily intakes of <10 mg have no effects on mucosal histology, whereas adverse alterations are caused by a daily intake of 500 mg with observable changes at 100 mg. In another review of the published literature that included thirteen studies (three randomised controlled, one cohort, two crossover, and seven cross-sectional) Akobeng & Thomas (2008) reached a similar conclusion. They found that while some patients tolerated an average of 34–36 mg of gluten per day, other patients who consumed about 10 mg of gluten per day developed mucosal abnormalities. They found that the consumption of 'gluten-free' products with different degrees of gluten contamination was also inconsistent.

Since mucosal deterioration is the most sensitive measurement of a reaction to gluten in CD patients, a gluten threshold dietary exposure estimate based on that endpoint in a prospective, double-blind, and placebo-controlled gluten challenge study would be most informative. For the study, 49 adults with biopsy-proven CD and who had adhered to a gluten free diet for more than 2 years were challenged for 3 mo with a placebo or with either 10 or 50 mg gluten/day. Most exposed to 50 mg gluten/day showed a worsening of morphometric variables based on small intestinal biopsy testing. Dietary exposure to 50 mg/day was therefore taken as the LOAEL. Since there was an absence of any statistical differences in morphometric variables in the group exposed to gluten at 10 mg/day, this level of dietary exposure was considered to be the NOAEL (Catassi et al., 2007).

A clinical study on wheat-based starch hydrolysates, including glucose syrup, was published by Kaukinen et al (2008). The randomised DBPCFC prospective follow up study, involved ninety coeliac patients in remission. The patients were advised to use one sachet daily or seven weekly during the study period. Each glucose sachet contained 27.7 g glucose syrup (DE 41). The amount of glucose syrup used in the study was chosen to cover the high end of the realistic mean exposure in European countries. The total protein and gluten content of one sachet measured by high-performance liquid chromatography was 4.3 mg/kg and 1.7 mg/kg respectively. Gluten levels were under detection limits by sandwich and competitive R5 ELISAs (LOD <3.1 mg/kg and <2.4 mg/kg, respectively). Laboratory analysis, gastroscopy and duodenal biopsy and clinical evaluation, including gastrointestinal symptoms, were carried out. The primary outcome measurements were changes in small-bowel mucosal morphology or inflammation. Safety assessments included monitoring of adverse events, vital signs, physical examinations, laboratory evaluations (haematology, malabsorption parameters and coeliac disease serology) and a symptom questionnaire. The results show that daily ingestion of glucose syrup containing no more than 0.085 mg gluten had no deleterious effect on small-bowel mucosal villous architecture or inflammation in coeliac disease patients when compared to the placebo group.

In 2013 Gibert et al reported that the gluten content in 193/205 (94%) of gluten-free labelled products in 6 key food groups (bread, pasta, pastry, biscuits, pizza, and breakfast cereals) in Italy, Spain, Germany, and Norway was less than the quantification limit of 5 mg/kg in R5 sandwich ELISA. Only 3% of the samples had levels in excess of 10 mg/kg.

Based on the evidence that very few products had detectable gluten they concluded that the risk of mucosal damage resulting from consumption of gluten free labelled products ranged between 0.01% in Germany and 0.15% in Italy and it remained very low (1%), even in if they included the worst-case scenario involving the highest measured gluten level of 27.8 mg/kg.

3.5.2 Wheat allergy

In 2004, the European Academy of Allergology and Clinical Immunology published guidelines on oral food challenges in patients with immediate reactions to food (Bindslev-Jensen et al., 2004). In an extensive study with 60 patients, Battais et al (2005) showed that different antigenic profiles could be detected in food allergy to wheat, according to age and symptoms. Alpha, beta and gamma-gliadins and albumins/globulins appeared to be more important allergens for children with atopic dermatitis with or without asthma, while ω-gliadins were major allergens for adults with food dependent, exercise-induced anaphylaxis and/or anaphylaxis (100%) or urticaria (55%). LMW glutenin subunits also featured in anaphylaxis cases in adults. Only 23% of patients with AD and 8% of those with atopic dermatitis and asthma reacted to ω-gliadins.

A study on the safety of barley starch syrup in patients with allergy to cereals was published by Nermes et al (2009). Fifteen patients with allergy to at least one of the cereals wheat, rye, barley or oats, confirmed by DBPCFC, were enrolled in DBPCFC with barley starch syrup. The age range of the study subjects allergic to wheat, barley, rye or oats was 0.9 -13.8 years (median 2.1 years). The daily challenge dose of 30 g syrup was estimated to correspond to maximal daily consumption in the European diet. The patients were challenged (DBPCFC) with barley starch hydrolysate, none showed objectively detectable symptoms. The authors noted that the only patient who was highly sensitised to cereals on skin test and previously had anaphylaxis when exposed to cereal, developed no symptoms on the glucose syrup challenge. Analytical testing suggests the amount of syrup used in the challenge contained a maximum of 0.9 mg N calculated to equal 5.6 mg of protein. However, no conclusions could be drawn due to study limitations, including the small number of patients.

3.6 Dose response in wheat allergic patients

There was limited information in the literature on the prevalence of wheat allergy and the dose response to wheat protein among wheat allergic individuals. Hischenhuber et al (2006) reviewed the available evidence on the eliciting dose for wheat in wheat allergic and CD patients. The review noted regional variability in the frequency of wheat allergy, being more frequent in northern than in southern Europe. In relation to available data on food challenges in wheat allergic individuals, the review noted that most studies suffer from design limitations including small number of patients and the use of wheat flour with undetermined protein or gluten content. The review also noted the lack of challenge data on patients with a convincing history of anaphylaxis to isolated wheat ingestion.

In 2007 EFSA reported that there were two published studies in which wheat flour was used in diagnostic testing of wheat allergic patients (EFSA, 2007d; Moneret-Vautrin et al., 2003; Scibilia et al., 2006). In the study by Moneret-Vautrin et al, up to 83% of allergic children (n=38) reacted to <2 g, while only 18% of wheat allergic adults (n=41) responded at this level. The results suggested differences between wheat-allergic adults and children in their reaction to controlled oral challenge with wheat protein. In 20% of children, more than 2 g of wheat protein was needed to provoke a reaction; and 6% (2 of 32) of the children reacted to <10 mg of protein. On the other hand, the entire adult allergic population in the study required at least 1 g of wheat protein and half required more than 6 g to induce an allergic response. EFSA (2007d) reported that it was unclear how the figures reported for wheat proteins were calculated from the amount of wheat flour used in the clinical challenges despite the authors considering the protein content of wheat flour to be 12% by weight.

