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Scientific Opinion related to a notification from Lyckeby Starch AB on barley starch to be used in the manufacturing of several foods as ingredient, of the food additive modified starch and of glucose syrups pursuant to Article 21(2) of Regulation (EU) No 1169/2011 – for permanent exemption from labelling

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver a scientific opinion on barley starch to be used in the manufacturing of several foods as ingredient, of the food additive modified starch and of glucose syrups pursuant to Article 21(2) of Regulation (EU) No 1169/2011, as notified by Lyckeby Starch AB. The applicant provided information on the manufacturing process and data on the content of total protein, gluten and allergenic proteins in barley starch. The applicant also performed IgE-binding *in vitro* tests, which were considered inconclusive by the Panel. No human intervention studies with barley starch or food products thereof were provided by the applicant, except for a DBPCFC with barley starch hydrolysate in cereal allergic individuals. The Panel notes that glucose syrups based on barley have been already exempted from allergen labelling as per Annex II of Regulation (EU) No 1169/2011 and that the current application is for the exemption from labelling of all foods manufactured from barley starch. In all the scenarios considered for the anticipated intake, the calculated total protein intake from barley starch was above the MED/MOED for wheat (expressed in mg of wheat protein) in adults (10 mg) and children (2 mg). The Panel concludes that the data available are insufficient to conclude on the likelihood of adverse allergic reactions in cereal-allergic individuals upon consumption of barley starch under the conditions of use proposed by the applicant, and that the consumption of foodstuffs produced from barley starch as starting (raw) material or foodstuffs containing barley starch as an ingredient is unlikely to cause an adverse reaction in individuals with coeliac disease who are not allergic to cereals, provided that the value of gluten for 'gluten-free' foods (20 mg/kg) is not exceeded.

Keywords: Barley starch, cereal allergy, coeliac disease, wheat-induced exercise-dependent anaphylaxis, allergen labelling

Requestor: European Commission

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Summary

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver a scientific opinion on barley starch to be used in the manufacturing of several foods as ingredient, of the food additive modified starch and of glucose syrups pursuant to Article 21(2) of Regulation (EU) No 1169/2011, for permanent exemption from allergen labelling, as notified by Lyckeby Starch AB.

Since barley is known for its potential to elicit both coeliac disease and IgE-mediated food allergy and it is subject to mandatory labelling, it is appropriate to assess barley starch and products thereof for their potential to induce coeliac disease and/or food allergy.

Barley starch is not sold to consumers directly and it is not consumed as such. Relative amounts of barley starch in selected food categories vary depending on the type of food product and the desired function. The worst-case scenario for the anticipated intake assumes that all dietary digestible carbohydrates are consumed as barley starch. A 'realistic' scenario proposed by the applicant assumes an intake of barley starch of 5% of total energy or 12% of total carbohydrates, when 41% of all digestible carbohydrates are consumed on a single occasion. The Panel considers that a 'realistic' scenario for risk characterisation would rather focus on populations of high consumers of barley starch. This scenario assumes that all digestible carbohydrates in the diet come from gluten-free staple foods, which can contain up to 35% of digestible carbohydrates as barley starch, or the consumption of sports foods (75 g of barley starch per serving).

Owing to the manufacturing process, the protein content in barley starch will be lower than the protein content in the starting material. The highest protein content in different batches assessed using the Bradford method was 0.78 g/kg, and below the limit of quantification (0.5 g/kg) of the single amino acid method. The concentration of residual gluten was generally below the limit of detection (LOD), from < 4 to < 10 mg/kg, depending on the analytical procedures used. Each of the five barley allergens measured (Hor v 16, B1-hordein, Hor v 15, Hor v 17, and γ-hordein-3) was below the LOD of 1 mg/kg.

No human intervention studies with barley starch or food products thereof were provided by the applicant, except for a double-blind placebo-controlled food challenge (DBPCFC) with barley starch hydrolysates (glucose syrups) in cereal allergic individuals. The Panel notes that glucose syrups based on barley have been already exempted from allergen labelling as per Annex II of Regulation (EU) No 1169/2011 and that the current application is for the exemption from labelling of all foods manufactured from barley starch.

No human data have been provided on minimal (observed) eliciting doses (MED/MOED) for barley. The Panel notes that the lowest reported MOED in wheat allergic paediatric patients undergoing oral food challenges is about 2 mg of wheat protein (first dose tested). The lowest reported MED in adult wheat allergic patients undergoing DBPCFC is 100 mg of wheat flour, or about 10 mg of wheat protein. However, doses of wheat triggering allergic reactions in sensitive individuals may be lower because patients with a history of severe allergic reactions (e.g. wheat-allergic patients with wheat-induced anaphylaxis (WIA) or wheat-dependent exercise-induced anaphylaxis (WDEIA)) have been excluded from the challenge studies available. The Panel also notes that, whereas barley mono-allergic individuals may be rare, a relevant proportion of wheat allergic individuals reacts to barley upon food challenge.

The results of IgE-binding *in vitro* tests performed by the applicant were considered inconclusive by the Panel.

For all the scenarios considered for the anticipated intake, the calculated total protein intake from barley starch was above the MED/MOED for wheat (expressed in mg of wheat protein) in adults (10 mg) and children (2 mg). Using data published in the literature, the applicant re-calculated the MED/MOED for wheat in terms of 'allergenic protein content', rather than in mg of total protein in wheat, for adults (2.1 mg) and children (0.42 mg). In a 'realistic' scenario where 35% of digestible carbohydrates could come from barley starch, the margin of exposure for the MED/MOED calculated by the applicant for allergenic proteins in barley ranged from 1.6 to 3.8 in children, and from 2.2 to 5.2 in adults (from 2.8 to 6.8 considering sports foods only). These margins of exposure are insufficient to conclude on the likelihood of allergic reactions in cereal-allergic individuals, owing to the uncertainty associated with the assumptions made regarding the calculation of the wheat protein fraction that is allergenic and the insufficient characterisation of the risk for wheat-allergic patients with WIA or WDEIA.

Based on the data presented by the applicant and on data currently available in the literature, the Panel concludes that:

- The data available are insufficient to conclude on the likelihood of adverse allergic reactions in cereal-allergic individuals upon consumption of barley starch under the conditions of use proposed by the applicant.
- For coeliac disease, assessment of the evidence submitted indicates that the consumption of foodstuffs produced from barley starch as starting (raw) material or foodstuffs containing barley starch as an ingredient are unlikely to cause an adverse reaction in individuals with coeliac disease who are not allergic to cereals, provided that the value of gluten considered by Codex Alimentarius and implementing Regulation (EU) No 828/2014 for 'gluten-free' foods (20 mg/kg) is not exceeded.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background

Annex II to Regulation (EU) No 1169/2011[1] (hereafter "the Regulation") includes an EU list of food substances or products known as likely to trigger adverse reactions in sensitive individuals, and therefore their presence in foods must be indicated. This list has been established on the basis of the scientific opinions of the European Food Safety Authority (EFSA).[2] According to the latter, those substances are considered as part of the most common food allergens and there is sufficient evidence to support their inclusion into the list.

In order to ensure better information to consumers and to take account of the most recent scientific progress and technical knowledge, Article 21 paragraph 2 of the Regulation requires the Commission to systemically re-examine, and where necessary, update the list in Annex II, in accordance with Article 51 of the Regulation.