Scibilia et al (2006) reported that 1 of 13 responders reacted to the lowest dose of wheat flour tested (100 mg of a mix of bread and durum flour, approximately 10 mg protein) in DBPCFCs. The challenge dose was in the range of 0.1−25 g wheat flour. The results indicate that 27% of the wheat allergic subjects reacted to ≤1.6 g wheat flour. However, EFSA considered that the Moneret-Vautrin et al and Scibilia et al studies to only be of indirect relevance to a determination of an eliciting dose, since the protein content of the wheat flour was not directly quantified.

Since the EFSA (2007d) review was finalised four additional studies have reported food challenges in wheat allergic patients. Pastorello et al (2007) reported an open challenge in three children aged ≤ 3 years. The children reacted at doses between 0.75g, 1 g and 2 g cooked wheat pasta, but the protein content was not indicated. In the study reported by Ito et al (2008), thirty five wheat-sensitised Japanese children were openly challenged with increasing amounts from 0.1 g to 68 g udon noodles (cumulative wheat protein equivalent of 2.6 mg−1771 mg, based on 2.6% wheat protein reported by the investigators). Of the twenty one children who had previously been challenge positive, eighteen children (86%) reacted to cumulative doses of wheat protein ≥ 50 mg. Two children reacted to 29 mg and one reacted to a wheat protein equivalent dose of 2.6 mg indicating that the study did not establish a NOAEL (see Table 4).

In 2012, Rolinck-Werninghaus et al investigated the symptom-eliciting allergen doses, and specific IgE levels and severity of symptoms during challenges with milk, egg, wheat, or soybean in children. The study performed a total of 1671 challenges on 869 food allergic children (median age 1.2 years) with milk egg, wheat (n=88) or soybean. The oral challenge protocol comprised seven doses starting with 3–5 mg of protein with semi-log increases every 30 minutes. Symptoms were graded I to V based on increasing severity. The study results show that 3% (+ve=3) of wheat allergic individuals, compared with 2% for soy (+ve=1), 10% (+ve=30) for milk and 9% (+ve=28) for egg allergic individuals, responded to the first challenge dose of 3 mg wheat gluten (ie. no NOAEL). For all allergens, objective symptoms occurred at all dose steps. As most wheat allergic children have IgE activity against wheat water/salt-soluble albumins and globulins in addition to gluten it is unclear whether the use of gluten rather than wheat flour (protein) would influence the outcome of this study.

In contrast to the approach by EFSA, Taylor et al (2014) and Allen et al (2014) assumed that the natural variability in wheat flour is essentially zero and applied a single conversion factor of 10% to estimate 'protein equivalent' doses in studies for which the actual wheat protein levels had not been quantified. In their review they summarised protein elicitation doses in both published and unpublished data and applied a parametric Survival Analysis[2] (involving log-normal, log-logistic, Weibull models) to estimate the 95% confidence interval of the eliciting dose in 95% of the allergic population (ED_{05}) for a range of different allergens including wheat protein. In their calculated estimate of the ED_{05} for wheat protein they included the published studies by Sciblia et al (2006), Ito et al (2008) and Pastorello et al (2007) along with some unpublished clinical data. Using all three dose distribution models they estimated the Reference Dose for wheat (protein) to be 1 mg based on the data from a total of 40 individuals (12 adults, 28 children) in the various DBPCFC trials. For discrete dosing they reported the ED_{05} to be 1.5, 1.6 & 0.44 mg for the log-logistic, log-normal and Weibull distributions respectively whereas for cumulative dosing it was 1.3, 1.4 and 0.41 mg.

[2] In DBPCFC trials the exact dose at which an objective symptom is observed is usually not known. Typically, the oral dose causing an adverse event will fall somewhere between the dose causing no effect and the next higher dose which causes a response; such data are said to be censored. These data are analysed using Survival Analysis. Left-censored data occurs when an allergic response occurs at the first tested dose. Right-censored data involve those who did not respond at the highest tested dose.

It is unclear whether the Rolinck-Werninghaus et al (2012) study which tested 88 wheat allergic children was excluded from Survival Analysis on the grounds that the challenge material was reported to be “wheat/gluten powder” and its relationship to wheat flour protein not specified or whether it was simply overlooked. Reference to the Rolinck-Werninghaus et al (2012) study is made by Allen et al (2014) but only in the context of generally milder reactions being observed at low doses for most allergens. However, Ito et al (2008) reported a severe reaction in a child at the lowest wheat protein dose (2.6 mg) tested (see Table 4).

Allen et al (2014) noted that the allergic individuals who underwent controlled low-dose oral challenges in trials tended to need higher elicitation doses than from community exposures because the clinical challenges were controlled with respect to factors that may reduce reactivity, such as overall health status, medications, and exercise. Similarly, those enrolled in these trials were not necessarily representative of the entire group of children with a specific food allergy but were generally a reactive subgroup, but with a lower proportion of highly reactive individuals. Since it is also not possible to estimate the number of highly sensitive who might be advised not to participate in DBPCFC trials these threshold estimates have uncertainty attached to them. Considering all such confounders, including the effect of using different foods in the trials, (eg chocolate bar, cooked/uncooked flour) might suggest the application of an additional uncertainty factor beyond the estimated reference dose which is likely to protective. However, Allen et al suggested that those sensitive to very low levels of protein should avoid exposure to wheat protein during times of ill health or vigorous exercise and be encouraged to access appropriate treatment rather than adjust the reference dose.

Table 4: Threshold data from published double-blind, placebo-controlled wheat flour challenge studies

Patients* (no. and age range)	Cumulative Wheat Flour Dose (mg protein equiv.)	Interval Between Challenge Doses (min)	Lowest Cumulative Eliciting Dose (mg)	Highest Cumulative Eliciting Dose (mg)	Left Censored Data Points[#]	Reference
12 adults (28-60 y) + 1 child (16 y)	10-2410	20	10	2410	1	Scibilia et al (2006)
21 children (1-8.7 y)	2.6-1771	20	2.6	1771	1	Ito et al (2008)
88 children (0.1-16 y)	3-4032^	30	3	4032	3	Rolinck-Werninghaus et al (2012)
6 children (5-11 y)	0.11-12521	20	821	12521	0	Rodríguez del Río et al (2014)
Overall						
12 adults & 116 children	0.11-12521	-	2.6	12521	5	

* With objective symptoms
^ Gluten dose only
[#] Number responding to lowest tested dose

Six children between 5-11 years old with atopic dermatitis underwent a DBPCFC with wheat protein, and none reacted to the placebo (Rodríguez del Río et al., 2014). Immediate objective symptoms were recorded at a dose of 0.82 g of wheat protein. No patients showed worsening of their atopic dermatitis, and 4 out of 6 (66.7%) experienced anaphylaxis. The DBPCFC involved administering 9 separate doses at 20-minute intervals.

For the active challenge, the first 5 doses were administered as durum wheat semolina porridge (10.6% protein), and the remaining doses were administered as boiled durum wheat semolina pasta (13.0% protein) until a cumulative dose of 100 g of dry wheat semolina (12.52 g of wheat protein) was reached. The doses of porridge were 0.001, 0.01, 0.1, 0.5, and 1 g; the doses of pasta were 5, 10, 20, and 60 g. For the placebo, a gluten-free porridge made of rice and maize was used for the first 5 doses, and boiled rice pasta for the last 4 doses. Serum IgE activity to a panel of wheat proteins (α-amylase inhibitors, wheat lipid transfer protein, peroxidase, gliadins, glutenins, and 5-ω-gliadin) was assessed using ELISA. None of the patients showed IgE binding activity to 5-ω-gliadin but α-amylase inhibitors were recognised in all patients. 5-ω-Gliadin is considered to be an effective marker of not only WDEIA, but also for wheat allergy (Palosuo et al., 2001; Matsuo et al., 2004, Sapone et al., 2012).

To determine the clinical threshold to wheat a DBPCFC was performed on 21 challenge positive (15 children, 6 adults) patients. All children had atopic dermatitis, and most (13/15) outgrew their wheat allergy. Most children (13/15) had other food allergies. Material for DBPCFC was produced by masking 25/50 gram wheat or placebo in a chocolate bar weighing in total 155–175 gram. Increasing doses of 0.5, 1, 4, 8, 16, 32 and 95–115 gram bar were given at 30 minute intervals. Since the protein content of wheat flour was not reported it was not possible to calculate actual wheat protein used in this study. The clinical threshold for elicitation of objective signs varied between 25 mg and 50 g but there was no correlation between age of the patient and threshold (Christensen et al., 2014).

Food Allergy and Intolerance Scientific Advisory Group (FAISAG) Comments

The FAISAG indicated that currently only limited information has emerged regarding the relationship between food challenge data and clinical reactions in daily life. Thus, the allergic individuals undergoing low-dose challenges in DBPCFC are not necessarily representative of the entire group with a specific food allergy but are generally a reactive subgroup, but with a lower proportion of highly reactive individuals. Data are lacking, however, to quantify the relationship between this challenged population in trials and the overall allergic population. Data are scarce about the existence of subpopulations with different thresholds. Similarly another potential confounder to derive a threshold eliciting dose is the use of different forms of food in clinical DBPCFCs for wheat (eg chocolate bar, cooked/uncooked flour).

In low dose food challenge tests, patients are closely monitored and once a reaction has clearly taken place (usually if objective symptoms occur) the challenge is stopped and the patient treated (if necessary). Consequently the reaction during challenge will usually not be as severe as reactions can be in daily life. There is also no clear relationship between minimum eliciting dose and severity of reaction (Ito et al, 2008; Rolinck-Werninghaus et al, 2012).

FSANZ sought additional comments from FAISAG on the best estimate for a NOAEL of wheat protein in allergic children. In earlier discussions the FAISAG indicated that the Rolinck-Werninghaus et al (2012) study was a suitable study on which to base an estimate of NOAEL. However, the group noted that while the Rolinck-Werninghaus study involved 88 wheat allergic children, it did not establish a NOAEL at the lowest tested dose of 3 mg, hence a level of 1 mg was considered by FAISAG to be a reasonable estimate of the NOAEL. Recent reviews by Taylor et al (2014) and Allen et al (2014) reported that the 95% lower confidence interval for the eliciting dose (ED_{05}) of wheat protein to be 1 mg. The three chosen statistical dose distribution models (log-normal, log-logistic, Weibull) yielded values ranging between 0.44 and 1.6 mg for discrete dosing and 0.41–1.4 mg for cumulative dosing. However, this estimate did not include either the Rolinck-Werninghaus et al (2012) or Rodríguez del Río et al (2014) studies but instead three older published studies and some unpublished data involving a cumulative total of 40 allergic individuals.

In consideration of the Australian consumption data for high consumers (97.5 percentile) of products containing glucose syrup outlined below, the FAISAG concluded that the majority of wheat allergic patients are likely to be protected if glucose syrups are prepared according to the scheme shown in Appendix 2. In Europe such syrups have been shown to contain a total protein concentration ranging between 0.3–1.4 mg/kg (EFSA 2007b). ELISA kits have shown that the residual gluten content in these glucose syrups is usually lower than the limit of detection (<3 mg/kg).

3.7 Dietary exposure to protein from glucose syrup

The main use of glucose syrup in Australia and New Zealand is in confectionery, chocolate filling and icecream. FSANZ generated consumption data for these food types for Australia and New Zealand populations. Since the last assessment consumption data for the New Zealand population from the 2002 New Zealand Childrens' Nutrition Survey (NZ CNS) and the 2008 New Zealand Adults' Nutrition Survey (NZ ANS) have been incorporated in the assessment, as has the recently released data from the 2011-12 National Nutrition and Physical Activity Survey (NNPAS) component of the 2011-13 Australian Health Survey. The consumption data was derived for consumers of the food of interest only, therefore consumption amounts for confectionery, chocolate or icecreams should not be added since consumer groups will usually be non-identical (i.e. not everyone who consumed confectionery also consumed chocolate or icecream). Since consumption data per meal is not available, the daily consumption data at the 97.5th percentile was taken to represent acute exposure (single meal) for a high consumer, which is likely to be an overestimate.

3.7.1 Australia

The consumer mean, 90th percentile, 95th percentile and 97.5th percentile food consumption amounts for Day 1 from the 2011-12 NNPAS for the selected food types are presented in Table 5 for the 2-4 year and 5-14 years and 15 years and over age groups, to enable comparison with New Zealand data set, which does not cover 2-4 year olds..