Pursuant to Article 5 of the same legislation, any Union measure in the field of food information law which is likely to have an effect on public health shall be adopted after consultation of EFSA on the basis of Article 29 of Regulation (EC) No 178/2002[3].

The update of the list may also consist in the deletion of food allergens for which it has been scientifically established that it is impossible to cause adverse reactions. To this end, the interested parties may communicate to the Commission studies establishing that certain products derived from substances listed in Annex II are unlikely to trigger adverse reactions in individuals.

The EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA) has previously delivered a scientific opinion on Scientific and technical guidance for the preparation and presentation of such applications (EFSA NDA Panel, 2013). It may be confirmed that this request is in line with this guidance.

1.1.2. Terms of Reference

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission requests the European Food Safety Authority to evaluate the scientific data submitted by the *Lyckeby Starch AB* in the context of the re-examination of the Annex II to Regulation (EU) No 1169/2011 as requested in Article 21(2) of the latter.

EFSA is requested to issue an opinion on the information provided, and particularly to consider the likelihood of adverse reactions triggered in susceptible individuals from the consumption of barley starch used as an ingredient or when used to make modified starch.

2. Data and methodologies

2.1. Data

Notification from Lyckeby Starch AB on barley starch to be used in the manufacturing of several foods as ingredient, of the food additive modified starch and of glucose syrups pursuant to Article 21 (2) of Regulation (EU) No 1169/2011 for permanent exemption from labelling, presented in a common and structured format as outlined in the Guidance on the preparation and presentation of applications pursuant to Article 21 Paragraph 2 of Regulation (EU) No 1169/2011 (EFSA NDA Panel, 2013). As outlined in that guidance document, it is the duty of the applicant to provide all pertinent scientific data in the application (published and unpublished, data in favour and not in favour) relative to the assessment of the likelihood of adverse reactions being triggered in sensitive individuals by the oral

[1] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004. OJ L 304 of 22.11.2011, p. 18–63.

[2] EFSA Journal http://www.efsa.europa.eu/EFSA/Scientific_Opinion/opinion_nda_04_en1,1.pdf

[3] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31 of 1.2.2002.

consumption of the food allergen-derived preparation/foodstuff(s) under the proposed conditions of use.

2.2. Methodologies

The general approach of the NDA Panel for the evaluation of applications on food ingredients or substances with known allergenic potential is outlined in the Guidance on the preparation and presentation of applications pursuant to Article 21 Paragraph 2 of Regulation (EU) No 1169/2011 (EFSA NDA Panel, 2013).

3. Assessment

3.1. Introduction

Barley grain (*Hordeum vulgare L.*) is a gluten-containing cereal subject to mandatory allergen labelling as per Annex II of Regulation (EU) No 1169/2011.

Wheat is the gluten-containing cereal most often reported to induce cereal allergy, as compared to barley, rye and oats. The prevalence of wheat allergy based on clinical history and positive food challenges is as low as 0.4% in young children (1–3 years of age). The prevalence of barley allergy based on clinical history and positive food challenges is unknown, but most likely to be lower than the prevalence of wheat allergy. Wheat allergy frequently resolves during adolescence (EFSA NDA Panel, 2014).

Wheat may trigger severe anaphylactic reactions in children suffering from wheat allergy. In Japan, wheat is often reported among the top three foods responsible for food-induced anaphylaxis in adults and children. In Europe, anaphylaxis to wheat seems to be less frequent than in Asia. Wheat has also been reported to be an important triggering factor for food-dependent exercise-induced anaphylaxis (FDEIA) and is considered the most frequent cause of FDEIA in Japan (EFSA NDA Panel, 2014). Tissue transglutaminase, an intestinal enzyme locally activated during exercise, is able to cross-link to ω-5-gliadin-derived peptides in wheat causing a marked increase in IgE binding, which may explain the role of this gliadin (a component of gluten) in wheat-dependent exercise-induced anaphylaxis (WDEIA). WDEIA (also known as ω-5-gliadin allergy) is nevertheless considered to be a rare condition. One allergenic protein present in barley (Hor v 20, γ-hordein-3) has been reported to cross-react with ω-5-gliadin in wheat (Palosuo et al., 2001).

Individuals allergic to wheat and experiencing wheat-induced anaphylaxis (WIA) or WDEIA have, in most cases, high levels of IgE antibodies specific to ω-5 gliadin (and high molecular weight-glutenin),[4] but only in about 50% of cases develop serum IgE specific to wheat.[5] This is in sharp contrast with wheat allergic individuals without WIA or WDEIA (e.g. presenting only with atopic dermatitis or urticaria), who systematically have high serum IgE specific to wheat and most often have very low or no ω-5 gliadin-specific IgE (Matsuo et al., 2005; Park et al., 2012; Takahashi et al., 2012).

In China, among 283 clinically diagnosed WDEIA patients, anaphylaxis was reported to be rare (number of cases not specified) during one year of strict avoidance of wheat before exercise or other triggers, such as aspirin (Jiang et al., 2018). In a study conducted in the UK in 132 adult patients with WDEIA, the most effective strategies to avoid anaphylaxis were avoidance of wheat before exercise or a gluten-free diet, which were effective in only two-thirds of the patients who received this advice (Kennard et al., 2018).

Coeliac disease is a life-long autoimmune systemic disorder triggered by cereal storage proteins (prolamins) present in gluten from wheat, rye and barley. Its prevalence is estimated to be 0.5 to 1% (Fasano AaC, 2001; Rubio-Tapia et al., 2009; Virta et al., 2009) A gluten-free diet is the conventional treatment. The limit values of 20 and 100 mg/kg of gluten in 'gluten-free' and 'very low gluten' foods, respectively, help in managing the diet of most patients with coeliac disease efficiently (EFSA NDA Panel, 2014).

Since barley is known for its potential to elicit both coeliac disease and IgE-mediated food allergy (EFSA NDA Panel, 2014) and it is subject to mandatory labelling, it is appropriate to assess barley starch and products thereof for their potential to induce coeliac disease and/or food allergy.

Barley starch is used in a wide category of food products as an ingredient but also as raw material to make the food additive modified starch, maltodextrins and glucose syrups.

[4] Based on test kits including the salt-insoluble protein fraction.
[5] Based on test kits including the salt-soluble protein fraction.

In 2004, the NDA Panel issued an Opinion on a notification submitted by Finnsugar Ltd. to the European Commission for temporary exemption from labelling of glucose syrups produced from barley starch (EFSA NDA Panel, 2004). In 2007, the NDA Panel issued another opinion based on an updated dossier submitted by Finnsugar Ltd, for the permanent exemption from labelling of glucose syrups produced from barley starch, namely starch hydrolysates (EFSA NDA Panel, 2007). Glucose syrups based on barley have been exempted from allergen labelling as per Annex II of Regulation (EU) No 1169/2011.

The present Opinion is based on a dossier submitted by Lyckeby Starch AB on barley starch to be used as ingredient in the manufacturing of several foods, of the food additive modified starch and of glucose syrups pursuant to Article 21(2) of Regulation (EU) No 1169/2011 for permanent exemption from labelling.

3.2. Food allergen-derived preparation

The food allergen-derived preparation for which the exemption from labelling is requested is barley starch.