Table 5: Consumption for individuals aged 2-4, 5-14, 15 years & above, 2011-12 NNPAS

Food	Number of consumers	% consumers to respondents	Consumption of food for consumers[1] only (g/day)[2, 3]			
			Mean	90th percentile	95th percentile	97.5th percentile
Age: 2 – 4 years						
confectionery[4]	80	16	13	25	29	**52**
chocolate[5]	84	17	28	59	80	**100**
ice cream[6]	77	16	62	100	132	**165**
Age: 5 – 14 years						
confectionery	273	17	25	55	83	**100**
chocolate	318	20	40	70	110	**183**
Ice cream	368	23	113	198	267	**276**
Age: 15 years and above						
confectionery	867	9	30	65	95	**125**
chocolate	1619	16	43	90	124	**190**
Ice cream	1290	13	113	198	273	**348**

[1] Consumers' are those respondents from the survey who ate the food(s) of interest.
[2] Derived using Day 1 food consumption amounts and using the appropriate weighing factors (12,153 survey respondents)
[3] All food consumption amounts have been rounded to 2 significant figures.
[4] Confectionary food group includes boiled sweets, jelly-type lollies, fudge, marshmallows, liquorice, toffee, Turkish delight, but excludes confectionary used in mixed foods.
[5] Chocolate food group includes filled chocolates and chocolate bars but excludes chocolate used as an ingredient in bakery products, cocoa, chocolate beverages.
[6] Ice cream food group includes ice cream from container, ice cream on stick or bar, soft serve and sundae style but excludes ice cream used in mixed foods (e.g. milkshakes, thickshakes, smoothies)

3.7.2 New Zealand

Consumption data for confectionery, chocolate and icrecream were avalable from Day 1 records for children aged 5-14 years from the 2002 NZ CNS and for the population aged 15 years and over from the 2008 NZ ANS, presented in Table 6.

Table 6: Consumption for individuals aged 5-14 years, 2002 NZ CNS and 15 years & above, 2008 NZ ANS

Food	Number of consumers	% consumers to respondents	Consumption of food for consumers[1] only (g/day)[2, 3]			
			Mean	90th percentile	95th percentile	97.5th percentile
Age: 5 – 14 years						
confectionery[4]	637	19	44	116	145	**232**
chocolate[5]	590	18	35	69	95	**100**
ice cream[6]	703	21	129	215	273	**348**
Age 15 years and above						
confectionery	317	7	45	120	174	**240**
chocolate	722	15	48	105	150	**180**
Ice cream	559	12	104	213	286	**305**

[1] Consumers' are those respondents from the survey who ate the food(s) of interest.
[2] Derived using Day 1 food consumption amounts and using the weighing factors (3275 surveys respondents in the 2002 NZ CNS, 4721 respondents in the 2008 NZ ANS)
[3] All food consumption amounts have been rounded to 2 significant figures.
[4] Confectionary food group includes boiled sweets, jelly-type lollies, fudge, marshmallows, liquorice, toffee, Turkish delight, but excludes confectionary used in mixed foods.
[5] Chocolate food group includes filled chocolates and chocolate bar but excludes chocolate used as an ingredient in bakery products, cocoa, chocolate beverages.
[6] Ice cream food group includes ice cream from container, ice cream on stick or bar, soft serve and sundae style but excludes ice cream used in mixed foods (e.g. milkshakes, thickshakes, smoothies)

3.7.3 Summary of food consumption of foods containing glucose syrup

Icecream is consumed in larger quantities than either chocolate or confectionery in both Australia and New Zealand with little difference between children and adults: the estimated 97.5th percentile amount for consumers of icecream was 165 g/day for Australian children aged 2–4 years, 276 g/day for Australian children aged 5–14 years and 348 g/day for New Zealand children aged 5–14 years. For populations aged 15 years and over the estimated 97.5th percentile amount for consumers of icecream was 348 g/day in Australia and 305 g/day in New Zealand.

The amount of chocolate (bars of chocolate and filled chocolates) estimated to be consumed by high consumers (97.5th percentile) was 100 g/day for Australian children aged 2–4 years, 183 g/day for Australian children aged 5–14 years and 100 g/day for New Zealand children aged 5–14 years. For populations aged 15 years and over the estimated 97.5th percentile amount for consumers of chocolate was 190 g/day in Australia and 180 g/day in New Zealand.

For confectionery, the patterns of consumption were different for the Australian and New Zealand populations, with approximately double the amount of confectionery being consumed in New Zealand compared to Australia for children and adults, however the proportion of consumers of these products was similar. The amount of confectionery estimated to be consumed by high consumers (97.5th percentile) was 52 g/day for Australian children aged 2–4 years, 100 g/day for Australian children aged 5–14 years and 232 g/day for New Zealand children aged 5–14 years. For populations aged 15 years and over the estimated 97.5th percentile amount for consumers of confectionery was 125 g/day in Australia and 240 g/day in New Zealand.

3.7.4 Comparison of estimated food consumption amounts with the maximum amount of food that can be consumed to reach the threshold level for wheat protein

Updated information from the confectionery industry indicates that when used in ice cream, glucose syrup would represent around 8–10% of the ingredients. There is a range of use of glucose syrup in confectionery, with 50% being a very conservative estimate as it is higher then most uses. For example, gums and jellies typically contain 30-40% glucose syrup, hard boiled confectionery 30–40%, Turkish delight 5–28%, fudge 22%, hundreds and thousands 1–7%, marshmallow 25–70%, fondant 17%, and caramel topping 20–25%. For glucose syrup used in the centre of filled chocolates (centre may be one of the confectionery types above) the finished chocolate product will be at a maximum of 30% glucose syrup. It is also noted that in some of these products the source of glucose syrup may also be from other grains, such as corn, thus would not contain wheat protein. For example, 90% of the glucose syrup from the sole producer in New Zealand is corn based, some of this corn based product is also sold to Australian manufacturers. However Australian made products are also sold in New Zealand.

Taking into account the threshold level for wheat protein, the maximum amount of food containing 1 mg wheat protein at different gluten concentrations in glucose (10% to represent the likely level for 90% product, 15% for the remaining 10% of glucose syrup and 20% the theoretical maximum) was calculated. Table 7 illustrates the inverse relationship between the level of protein in glucose syrup and the corresponding amount of food that would contain 1 mg wheat protein when glucose syrup comprises 50% (limited number of confectionery products), 30% (filled chocolates, confectionery) and 10% (icecream) of the food product.

Table 7: Calculation of the maximum amount of food that can be consumed before the threshold level of 1 mg wheat protein is achieved at three different levels of gluten in glucose syrup

Level of gluten in glucose syrup	**Amount of glucose syrup containing 1 mg wheat protein** (assuming gluten is 75% total wheat protein)	**Amount of food containing 1 mg wheat protein** (assuming maximum 50% glucose syrup, as in some confectionery)	**Amount of food containing 1 mg wheat protein** (assuming maximum 30% glucose syrup, as in confectionery, filled chocolates)	**Amount of food containing 1 mg wheat protein (**assuming maximum 10% glucose syrup, as in icecream)
20 mg/kg	37 g	75 g	125 g	375 g
15 mg/kg	50 g	100 g	167 g	500 g
10 mg/kg	75 g	150 g	250 g	750 g

Estimated food consumption amounts for Australian and New Zealand populations given in Tables 5 and 6 were compared with the maximum amounts calculated in Table 7 to determine the potential risk of exceeding the threshold level for wheat protein.