Barley starch is a powder obtained from barley kernels following a manufacturing process claimed as confidential by the applicant. A brief description of the manufacturing process is given in Section 3.5.1.2.

3.3. Conditions of use

The applicant specifies that barley starch is not sold to consumers directly and it is not consumed as such. Barley starch is used by the food industry as raw material for further processing or as an ingredient.

Barley starch can be used as raw material for preparing modified starches, i.e. food additives as per Regulation (EC) No 1333/2008[6], defined as substances obtained by one or more chemical treatments of edible starches, which may have undergone a physical or enzymatic treatment, and may be acid or alkali treated or bleached. Barley starch can also be hydrolysed into glucose syrup or maltodextrins.

Glucose syrups based on barley starch and maltodextrins based on wheat starch have been exempted from allergen labelling as per Annex II of Regulation (EU) No 1169/2011. Maltodextrins from barley starch are currently subject to mandatory allergen labelling.

The applicant states that barley starch is used as ingredient in several foods. These include fruit fillings and jams, yoghurt and yoghurt fillings, cheeses, milk desserts, processed meat products, sports nutrition products, sweets and confectionary, dressings and sauces, bread, bakery products and bakery fillings, baby foods, breast milk substitutes and nutrition solutions among others. In this context, barley starch is included in the list of ingredients as 'barley starch' or 'modified barley starch'. The applicant also stated that barley starch is also used in food products meeting the labelling requirements for 'gluten-free' as required in Regulation (EU) 828/2014[7] (i.e. containing no more than 20 mg of gluten/kg of food).

3.4. Anticipated intake/exposure

Barley starch can be used in different food categories with different amounts depending on the type of food product and the desired function. The food categories and the relative amounts of barley starch indicated by the applicant are presented in Table 1.

Table 1: Relative amounts of barley starch in selected food categories as indicated by the applicant

Food category	Relative amount of barley starch
Beer	< 2%
Fat emulsions and blended fats	≤ 10%
Composite dishes	1–7%
Eggs and egg products	≤ 2%

[6] Regulation (EU) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

[7] Regulation (EU) No 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food. OJ L 228, 31.7.2014, p. 5–8.

Food category	Relative amount of barley starch
Processed fish and sea food	≤ 2%
Foods for infants and young children	3–15%
Processed fruits and fruit products	≤ 6%
Extracts of plant origin	≤ 50% of the fat-free carrier
Bread and rolls, pasta, doughs and similar	≤ 35%
Breakfast cereals	≤ 10%
Fine bakery wares (biscuits, pastries and cakes)	≤ 50%
Processed legumes, nuts, oilseeds and spices	≤ 5%
Isolated ingredients, additives, flavours, baking and processing aids	30–100%
Processed meat products	≤ 4%
Milk and dairy products	≤ 3–7%
Meat and dairy imitates	≤ 20%
Artificial sweeteners	≤ 80%
Food supplements	≤ 80%
Sports foods	75 g/serving
Seasoning sauces and condiments	≤ 5–30%
Snacks	≤ 7–20%
Confectionary	≤ 5–50%
Glucose syrups	100%
Processed vegetable and vegetal products	≤ 5%
Water-based beverages	≤ 1%

The applicant has identified individuals eating gluten-free bread and individuals performing strenuous exercise/training as those who would consume the highest daily doses of barley starch. One slice of gluten-free bread contains about 6 g of barley starch, whereas one serving of sports foods contains about 75 g of barley starch. Assuming a daily consumption of six to seven slices of gluten-free bread by individuals on gluten-free diets and of two servings of sport foods by individuals performing strenuous exercise/training, the applicant estimated a daily intake of 36–42 g and 150 g of barley starch in these two population groups, respectively.

The applicant proposes two scenarios for the anticipated intake that could be used in the risk characterisation: a worst-case scenario, and a 'realistic' scenario.

Worst-case scenario

The applicant states that an intake of all dietary digestible carbohydrates as barley starch could be considered as the worst-case scenario for the anticipated intake.

In 2010, EFSA proposed Dietary Reference Values (DRVs) for carbohydrates and dietary fibre. For digestible carbohydrates, 45–60 E% was proposed as the Reference Intake (RI) range (EFSA NDA Panel, 2010). As reported in the same Scientific Opinion, average carbohydrate intakes in adults varied from approximately 38–54 E%. In the various age categories, average intake of digestible carbohydrates ranged from approximately 41 to 51 E% (19–34 years), 38 to 49 E% (35–64 years) and 40 to 53 E% (65 years and over), respectively. More than half (53%) of the reported mean values were between 45 and 50 E%. Average carbohydrate intakes of 50 E% and higher were reported in 16% of the adult groups belonging to the age categories 19–34 years and 65 years and over. Within population ranges varied from 31 to 34 E% at the lower (5th percentile) and from 58 to 61 E% at the upper end (95th percentile) of the distributions. In countries in which intakes of mono- and disaccharides were reported separately, they varied between 23 and 36 E% in children and adolescents, with the highest intakes in infants, whereas the intake of digestible polysaccharides was between 23 and 25 E%. The Panel notes that the upper bound of the RI range for digestible carbohydrates (60 E%) indeed corresponds to the highest 95th percentile observed in distributions of intake in European countries.

In 2013, EFSA set DRVs for energy based on age, sex and physical activity level (PAL) (EFSA NDA Panel, 2012). The highest average requirements (AR) for energy were established for 17-year-old men with a very active lifestyle (PAL = 2.0; AR = 15.4 MJ/day or 3,678 kcal/day). Using the upper bound of

the RI range for digestible carbohydrates (60 E%), this would lead to a mean intake of 2,207 kcal from digestible carbohydrates/day, or 552 g/day of digestible carbohydrates. The applicant states that using an estimated average weight of 68.1 kg for adult men, this would correspond to 8.1 g of digestible carbohydrates/kg body weight per day.

Following the same reasoning, the applicant estimates an intake of 616 kcal/day from digestible carbohydrates, or 154 g/day, corresponding to 12.6 g/kg body weight per day, for 2-year-old boys for which the AR for energy is 4.3 MJ/day, corresponding to 1,027 kcal/day.

The Panel notes that, while the upper bound of the RI range for digestible carbohydrates (60 E%) indeed corresponds to the highest 95th percentile observed in distributions of intake in European countries, DRVs for energy are derived to cover energy requirements of individuals at the 50th percentile of reference body weights and heights for EU children, and of adult individuals with a body mass index BMI of 22 kg/m^2. Hence, DRVs for energy are below observed energy intakes of overweight and obese individuals. Having said this, the Panel also notes, however, that these individuals are unlikely to have very active lifestyles (PAL = 2).

The Panel agrees with the applicant that an intake of all dietary digestible carbohydrates as barley starch could be considered as the worst-case scenario for the anticipated intake on the assumption that all carbohydrates are consumed on a single occasion. This would mean an intake of barley starch of 552 g/d in adults and of 154 g/d in young children.

'Realistic' scenario

The applicant also proposes a 'realistic' scenario for the anticipated intake, which assumes that, overall, 5% of total energy intake and 12% of all digestible carbohydrates could be consumed as barley starch (based on applicant's own calculation and assumptions from the average dosage levels used in different foods), and that 41% of all digestible carbohydrates in the diet could be consumed on a single occasion. This would lead to a consumption of 66 g of barley starch per day and 27 g of barley starch per meal in adults, and to a consumption of 18.5 g of barley starch per day and 7.6 g of barley starch per meal in young children.