For icecream, assuming a maximum of 10% glucose syrup content, the threshold level of 1 mg wheat protein was unlikely to be reached by high consumers of ice cream for products with 10 mg/kg, 15 mg/kg or 20 mg/kg gluten content. The 97.5th consumption ranged from 275–348 g/day,whereas the maximum amount before threshold is reached at 20 mg gluten/kg is 375 g, at the gluten level of 15 mg/kg the maximum amount of icecream able to be consumed is 500 g.

For filled chocolates, assuming a maximum of 30% glucose syrup content, the threshold level of 1 mg wheat protein was unlikely to be reached by children who were high consumers of chocolates for products with 10 mg/kg or 15 mg/kg gluten content or adults who were high consumers of chocolates for products with 10 mg/kg gluten content. However, in the worse case scenario it may be possible for a high consumer to exceed the 1 mg threshold level of wheat protein, if the filled chocolate product contained the maximum level of wheat based glucose syrup and that syrup contained more than 10 mg/kg gluten for adults and more than 15 mg/kg for children 5–14 years. The maximum amount before the threshold is reached at 10 mg gluten/kg is 250 g, at the gluten level of 15 mg/kg the maximum amount of filled chocolates able to be consumed is 167 g and at 20 mg/kg gluten 125 g. There were low numbers of children 5–14 years in the Australian survey who reported consuming over 125 g filled chocolates per day (for example, 16 out of 318 Australian children aged 5–14 years reporting consuming filled chocolates at or over the 95th percentile of 110 g/day and of these 8 children were at or over the 97.5th percentile of 183 g/day).

For confectionery consumed by the Australian population, assuming a maximum of 30% glucose syrup content that applies to most confectionery products, the threshold level of 1 mg wheat protein was unlikely to be reached by children or adults who were high consumers of products with 10 mg/kg, 15 mg/kg or 20 mg/kg gluten content. However, in the worse case scenario it may be possible for a high consumer to exceed the 1 mg threshold level of wheat protein, if the confectionery product contained the maximum level of wheat based glucose syrup (50%) and that syrup contained more than 10 mg/kg gluten. The 97.5th consumption amount for confectionery ranged from 91-125 g/day,whereas the maximum amount before the threshold is reached at 10 mg gluten/kg and 50% glucose syrup content is 150 g, at a gluten level of 15 mg/kg and 50% glucose syrup content the maximum amount of confectionery able to be consumed is 100 g. There were low numbers of children under 14 years in the NNPAS survey who reported consuming confectionery at levels over 90 g per day (for example, 9 out of 353 Australian children consuming confectionery at or over the 97.5th percentile of 91 g). FSANZ is also aware that the New Zealand manufacturer of glucose syrups provides New Zealand industry with 90% of its glucose syrup requirements, and this New Zealand glucose syrup is corn based. FSANZ also understands imported syrups and imported confectionery typically comprise corn or tapioca based syrups.

For ice cream, chocolates and confectionery containing glucose syrup with 10 mg/kg gluten all populations have estimated food consumption amounts lower then the maximum amount of food that can be consumed before the threshold level of 1 mg wheat protein is reached, the one possible expection being New Zealand children aged 5–14 years if it is assumed confectionery has 50% glucose syrup, which is not the case for most of these products.

3.8 Summary

Glucose syrup is used as an ingredient in food, mainly confectionery, filled choclates, icecream and some desserts. The manufacturing process of glucose syrup from wheat starch involves a number of steps aimed at separating and hydrolysing the starch. Purification removes most of the proteins and other nitrogen-containing compounds. Gluten is a major constituent of wheat protein (approx. 75%) and as such, analysis for gluten serves as a useful marker for residual protein. Recent analytical data on glucose syrup manufactured in Australia indicate that all batches contain gluten at levels <20 mg/kg (ie 90% have <10 mg/kg, the remainder less than 20 mg/kg).

In relation to wheat allergy, clinical evidence available suggests that consumption of 1 mg of wheat protein represents the limit that would protect the majority of wheat allergic individuals. It would also be protective for non-IgE immune mediated CD.

Consumption data indicates that the amount of food (confectionery or chocolate) consumed per day for high consumers was between 52-100 g for Australian 2–4 year olds; between 100–183 g/day for 5–14 year olds in Australia and between 100–232 g/day for 5–14 year olds in New Zealand. Confectionery products contain glucose syrup in various amounts between 1–70% of the final product, however not all the glucose syrup is derived from wheat, particularly in New Zealand where the sole manufacturer meets 90% of its glucose syrup requirements, and this glucose syrup is corn based.

Wheat protein exposure from glucose syrup will depend on its protein level (measured as gluten). For example, at a residual protein level of 15 mg/kg, 50 g glucose syrup would contain the upper limit of 1 mg wheat protein; and at a residual protein level of 10 mg/kg, 75 g of glucose syrup would contain 1 mg wheat protein. The main Australian glucose syrup manufacturer has indicated that the filtration steps are designed to reduce gluten content in the majority of batches to <10 mg/kg (or approx. <13 mg/kg wheat protein assuming the ratio of gluten to total protein in purified glucose syrup is similar to that in wheat).

For ice cream, chocolates and confectionery containing glucose syrup with 10 mg/kg gluten all populations have estimated food consumption amounts lower then the maximum amount of food that can be consumed before the threshold level of 1 mg wheat protein is reached, the one possible expection being New Zealand children aged 5–14 years if it is assumed confectionery has 50% glucose syrup, which is not the case for most of these products.

3.9 Conclusion

Based on the available clinical evidence and likely single meal consumption, wheat-derived glucose syrup with a gluten content of 10–20 mg/kg is likely to present a negligible risk to the majority of wheat allergic individuals. However, to ensure that gluten levels in glucose syrup are as low as technically achievable, the FAISAG has recommended that manufacturers of glucose syrup from wheat starch should be encouraged to prepare syrups with the lowest possible gluten levels.

4 Alcohol distilled from whey and from wheat

Distillation is one of the oldest methods of separating and purifying substances. Distillation is used to separate liquids and volatile substances from non-volatile substances, or to separate two or more liquids that have different boiling points. Alcohol distillation is used mainly to achieve higher alcohol content, but it also removes proteins and other substances present in the fermentation. There is general scientific agreement that non-volatile substances such as sugars (e.g. lactose from whey) and proteins do not distil and therefore, would not be present in the distilled product.