The Panel considers, however, that 'realistic' scenarios to be used for risk characterisation should be more conservative and rather focus on populations of high consumers of barley starch, i.e. individuals consuming gluten-free food products and sports foods. On the assumption that all digestible carbohydrates in the diet come from gluten-free staple foods such as bread and rolls, pasta, doughs and similar, which can contain up to 35% of digestible carbohydrates as barley starch (Table 1), this would lead to a consumption of 193 g/day and 79 g/meal of barley starch in adults, and to a consumption of 54 g/day and 22 g/meal of barley starch in young children. Consumption of sports foods would lead to a consumption of 75 g of barley starch per meal (one serving), and of 150 g of barley starch per day (two servings). The Panel notes that this last scenario could be particularly relevant for individuals with WDEIA.

3.5. Characteristics of the food allergen-derived preparation/foodstuff(s)

3.5.1. Food allergen-derived preparation (barley starch)

3.5.1.1. General specifications

The general specifications of barley starch, which is produced as a white powder to be stored at ambient temperature in clean and dry conditions, away from odorous materials, are depicted in Table 2.

Table 2: Chemical and physical data for barley starch

Chemical and physical properties	Unit	Specific range	Analytical methods
Moisture	%	Max. 15 (as packed)	
Gluten	mg/kg	< 20	ELISA R5-Mendez (Sandwich)
Nutrients			
Energy	kJ/kcal/100 g	1,445/340	–
Fat Of which saturated fat	g/100 g	≤ 5 0	–
Carbohydrates Of which sugars	g/100 g	85 0	–
Protein	g/100 g	≤ 0.5	Kjeldahl Nitrogen
Salt	g/100 g	0	–
Microbiological status			
Total plate count	cfu/g	≤ 10 000	NMKL no 86
Coliform	cfu/g	≤ 10	NMKL no 44, 2004 edition 6
Yeast	cfu/g	≤ 100	NMKL no 98
Mould	cfu/g	≤ 100	NMKL no 98
Salmonella, in 25 g	cfu/g	Negative	NMKL no 71

cfu: colony-forming units.

The applicant states that all food additives used to manufacture barley starch fulfil purity criteria as a per Regulation (EU) No 231/2012[8], and that the heavy metal content meets the requirements in Regulation (EU) No 1881/2006[9].

The Panel notes that the total protein content in barley starch given in the specifications is ≤ 5 g/kg. The applicant indicates that the specifications for protein in barley starch are given in accordance with the requirements of Regulation (EU) No 1169/2011 on food labelling and the relative guidelines, namely as the result of Kjeldahl nitrogen determination (Regulation (EU) No 231/2012[8]), but it does not represent the actual protein content as measured by two reference methods (see Section 3.5.1.3).

3.5.1.2. Manufacturing process

The applicant states that the Food Safety Management System of Lyckeby Starch AB complies with the requirements of the Food Safety System Certification FSSC 22000 v4.1.

The barley starch production process exclusively uses barley free from germinated seeds, in which the amount of α-amylase is negligible (Macgregor et al., 1984). The production process follows several steps. Barley kernels are first cleaned and checked for the absence of contaminating crops. Kernels are then de-hulled, crushed and finely milled. The resulting material is repeatedly dispersed in fresh tap water, decanted and then separated from fibres. After an alkalinising step and a subsequent acidification step, both basic-soluble and acid-soluble proteins are washed off and the remaining starch suspension is neutralised, dewatered and dried to a powder in a flash dryer to a moisture content of < 15%. The powder is stored in sacks or big bags.

The applicant claims that, during the manufacturing process, the water- and salt-soluble proteins are eliminated in the washing procedure. The applicant also claims that the washing conditions are adjusted to solubilise the prolamin protein fraction of the gluten proteins in barley flour which are not soluble in water or salt, so that this protein fraction is also removed during the manufacturing process of barley starch.

The Panel notes that the protein content in barley starch will be lower than the protein content in the starting material due to the manufacturing conditions.

[8] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1–295. http://data.europa.eu/eli/reg/2012/231/oj

[9] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24. http://data.europa.eu/eli/reg/2006/1881/oj

3.5.1.3. Allergen specifications

The applicant assessed total (crude) protein, gluten and specific barley allergens in 42 different production batches of barley starch and one reference batch of maize starch (control).

Total protein

The total amount of protein in barley starch was assessed by analysing total nitrogen by the Kjeldahl method and using a conversion factor of 5.83 for barley (FAO, 2003).

The results show a residual nitrogen content between 0.0060% and 0.0218%, and thus a residual protein content between 0.035% and 0.127% (0.35 g/kg and 1.27 g/kg) in all samples of barley starch analysed. The applicant claims that one batch (#1) at the upper end value of 0.11% (1.1 g/kg) protein is likely to have been contaminated with the starting material (barley flour) during sample handling after the production process.

The Panel notes that the total protein content in barley starch using the Kjeldahl method was between 0.35 g/kg and 1.27 g/kg in most of the batches analysed, which is well below the < 5 g/kg value provided in the product specifications. The Panel also notes that the Kjeldahl method also detects non-protein nitrogen, and therefore, it may overestimate the protein content in barley starch.

Upon EFSA's request for clarification, the applicant also provided a quantification of proteins in five barley starch samples and one maize sample (control) using two different methods of protein analysis: a) quantification of single amino acids which are then converted to proteins using the Swedish National Food Agency nutrient composition database (Livsmedelsverket, 2015), and b) the Bradford method (Bradford, 1976). The five barley starch samples were selected at the highest and lowest protein content as assessed with the Kjeldahl method.

The residual protein content in the barley starch samples analysed was at the limit of quantification (LOQ = 0.5 g/kg) of the single amino acid method and between 0.13 and 0.78 g/kg using the Bradford method.

The Panel notes that the protein content in barley starch differs depending on the methods used for protein analysis and considers that both the upper bound of the protein content assessed using the Bradford method (0.78 g/kg) and the LOQ of the single amino acid method (0.5 g/kg) could be used for risk characterisation.

Gluten

Gluten is widely used as a collective term for the complex protein mixture of wheat, rye or barley which is not soluble in water or salt solution.[10] It consists of prolamins, which are seed storage proteins that have been shown to trigger the pathophysiological changes and clinical features in patients with coeliac disease.

In barley, prolamins are called hordeins and are classified in four groups according to their molecular weight, i.e. D, C, B and gamma (γ) (Tanner et al., 2019). B-hordeins have at least two subfamilies (B-1 and B-3) and account for about 70–90% of total hordeins, whereas C-hordeins account for 10–30% of the total. D- and γ-hordeins are minor components, accounting for 1–2% and 2–4% of total hordeins, respectively (Shewry et al., 1985). Hor v 15 is an α-amylase inhibitor which belongs to the prolamin family.

The following methods were used to analyse gluten in barley (and maize-control) starch samples:

- ELISA R5-Sandwich Mendez method, validated as AACCI 38.50.01, AOAC-RI 120601 and AOAC-OMA 2012.01;
- ELISA R5-Competitive method, validated as AACCI 38.55.01 and AOAC-OMA 2015.05;
- ELISA G12 method**,** validated as AACCI 38-52.01 and AOAC-OMA 2014.03;
- PCR (Polymerase Chain Reaction) using the method as reported in Dahinden et al. (2001). This is a non-officially validated analytical protocol.

The results obtained with the ELISA R5-Sandwich Mendez method showed gluten levels < 5 or < 7 mg/kg for all barley starch samples except for one batch (#1), which showed an amount of gluten of 25 mg/kg.

The results obtained with the ELISA R5-Competitive and the ELISA G12 methods showed gluten levels below the limit of detection (LOD) of the methods (LOD of 10 mg/kg for the ELISA

[10] Codex Alimentarius Commission (2015) Codex Standard 118-1979: Codex Standard for Foods for Special Dietary use for Persons Intolerant to Gluten. Revision 1. Amendment 2.

R5-Competitive and of 4 mg/kg for the ELISA G12) with the exception of one sample (#1), which showed an amount of gluten of 5.1 mg/kg with the ELISA G12 method. This is the batch which had also a higher crude protein content using the Kjeldahl procedure. The applicant claims that the barley starch sample had been accidentally contaminated with the starting material (barley flour) during handling after production because gluten was not detected in the pre-gelatinised version of this batch (batch 1004000151).

In order to have a comparison between the different procedures, the same 42 batches of barley starch and the one reference batch of maize starch were also analysed by the indirect nucleic acid PCR procedure as described by Dahinden et al. (2001), which detects genetic material (specific DNA) of wheat, rye and barley as specific marker for potentially allergenic protein residuals from the raw material (Zeltner et al., 2008). No barley specific DNA was detected (LOD of 10 mg/kg of barley flour) in all batches but two. The applicant attributes the two positive results to a non-specified error at the laboratory where the analyses were performed.

Although the ELISA and PCR methods used had different sensitivity and LODs, the Panel notes that the concentration of residual gluten in the batches analysed using well-accepted methods was generally below the LOD, from < 4 to < 10 mg/kg, depending on the analytical procedures used. The Panel also notes that these levels are lower than 20 mg/kg, the cut-off given in the specifications and considered by Codex Alimentarius and implementing Regulation (EU) No 828/2014 for the labelling of gluten-free foods.

Allergenic proteins

The allergenic proteins listed in the International Allergen Nomenclature reported in the WHO/IUIS database for barley[11] are shown in Table 3.

Table 3: Allergens in barley

Allergen	Biochemical name	Superfamily/family
Hor v 5	B-1 hordein	
Hor v 12	Profilin	Profilin
Hor v 15	α-amylase inhibitor BMAI-1 precursor	Prolamin
Hor v 16	α-amylase	
Hor v 17	β-amylase	
Hor v 20	γ-hordein-3	

BMAI: Barley monomeric α-amylase inhibitor.

According to the WHO/IUIS database, Hor v 5 was recognised in immunoblotting experiments by IgE antibody preparations from individuals allergic to barley pollen allergens. Hor v 12 is a profilin, a pan-allergen,[12] for which no specific data on allergenicity has been reported. Hor v 15 has been identified as a major IgE-binding component in sera from patients with bakers' asthma. Hor v 17 has been identified as potential allergen in barley by Sotkovsky (2008). When tested by ELISA, 91% of patients with WDEIA had IgE-antibodies to Hor v 20 (γ-hordein-3) and skin prick tests (SPTs) were positive in about 47% of the patients tested (WHO/IUIS database). This is because γ-hordein-3 in barley cross-reacts with ω-5 gliadin, a major allergen in wheat triggering WDEIA (Palosuo et al., 2001).

The applicant provided several references in which allergenic proteins in barley were identified. Lexhaller et al. (2019) found three allergenic proteins (Hor v15, Hor v 17 and Hor v 20) to be present in barley, while Hor v 16 was not detected. Considering published data (Weselake et al., 1983; Macgregor et al., 1984; Rahman et al., 1984; Macgregor and Bhatty, 1993; Tanner et al., 2019), the applicant calculated the contribution of individual allergenic proteins to the total protein content in barley seeds as follows: Hor v 15 = 0.1–0.5%, Hor v 17 = 1% and Hor v 20 = 0.6%. The amount of Hor v 16 (α-amylase) was found to be very low in non-germinated seeds and that of Hor v 12 (profilin) was below the limit of detection.

The applicant estimates that a minimum of 21% of proteins in wheat (considering only the gluten fraction) is allergenic, whereas only < 3% of proteins in barley are allergenic. The Panel considers that

[11] Allergen nomenclature: The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee. Barley. Last updated 2014-11-13. http://www.allergen.org/search.php?allergenname=&allergensource=barley&TaxSource=&TaxOrder=&foodallerg=all&bioname=

[12] Panallergens are homologous molecules that originate from a multitude of organisms and cause IgE cross-reactivity between evolutionarily unrelated species.

these calculations are acceptable approximations that reflect current knowledge on the allergenic profile of barley.

The presence of specific barley allergenic proteins in barley starch was investigated by analysis of the peptides resulting after enzymatic digestion, using a proteomic approach and Ultra High-Performance Liquid Chromatography-tandem Mass Spectrometry (UHPLC-MS/MS).

The applicant investigated all allergenic proteins listed in Table 3 except Hor v 12, which was found to be absent in barley flour, and thus, it was not analysed in barley starch.

In addition, the applicant assessed B3-hordein because of its potential relevance to individuals with coeliac disease.

The extraction procedure of allergenic proteins in barley starch used for the UHPLC-MS/MS analysis combines two methods: proteins are extracted at alkaline pH and digested by the proteolytic enzyme trypsin as described by Seung-Taik et al. (1999). The tryptic digest undergoes reduction and alkylation according to a protocol described by Lock (2014), which includes the description of the UHPLC-MS/MS method for the detection of the resulting peptides in the tryptic digest.

An extract of barley flour prepared according to this protocol was used to identify the peptides present. The two most prominent peptides from each protein (Hor v 16, B1-hordein, B3-hordein, Hor v 15, Hor v 17 and γ-hordein-3) were used to identify and quantify the proteins in the barley starch extracts. In order to identify the proteins, the spectra obtained were matched against a library database with different peptide fractions from different protein sources. The LOQ for every single allergenic protein residue was found to be at 1 mg/kg, and LOD at 1 mg/kg, although a lower LOD of 0.5 mg/kg could be obtained when analysing low viscosity samples.

The six proteins were analysed in 42 batches of barley starch and one batch of maize starch. The analysis of every single protein residue gave results below the LOD, except for two batches in which residues of B3-hordein were found: one (#2) just at the LOD of 1 mg/kg and one (#1) above the LOD at 7 mg/kg. The latter is the same batch that had the highest crude protein content and gave positive results for gluten.

The Panel notes that the only barley protein that could be detected in barley starch by UHPLC-MS/MS was B3-hordein, which may be relevant for individuals with coeliac disease but not for individuals with cereal (including barley) allergy.