4.1 Production process

The first step in producing alcohol is yeast fermentation of sugars from various sources, including cereal grains and milk whey which also contain protein. Fermentation itself does not eliminate the allergenic proteins present in the mixture, and fermentation products contain proteins and protein fragments. The alcohol content is maintained up to 15% because the fermenting yeast is destroyed at higher alcohol concentrations. To purify and concentrate ethanol, the aqueous fermentation solution is distilled.

Distillation relies on the difference in the boiling points of the components in the fermentation broth. Both continuous and batch distillation are used to produce alcohol. The fermented liquid is heated so that components with lower boiling temperature will preferentially vaporise first. The alcohol starts to vaporise at 78°C, taking volatile substances with it.

The vapour passes into a distilling head and then into a condenser, where it is cooled to liquefy. In batch distillation, initially low boiling components are collected but as the distillation proceeds, these components are depleted from the starting mixture and higher boiling components begin to distil over. In commercial distillation, the operation is usually continuous and well controlled to prevent higher boiling components in the starting material from being carried over to the distilled product. The vaporised alcohol is then condensed forming clear liquid.

4.2 Uses in food

Distilled alcohol derived from cereals and from whey is commonly used in alcoholic beverages and for use as a solvent in the formulation of flavours and other food ingredients. Distilled alcohol may be further processed to produce vinegar.

4.3 Adverse effects of alcohol and relevance to allergy

Adverse reactions to alcoholic beverages and their potential causes were reviewed by Vally and Thompson (2003). Symptoms reported in the literature include sneezing, rhinitis, itching, flushing, headache and asthma. The review considered reports of sensitivities to a range of alcoholic beverages including beer, wine, spirits and distilled liquors. The review authors concluded that most sensitivities to alcoholic beverages do not appear to be immune-mediated. Adverse reactions are more likely to result from a pharmacological intolerance to specific components such as biogenic amines (eg. histamine) and/or sulphites in some alcoholic beverages.

4.4 Alcohol derived from whey

Whey is a by-product of cheese and casein manufacturing, with approximately 9 kg of whey resulting from the production of 1 kg of cheese or casein. Disposal of whey in effluent streams is problematic due to its high lactose content. Protein can be extracted from whey to produce whey protein concentrate, and the remaining liquid, containing 4-5% lactose, can be fermented by yeast and distilled to produce ethyl alcohol (Cressey et al 2010). Beta-lactoglobulin (BLG), the most abundant whey protein, is a major milk allergen with conformational and linear epitopes. Alpha-lactalbumin (ALA) ranks second in abundance in whey but its role in milk allergy is less clear (Fiocchi et al., 2010).

4.4.1 Clinical studies with alcohol from whey

No clinical studies have been performed on distilled alcohol from whey in milk allergic individuals.

4.4.2 Analytical studies on protein and lactose content in distilled alcohol from whey

As FSANZ did not have access to the unpublished data that had been provided to EFSA, a summary of these data is taken from their report (EFSA, 2007e). The analytical data related to 24 samples of whey ethanol, produced by two companies from three distilleries in Europe. The Bradford microassay method was calibrated with bovine serum albumin (BSA) to a concentration of 0.6 mg/L, and the LOD was 0.5 mg/L. Since ethanol interfered with the protein detection all samples were dried and reconstituted in water. In addition, a commercial whey protein ELISA specific to BLG validated for a LOD of 0.5 mg/L was used. No protein was detected in any of the samples above the LOD using these methods. On the ELISA method, EFSA noted that the kit instructions indicated that it could detect native and processed protein and fragments thereof. However, no evidence was provided to support this claim.

To quantify lactose in various batches, ion chromatography HPLC with amperometric detection and a LOD of 0.01 mg/L was used. Lactose was not detectable in 20 of the 24 samples. However, in four samples, lactose levels ranged between 0.02 mg/L and 0.150 mg/L. When adjusted for alcohol content (40% by volume), the lactose levels were 0.008–0.04 mg/L.

It was concluded that whey proteins/peptides and lactose are not found in products of distillation above 0.5 mg/L for protein and 0.04 mg/L for lactose.

4.4.3 Analytical data on whey alcohol and vinegar produced in New Zealand

Cressey et al. (2010) reported on the analysis of distilled ethanol from whey provided by a New Zealand manufacturer.

Thirty-five samples were analysed for residual protein using ELISA specific for the milk whey protein BLG with an LOD of 2.5 mg/L. No samples contained detectable BLG. Absence of whey proteins was further confirmed by liquid chromatography-mass spectrometry (LC-MS) analysis.

Distillation products may be processed further to produce foods and ingredients. Down-stream products, such as vinegar, are made from distilled alcohol. To confirm absence of whey proteins in vinegar, Cressey et al. (2010) analysed seven commercial samples of vinegar produced in New Zealand by secondary fermentation of distilled whey ethanol. Based on ELISA method, no samples contained detectable BLG at the LOD (2.5 mg/L), or whey protein detected by LC-MS.

4.5 Alcohol derived from wheat starch

Wheat starch undergoes enzyme hydrolysis, fermentation and distillation to produce alcohol.

4.5.1 Analytical data on protein content in distilled alcohol from wheat

The following is a summary of analytical data as reported in the EFSA Opinion (EFSA, 2007f). The analytical studies described report the examination of 39 bottled products and 76 samples of distillates produced using cereal as a raw material. Of these, 86 samples were analysed for total protein content and 45 samples were analysed for gluten.

The presence of proteins in the samples was determined using two analytical methods. The first is the Bradford microassay, which accounted for a LOD of 1 mg/L for wheat proteins. Positive samples were tested with the AAA-Direct Analysis which involves hydrolysis of protein to amino acids prior to quantitation (LOD of 0.05 mg/L for free amino acids and 0.5 mg/L for proteins). Fifteen positive responses with the Bradford microassay were confirmed by the AAA-Direct Analysis at a protein level of 0.5–1 mg/L. The protein content in only one sample was higher than 1 mg/L (1.3 mg/L), but it was not confirmed in a repeated experiment. These two analytical methods were deemed appropriate for this purpose.

No gluten was detected in any of the samples analysed by ELISA (type of ELISA not specified), LOD of 10 mg/kg. With a pre-concentration step, the LOD can reach 1 mg/kg, and 0.4 mg/kg when the concentration factor is taken into account. The calibration and recovery factor experiments were satisfactory.