The Panel considers that the five barley allergens Hor v 16, B1-hordein, Hor v 15, Hor v 17 and γ-hordein-3 may be present in barley starch at doses of < 5 mg/kg (LOD of 1 mg/kg for each of the 5 barley allergenic proteins assessed). Owing to the fact that Hor v 16 in non-germinated seeds may be negligible and that only three allergenic proteins (Hor v 15, Hor v 17, and γ-hordein-3) were detected in measurable amounts in barley (Lexhaller et al., 2019), the Panel considers that 5 mg/kg is a conservative estimate of the content of allergenic proteins in barley starch.

3.5.1.4. Stability

No data has been provided by the applicant on the stability of the protein fraction in barley starch over the shelf-lifetime, which the applicant claims to be 4 years.

3.5.2. Barley starch-derived foodstuffs (food products)

Since the current application for exemption from labelling regards barley starch as an ingredient and/or as starting material for the manufacturing of foodstuffs, general specifications, manufacturing process, allergen specifications or stability data have not been provided for foodstuffs produced from barley starch as starting (raw) material or for foodstuffs containing barley starch as an ingredient.

3.6. Scientific data on allergenicity

3.6.1. Human intervention studies

3.6.1.1. Food challenges and skin prick testing studies

No food challenges in either cereal-allergic or barley-allergic individuals using the food-allergen derived preparation barley starch have been provided by the applicant.

The applicant initially designed a randomised, double-blind, placebo-controlled food challenge (DBPCFC) for the assessment of non-allergenicity of barley starch in cereal (wheat/barley/rye) allergic patients following the EFSA guidance document (EFSA NDA Panel, 2014). The applicant states, however, that the study could not be conducted due to problems with recruitment (i.e. few cereal-allergic patients

could be identified; DBPCFC or open-label oral food challenge for verification of diagnosis of food allergy and DBPCFC for allergenicity testing were refused by parents of young children).

Instead, the applicant provided one published DBPCFC study using barley starch syrup in 15 children with cereal allergy (Nermes et al., 2009). This publication by Nermes et al. (2009) reports on the clinical study reviewed by the NDA Panel in 2007 in the context of a notification by Finnsugar Ltd for the permanent exemption from labelling of glucose syrups produced from barley starch, namely starch hydrolysates (EFSA NDA Panel, 2007). The study was submitted as an unpublished study report and it was quoted as Nermes et al. (2006) in the opinion.

The study was a DBPCFC using barley starch hydrolysate for challenge in individuals with allergic reactions to a mixture of wheat, oats, rye and barley. The study did not specify clinical reactivity to barley alone and was not designed to assess the proportion of barley-allergic individuals that were likely to react to barley starch hydrolysates. Subjects with three or more food allergies were excluded from the study. It included 15 children (age range 0.9–13.8 years) with clinical allergic reactions at skin, intestinal and/or respiratory level. Skin prick test results were positive to barley in 5/15 patients and negative to barley starch hydrolysate in all patients. The Panel notes that 10 out of the 15 cereal allergic patients had negative skin prick tests to wheat, barley, rye, oats and gliadin. The 15 patients were challenged with 30 g of barley starch hydrolysate per day, corresponding to 5.6 mg of barley protein, over a period of 5 days. No allergic reaction was observed (EFSA NDA Panel, 2007).

The Panel notes that glucose syrups based on barley have been exempted from allergen labelling as per Annex II of Regulation (EU) No 1169/2011 following the scientific assessment of the application from Finnsugar Ltd by EFSA. The Panel also notes, however, that the current application is for the exemption from labelling of all foods manufactured from barley starch.

3.6.1.2. Other human intervention studies

In the absence of food challenges conducted with barley starch, the applicant provided published data on food challenges using other cereals in cereal-allergic individuals.

Out of 192 children with atopic dermatitis, 20% showed a positive DBPCFC for wheat with doses ranging from 0.4 to 10 g of wheat flour, corresponding to about 0.04–1 g of wheat protein (Sicherer et al., 2000).

In a study on 145 individuals with positive SPTs to one or more cereal grains, 31 patients (21%) reacted to a food challenge with doses up to 10 g of cereal flour (about 1.2 g of protein). A total of 25 individuals (81%) had positive challenge responses to only one grain (19 to wheat; 3 to corn; and 1 to either barley, rice or rye), whereas six had responses to two or more grains. In total, four patients reacted to barley (Jones et al., 1995).

Among 185 wheat allergic children, 24 (13%) reacted to barley and 18 (10%) reacted to oats upon food challenge. Both peak barley-specific IgE and the barley-specific/wheat-specific IgE ratio predicted clinical reactivity to barley (Keet et al., 2009). This publication is available as an abstract only and no details about the food challenge are provided.

In a study in 265 children with a clinical indication to undergo a food challenge with wheat, the majority of patients (number unspecified) reacted with objective symptoms only to the highest dose tested (2.8 g of protein), whereas only a few children (number unspecified) reacted to the starting dose of 3 mg protein (Rolinck-Werninghaus et al., 2012). No children with a history of anaphylactic reactions to wheat were included in this study.

In one study including 38 children and 41 adults with a convincing clinical history of wheat allergy, 6% of the children reacted to < 10 mg of wheat protein, 40% of children reacted to ≤ 1 g and 80% reacted to < 2 g (single-blind challenge). In adults, no subjects reacted to doses < 1 g of wheat protein, and 50% required at least 6 g to react. A minimal observed eliciting dose (MOED) was not derived by the authors (Moneret-Vautrin et al., 2003).

In a study conducted in Japan (Ito et al., 2008), 35 children sensitised to wheat underwent an oral food challenge (OFC) with noodles containing wheat protein (2.6%) in stepwise increasing amounts (0.1, 1, 2, 5, 10 and 20–50 g). Twenty-one patients (60%) reacted to the challenge at various doses. Children with a convincing history of wheat allergy were not challenged because of their high risk for anaphylaxis. One subject had a severe allergic reaction to 2.6 mg of wheat protein (MOED, first dose tested), whereas two reacted to 26 mg. Similarly, during wheat challenges in 62 children aged 7–103 months, four patients (3%) reacted to 2 mg of wheat protein (personal communication by D. Hill in (Hischenhuber et al., 2006)).

It has been reported that children respond to lower amounts of cereals in oral challenge studies than adults, with 62% of children reacting to $<$ 8–10 g of wheat flour (about 1.2 g of wheat protein).

However, most of these studies have been performed in children with atopic dermatitis and not in children with diagnosed wheat or cereal allergy, and only a few patients with a convincing history of wheat anaphylaxis have been challenged orally (Hischenhuber et al., 2006).

Adult subjects with suspected wheat allergy (convincing clinical history) were recruited in Italy (n = 24) and Denmark (n = 3) and underwent a DBPCFC with wheat flour (Scibilia et al., 2006). A minimum starting dose of 100 mg raw wheat flour was administered in a test meal, sequentially followed by 500 mg, 1 g, 1.5 g, 3 g, 6 g, 12 g until symptoms were reported or signs were observed, or until the entire test meal was eaten. The cumulative dose schedule was 100 mg, 600 mg, 1.6 g, 3.1 g, 6.1 g, 12.1 g and 25 g contained in the entire meal. Doses were administered at 20-min intervals. The same dose schedule was used for cooked wheat (pasta) DBPCFC in patients who had a positive result to raw wheat flour. The minimal eliciting dose (MED) was 100 mg of raw (3 patients) and cooked (2 patients) wheat flour, corresponding to about 10 mg of wheat protein.