Based on the analytical data, it was concluded that wheat proteins are unlikely to be carried over into the distillate at a level above 1 mg/kg during spirit manufacture. There were no data available, using appropriate sera, on the allergenic activity of residual protein.

4.5.2 Analytical data on wheat alcohol from Australia

Ten samples of finished grain ethanol were provided by an Australian manufacturer, in response to a request from FSANZ. Samples were taken during the period 21–27 April 2011 and included five samples of 95% alcohol and five samples of 100% alcohol. All samples were analysed for residual protein by ELISA and Bradford colourimetric method as reported by Cressey et al (2011) and summarised below.

The gluten ELISA method used in this analysis was previously validated and applicability of the method to grain alcohol was confirmed by adding the gluten control material provided with the kit to alcohol samples. Results were in the acceptable range for the gluten control. The gluten ELISA method has a LOD of 1 mg/L. No samples contained gluten above the detection limit of 1 mg/L.

Since ethanol interferes with the Bradford microassay, all samples were evaporated to dryness and residues extracted with phosphate-buffered saline, containing 0.2% Triton X. All samples were spiked with bovine serum albumin (BSA; approximately 4 mg/kg) to determine the efficiency of protein recovery. Spike recoveries were in the range 68–103%. Protein was not detected in any ethanol sample above the LOD of 0.12 mg/kg.

4.6 Summary

Alcohol distilled from wheat and whey is produced in Australia and New Zealand for use in alcoholic beverages and flavour carriers. There is general scientific agreement that non-volatile substances such as sugars (e.g. lactose from whey) and proteins, are unlikely to be found in the distillate. Reported analytical data, confirm that distilled alcohol from whey and wheat produced under proper controls, contain no detectable protein (i.e. <1 mg/kg). The data also confirmed the absence of detectable whey proteins in vinegar derived from whey alcohol.

4.7 Conclusion

Based on the available analytical evidence distilled alcohol and vinegar derived from whey present negligible risk to milk allergic individuals. Distilled alcohol derived from wheat presents negligible risk to wheat allergic and coeliac individuals.

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Appendix 1 – Production Process of soybean oil, tocopherols and phytosterols

Degumming

Crude soybean oil is processed in a series of steps to remove seed substances. The first of these steps is degumming, which removes phospholipids, seed particles, impurities, carbohydrates, **proteins** and traces of metals from the crude oil. The crude soybean oil is treated with processing aids and/or water, which leads to hydration of the phospholipids and other impurities. The hydrated substances precipitate from the oil and are removed by centrifugation.

Neutralisation

Degummed soybean oil is then neutralised, by treating it with an alkali solution. Neutralisation reduces the content of free fatty acids and their oxidation products, residual **proteins**, phosphatides, carbohydrates, traces of metals and a part of the pigments. Caustic soda is the most common alkali solution used in Australia. The oil is then washed with water and centrifuged to remove the impurities.

In many commercial soybean oil plants the degumming step is combined with neutralisation. This process is commonly termed ‘alkali refining’, or ’caustic refining’.

Bleaching

The term bleaching here does not refer to a chemical process, but a filtering process where the oil is streamed through activated clay or silica to absorb certain pigmented material such as carotenoids and chlorophyll from the oil. Bleaching also further removes residues of phosphatides, soaps, traces of metals, oxidation products, and **proteins**. These trace components interfere with further processing and reduce the quality of the final product necessitating their removal by adsorption with activated clay.

Deodorisation

Finally, a vacuum steam distillation step is applied to remove the relatively volatile components and colour pigments that give rise to undesirable flavours, colours and odours. Deodorisation improves the stability and colour of the oil, whilst preserving its nutritional value. Soybean oil-based fats are produced by interesterification and hydrogenation after bleaching and before deodorising.

In addition, a commercially valuable by-product of deodorisation of all vegetable oils, including soybean oil, is the vegetable oil deodoriser distillate (VOD). The distillate is a complex mixture of free fatty acids, triglycerols, cetones, peroxides, hydrocarbons, oleins, and rich in phytosterols and tocopherols. Both phytosterol and tocopherol are commercially valuable compounds used widely in food (also considered in this report).