Using published (Scibilia et al., 2006; Pastorello et al., 2007; Ito et al., 2008) and unpublished OFC data with wheat flour in wheat-allergic adults and children (Taylor et al., 2014) derived a reference dose of 1 mg of wheat protein based on the 95% lower confidence interval of the population eliciting dose ED_{05} due to the limited data available.

The Panel notes that the lowest reported MOED in wheat allergic paediatric patients undergoing OFCs is about 2 mg of wheat protein (first dose tested). The lowest reported MED in adult wheat allergic patients undergoing DBPCFC is 100 mg of wheat flour, or about 10 mg of wheat protein. However, doses of wheat triggering allergic reactions in sensitive individuals may be lower because patients with a history of severe allergic reactions (WIA, WDEIA) have been excluded from the challenge studies available. The Panel also notes that, whereas barley mono-allergic individuals may be rare, a relevant proportion of wheat allergic individuals reacts to barley upon food challenge (EFSA NDA Panel, 2014).

Owing to the lack of human data available on minimal eliciting doses for barley, the Panel considers that MEDs/MOEDs derived for wheat in wheat allergic individuals could be used for the risk characterisation in the present opinion.

3.6.2. Human observational studies

The applicant claims that case reports of allergic reactions to barley starch are available in the literature. The applicant states that few published reports identify barley (grain form) as a specific ingredient responsible for the observed allergic reactions to beer, such as urticaria or anaphylactic reactions. Some studies report that urticaria from beer is an IgE-mediated hypersensitivity reaction induced by a protein component of approximately 10 kDa deriving from barley malt during the brewing process. The applicant also claims that this detected beer allergen does not seem to be related to the major barley 16-kDa allergen (glycosylated subunit of α-amylase inhibitor) responsible for baker's asthma (Sanchez-Monge et al., 1992; Bonadonna et al., 1999) and may not be relevant for barley starch.

The applicant states that no allergic reactions have been reported to products containing barley starch during the years 2002–2018 and claims a history of safe use over that period.

3.6.3. Animal studies

No animal studies with the food allergen-derived preparation (barley starch) or the food allergen-derived foodstuff(s) (food with barley starch as an ingredient or manufactured from barley starch) have been provided.

3.6.4. *In vitro* studies

The applicant performed inhibition studies to analyse the IgE-binding capacity of some of these barley starch batches (selected based on the results of the above-mentioned protein analyses) using the same maize starch batch as control.

IgE-binding *in vitro* tests were performed by ImmunoSpot® assay using sera from individuals allergic to cereal, as described by Makinen-Kiljunen (1994). ImmunoSpot® is a radioimmunoassay in which the allergen extract is coated on a nitrocellulose membrane and incubated in serum. The IgE bound is detected with a radiolabelled antibody. The Panel notes that ImmunoSpot® assay is a semiquantitative method.

Five barley starch samples from five different batches and one maize starch sample (control sample) were tested against four different pools of sera from cereal allergic individuals and one pool of sera from non-allergic individuals (control sera).

The barley starch samples tested were the following:

a) One sample found to contain residues of B3-Hordein by UHPLC-MS/MS analysis (batch #2),
b) Three samples (batches #3, #4 and #5) selected from the higher and lower ends of total (crude) protein content assessed by the Kjeldahl method,
c) One sample originally planned to be used in the DBPCFC designed by the applicant on barley starch (batch #6)

The ImmunoSpot® was negative for the maize starch sample (control sample) against all five pools of sera. The pool of sera from non-allergic individuals (control sera) gave negative results for all (barley and maize) samples.

The ImmunoSpot® was negative for two barley starch samples (batches #4 and #5), positive for two other barley starch samples (batches #2 and #3), and the results were interpreted as 'weakly positive' for the fifth barley starch sample (batch #6). The applicant claims that the positive results obtained are likely to be due to residual amounts of B3-hordein in the samples, and that ImmunoSpot® analysis was only used for screening purposes.

Upon EFSA's request for clarification, the applicant reassessed the barley starch batch which resulted 'weakly positive' (batch #6) together with the maize starch sample using two pools of sera from cereal-allergic patients and one pool of sera from non-allergic individuals. The results of the ImmunoSpot® analysis were negative. The applicant also reanalysed allergenic proteins in the barley starch sample with UHPLC-MS/MS at an LOD of 0.5 mg/kg. The levels of allergenic proteins were below the LOD.

Upon EFSA's request to provide the clinical characteristics of the cereal-allergic individuals from which the sera used for the ImmunoSpot® analyses were obtained, the applicant answered that such information was not available. Pooled sera test sample #1 consisted of 22 test patients who attended a clinic for allergy testing (IgE antibody concentration in sera > 10 kU/L to cereals; 19 kU/L to barley, 17 kU/L to gluten and 32.1 kU/L to wheat), whereas pooled sera test sample #2 consisted of 37 patients (IgE antibody concentration in sera > 10 kU/L to cereals, 15 kU/L to barley and 29.4.1 kU/L to wheat). The Panel notes that the pooled sera have not been characterised with respect to IgE antibodies specific to ω-5 gliadin.

The Panel considers that the results of the ImmunoSpot® analyses are inconclusive, particularly for patients with WIA or WDEIA.

3.7. Exposure to the food allergen-derived preparation (barley starch)

Appendix A shows the calculated exposure to barley starch, to total protein from barley starch, both assuming a protein content of 0.78 g/kg (as estimated by the Bradford method) and of 0.5 g/kg (as estimated by the single amino acid method), and to barley allergenic proteins assuming a content of 5 mg/kg (LOD of 1 mg/kg for each of the 5 barley allergenic proteins assessed with ELISA). In all cases, intake estimates are given per day and per meal (41% of daily digestible carbohydrates), considering three scenarios for children (Appendix A.1) and four scenarios for adults (Appendix A.2). Such scenarios assume that:

a) 100% of digestible carbohydrates are consumed as barley starch, which is a worst-case but also a non-realistic scenario,
b) 35% of digestible carbohydrates are consumed as barley starch, as contained in staple foods such as bread and rolls, pasta, doughs and similar, which is a 'realistic' worst-case scenario,
c) 12% of digestible carbohydrates are consumed as barley starch, a 'realistic' scenario proposed by the applicant,
d) one portion per meal and two portions per day of sports foods containing 75 g of barley starch per portion are consumed, which is a 'realistic' worst-case scenario for adults.

For all these scenarios, the calculated total protein intake from barley starch was above the MED/MOED for wheat (expressed in mg of wheat protein) in adults (10 mg) and children (2 mg).

Using data published in the literature (Weselake et al., 1983; Macgregor et al., 1984; Rahman et al., 1984; Macgregor and Bhatty, 1993; Uvackova et al., 2013; Lexhaller et al., 2019; Tanner et al., 2019), the applicant estimates that a minimum of 21% of proteins in wheat (considering only the

gluten fraction) is allergenic, whereas only < 3% of proteins in barley are allergenic. Using these data, the applicant re-calculated the MED/MOED for wheat in terms of 'allergenic protein content', rather than in mg of total protein in wheat, for adults (2.1 mg) and children (0.42 mg). The margin of exposure in the 'realistic' scenario proposed by the applicant ranged from 4.5 to 11.1 in children and from 6.4 to 15.0 in adults. In a 'realistic' scenario where 35% of digestible carbohydrates could come from barley starch, the margin of exposure ranged from 1.6 to 3.8 in children and from 2.2 to 5.2 in adults, and from 2.8 to 6.8 for sports foods.