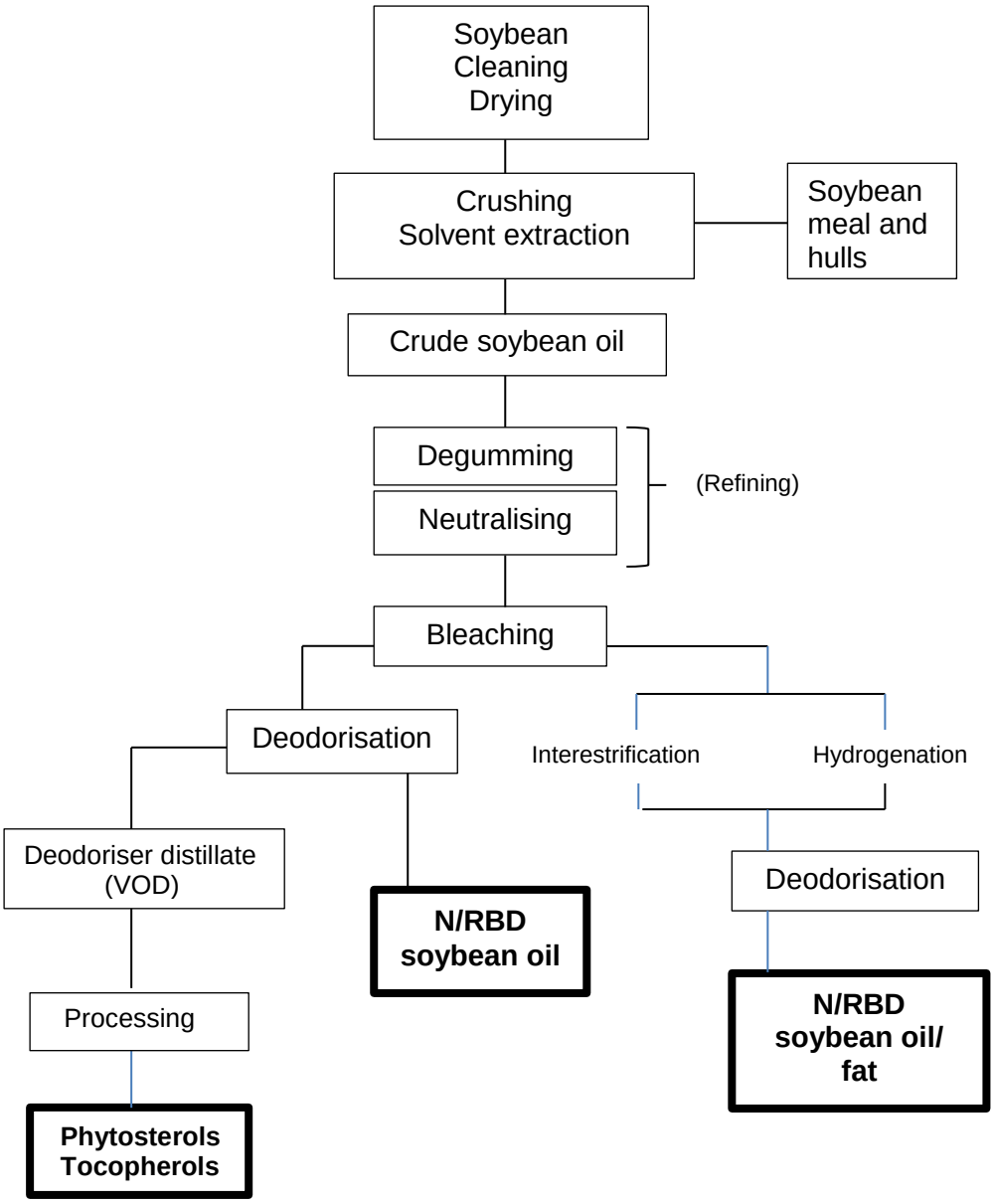

Figure 1: Production process – N/RBD soybean oil, phytosterols and tocopherols

Appendix 2 – Production process for glucose syrup from wheat starch

Commercial wheat starch is produced by mixing wheat flour and water into a homogenised slurry which is then separated into starch and gluten. Originally, glucose was produced by acid hydrolysis of the starch slurry in a batch process. However, the quality of the syrup was inconsistent between batches with traces of unconverted starch present and highly coloured syrup. Nowadays, the glucose production process is based on enzymatic hydrolysis of starch using a continuous converter, resulting in a consistent product.

In enzymatic hydrolysis, the bonds linking dextrose units of the starch chain are cleaved. Hydrolysis degrades the starch granules releasing the proteins and lipids. Further steps include centrifugation and/ or filtration, physical screening and ion exchange.

Enzyme hydrolysis

The refined A-starch slurry is pH-adjusted and enzymes are added. The prepared slurry is heated by direct steam in a steam jet.

Liquefaction: The liquefaction is typically a two stage process. The combination of heat and enzymes gelatinises and thins the starch. The enzyme does the work by cutting the long starch molecules into pieces by hydrolysis. A low dextrose equivalent (DE hydrolysate) is formed and at this point the starch has been converted into a maltodextrin.

Saccharification: The low DE hydrolysate is pH and temperature adjusted once again and new enzymes added to produce glucose with a higher DE. Glucose of different composition can be made depending on the enzymes added and the process applied - even products close to pure dextrose.

Filtration-protein removal

Starch granules naturally contain protein in their structure. The protein content varies depending on the starch source and is seen as undesirable since it gives rise to browning reactions both during the hydrolysis process to produce the glucose syrup and on storage of the finished syrup.

The glucose hydrolysate is heated and treated with activated carbon to remove impurities, including proteins and colour bodies, and then filtered. New technology allows cross-flow membrane filtration of the hydrolysate.

Ion exchange

The glucose hydrolysate is de-mineralised with ion exchange resins. Cation resins remove various ions as sodium, calcium, traces of iron and some amino acids. Anion resins remove ions like chloride, sulphate, phosphate and most residual amino acids.

Evaporation

The refined glucose syrup is concentrated by evaporation to its final commercial dry matter content. The final product, glucose syrup, is a clear, colourless, viscous sweet liquid.

A flow diagram of the process is at Figure 2 below.

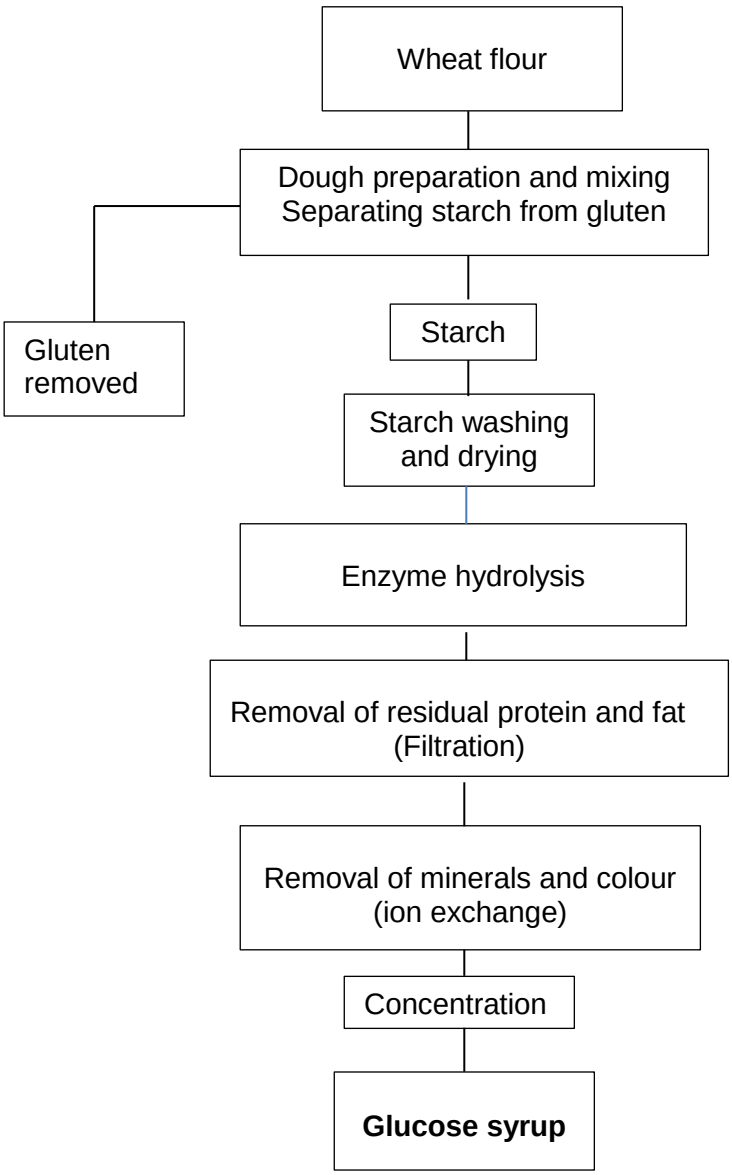

Figure 2: Production process – glucose syrup from wheat starch