3.8. Weighing of the evidence

In weighing the evidence, the Panel considered that:

a) Owing to the use of non-germinated barley seeds as starting material and to the manufacturing process, the amount of protein in barley starch is likely to be lower than that assessed by the Kjeldahl method and provided in the product specifications, possibly < 0.5 g/kg, whereas the amount of allergenic proteins is likely to be < 3 mg/kg rather than < 5 mg/kg (as used in the risk characterization);
b) Barley mono-allergic individuals may be rare; wheat-allergic individuals often manage their allergy by consuming gluten-free products while avoiding wheat, with no specific restriction of other gluten-containing cereals;
c) It is generally accepted that barley is less allergenic than wheat, and that MED/MOED derived for wheat may be lower than MED/MOED for barley;
d) Considering the MED/MOED calculated by the applicant for allergenic proteins in wheat, the margin of exposure could be from 11 up to 16 in the 'realistic' case scenario proposed by the applicant.

However, the Panel also considered that:

a) The exemption from labelling is requested for barley starch used as raw material for further processing or as an ingredient, and not for specific food products containing barley starch;
b) No human intervention studies have been provided with barley starch in individuals allergic to barley, wheat or other cereals;
c) MED/MOEDs for barley are not available in the literature;
d) Whereas barley mono-allergic individuals may be rare, a proportion of wheat allergic individuals react to barley upon food challenge;
e) Even if WDEIA is a rare condition, symptoms are life-threatening, and either avoidance of wheat and exercise, or a gluten-free diet, are effective strategies to avoid symptoms in about two-thirds of the patients only;
f) The results of the ImmunoSpot® test are inconclusive with respect to barley starch, particularly for patients with WIA or WDEIA;
g) The calculated total protein intake from barley starch was beyond the MED/MOED for wheat in adults (10 mg) and children (2 mg) for all the exposure scenarios considered;
h) In a 'realistic' scenario where 35% of digestible carbohydrates could come from barley starch, the margin of exposure for the MED/MOED calculated by the applicant for allergenic proteins in barley ranged from 1.6 to 3.8 in children, and from 2.2 to 5 in adults (from 2.8 to 6.8 considering sports foods only). These margins of exposure are insufficient to conclude on the likelihood of allergic reactions in cereal-allergic individuals owing to the uncertainty associated with the assumptions made regarding the calculation of the wheat protein fraction that is allergenic and the insufficient characterisation of the risk for wheat-allergic patients with WIA or WDEIA.

4. Conclusions

Based on the data presented by the applicant and on data currently available in the literature, the Panel concludes that:

- The data available are insufficient to conclude on the likelihood of adverse allergic reactions in cereal-allergic individuals upon consumption of barley starch under the conditions of use proposed by the applicant.

- For coeliac disease, assessment of the evidence submitted indicates that the consumption of foodstuffs produced from barley starch as starting (raw) material or foodstuffs containing barley starch as an ingredient are unlikely to cause an adverse reaction in individuals with coeliac disease who are not allergic to cereals, provided that the value of gluten considered by Codex Alimentarius and implementing Regulation (EU) No 828/2014 for 'gluten-free' foods (20 mg/kg) is not exceeded.

5. Documentation as provided to EFSA

Application for exemption from allergen labelling on barley starch pursuant to article 21 paragraph 2 of Regulation (EC) 1169/2011. February 2019. Submitted by Lyckeby Starch AB.

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Abbreviations

AACCI	American Association for Clinical Chemistry International
AOAC	Association of Official Agricultural Chemists
AR	Average requirement
BMI	Body mass index
BMAI	Barley monomeric α-amylase inhibitor
CFU	Colony-forming units
DBPCFC	Double-blind placebo-controlled food challenge
DNA	Deoxyribonucleic acid
DRV	Dietary Reference Values
E%	Percentage of energy
EC	European Commission
ED	Eliciting dose
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FDEIA	Food-dependent exercise-induced anaphylaxis
FSSC	Food Safety System Certification
Hor	Hordein
IgE	Immunoglobulin E
IUIS	International Union of Immunological Societies
kDa	KiloDalton
LOD	Limit of detection
LOQ	Limit of quantification
MED	Minimal eliciting dose
MoE	Margin of exposure
MOED	Minimal observed eliciting dose
NDA	Scientific Panel on Dietetic Products, Nutrition and Allergies
NKML	Nordic Committee on Food Analysis
OFC	Open food challenge
OMA	Official methods of analysis
PAL	Physical activity level
PCR	Polymerase Chain Reaction
RI	Reference Intake
SPT	Skin prick test
UHPLC-MS/MS	Ultra-High-Performance Liquid Chromatography-tandem Mass Spectrometry
UK	United Kingdom
WDEIA	Wheat-dependent exercise-induced anaphylaxis
WHO	World Health Organisation
WIA	Wheat-induced anaphylaxis

Appendix A – Calculated exposure to barley starch, to total protein from barley starch, and to allergenic proteins in barley starch

Table A.1: Young children

Scenario	Barley starch (g)		Barley total protein (mg), assuming a protein content of 0.78 g/kg					Barley total protein (mg), assuming a protein content of 0.5 g/kg					Barley allergenic proteins (mg), assuming a content of 5 mg/kg				
	Per day	Per meal	Per day	Per meal	MED/MOED (mg)	MoE		Per day	Per meal	MED/ MOED	MoE		Per day	Per meal	MED/ MOED	MoE	
						Per day	Per meal				Per day	Per meal				Per day	Per meal
1. 100% CHO	154	63	120	49	2	< 1	< 1	77	32	2	< 1	< 1	0.77	0.32	0.42	0.6	1.3
2. 35% CHO	54	22	42	17		< 1	< 1	27	11		< 1	< 1	0.27	0.11		1.6	3.8
3. 12% CHO	18.5	7.6	14.4	5.9		< 1	< 1	9.3	3.8		< 1	< 1	0.093	0.038		4.5	11.1

Table A.2: Adults

Scenario	Barley starch (g)		Barley total protein (mg), assuming a protein content of 0.78 g/kg					Barley total protein (mg), assuming a protein content of 0.5 g/kg					Barley allergenic proteins (mg), assuming a content of 5 mg/kg				
	Per day	Per meal	Per day	Per meal	MED/ (mg)	MoE		Per day	Per meal	MED/ MOED	MoE		Per day	Per meal	MED/ MOED	MoE	
						Per day	Per meal				Per day	Per meal				Per day	Per meal
1. 100% CHO	552	226	431	177	10	< 1	< 1	276	113	10	< 1	< 1	2.76	1.13	2.1	0.8	1.9
2. 35% CHO	193	79	151	62		< 1	< 1	97	40		< 1	< 1	0.97	0.40		2.2	5.2
3. 12% CHO	66	27	52	21		< 1	< 1	33	13.5		< 1	< 1	0.33	0.14		6.4	15.0
4. Sports foods	150	75	117	59		< 1	< 1	75	38		< 1	< 1	0.75	0.31		2.8	6.8

MED/MOED: minimal (observed) eliciting doses; MoE: margin of exposure, obtained dividing the MED/MOED by the intake.