codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 4 **CX/NFSDU 00/4-Add. 1**
April 2000

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES
Twenty-second Session
Berlin, Germany, 19-23 June 2000

DRAFT REVISED STANDARD FOR GLUTEN-FREE FOODS
- Comments at Step 6 of the Procedure -

Comments from:

FINLAND
KOREA, REPUBLIC OF
POLAND
SPAIN
SWEDEN

AOECS - ASSOCIATION OF EUROPEAN COELIAC SOCIETIES
ISDI - INTERNATIONAL SPECIAL DIETARY FOODS INDUSTRIES
PWG - WORKING GROUP ON PROLAMIN ANALYSIS AND TOXICITY

FINLAND

Finland proposes that oats should be removed from the list of cereals not suitable for patients with coeliac disease. Oats is mentioned in the draft standard (Alinorm 97/26, Appendix V), sections 2.1.a, 2.2.1 and 3.1. Regarding the reason, we would like to refer to the annexed overview of the recent clinical scientific research on oat and coeliac disease.

Finland supports the proposal for two levels of gluten. For foodstuffs that naturally do not contain gluten, the level should be determined so that it in a reliable way can be verified in laboratory tests. Finland considers that 40 ppm is easier to detect than the proposed 20 ppm by means of the present methods of analysis. Finland considers further the proposal for maintaining the gluten-free level at 200 ppm for wheat-, barley- or rye-based products appropriate.

In Finland wheat-starch based gluten-free products are widely available, the use of these products makes the baking easier and can even improve the dietary compliance. In Finland, coeliac disease and dermatitis herpetiformis patients and their doctors are satisfied with the diet based on gluten-free wheat starch and this diet has been in use for more than 30 years. According to the scientific evidence, no excess morbidity, malignant diseases nor extra mortality has been observed in individuals maintaining wheat-starch based but otherwise strict gluten-free.

Finland proposes that in the standard should be included a paragraph stressing the importance of the control of the points most critical for the gluten-free status of the product (HACCP). Special emphasis should be laid on the fact that during the production, processing, transportation, storage, serving and marketing of gluten-free foods, it should be ensured that gluten-free foods are not coming into contact with forbidden cereals and that the products are not mixed with foods containing gluten at any stage of the process.

Appendix

Oats and coeliac disease
- A report by the Finnish Coeliac Society on the suitability of oats for patients with coeliac disease

Oats is well tolerated by most patients with coeliac disease and dermatitis herpetiformis. All clinical research today speaks for the allowance of oats in the coeliac diet (Janatuinen et al., 1995; Srinivasan et al., 1996; Hardman et al., 1997; Feighery et al. 1998; Holm et al., 1998; Storsrud et al., 1998, Reunala et al., 1998, Withers et al, 1999; Urbonas V, 1999). Gliadin is the injurious constituent of wheat for individuals with coeliac disease and is found in the alcohol-soluble protein fraction called prolamin. Oats do not contain gliadin but have avenin in their prolamin fraction, and due to its different amino acid sequence avenin is considered to cause less, if not any, harm for coeliac patients. Furthermore, the total amount of avenin in the seed of oats is smaller than that of gliadin in wheat (1). In Scotland, oats was never forbidden in the diet of coeliac patients, and they have managed well (2).

A randomized trial in Finland in 1995 showed, that even newly diagnosed coeliac disease patients, when on an oat-containing, but otherwise gluten-free diet, recover as well as those on a traditional gluten-free diet when observing the architecture of their duodenal villi (3). Further, a study in Ireland in 1997, showed that oats do not cause any changes in the endomysial or gliadin antibody levels or in the densities of intraepithelial lymphocytes of coeliac patients (4). As the blistering rash occurring in dermatitis herpetiformis has been considered as a very sensitive marker to dietary lapses, a study of suitability of oats in the diet of these very sensitive patients with dermatitis

herpetiformis was performed in England in 1997. The results showed, that even the patients with dermatitis herpetiformis can include oats in their diet without getting any rash, changes in the villous architecture of small intestine, increase of antibody levels (antigliadin, antireticulin, antiendomysium), any abnormalities in the duodenal intraepithelial lymphocyte counts or in the levels of dermal IgA -antibodies (5). Similar results were recently obtained in Finland, the observation period was twice longer and no inflammation in the small-bowel mucosa was observed (6). Primary results of a study conducted in children in Finland in 1998, show that even newly diagnosed patients can include oats in their otherwise gluten-free diet, and that they recover as rapid as the children in the control group, who followed a normal gluten-free diet. The results express, that children with coeliac disease are able to ingest even high amounts of oats without harmful effects on the jejunal mucosal architecture, intraepithelial lymphocyte counts, antibody levels or clinical symptoms (7).

Other important issues concerning the allowance of oats in the diet of coeliac disease and dermatitis herpetiformis patients are, that oats would make the difficult coeliac diet much easier and comfortable to follow (1, 8), and what also important, reduce the high costs of the diet. Furthermore, the gluten-free diet is often rather poor in dietary fibre (9), and oats might bring more beneficial fibre in the diet in a normal way. So far the only problem with oats is, that it may be contaminated with other, gluten containing cereals. This could, however, be solved by separating the production of "coeliac friendly" oats from other production.

References:

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3. Janatuinen E, Pikkarainen P, Kemppainen T, Kosma V-M, Järvinen R, Uusitupa M, Julkunen R. A comparison of diets with and without oats in adults with coeliac disease. N Engl J Med 1995; 333:1033-37.
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6. Reunala T, Collin P, Holm K, Pikkarainen P, Miettinen A, Vuolteenaho N, Mäki M. Tolerance to oats in dermatitis herpetiformis. Gut 1998;43:490-493.
7. Holm K, Vuolteenaho N, Mäki M. No harm of oats in the diet of children with newly or previously diagnosed coeliac disease (CD). In: Lohiniemi S, Collin P, Mäki M, eds. Changing Features of Coeliac Disease. Tampere, Finland: Finnish Coeliac Society, 1998:116.
8. Storsrud S, Lenner R, Kilander A. The oat-coeliac study in Gothenburg. In: Lohiniemi S, Collin P, Mäki M, eds. Changing Features of Coeliac Disease. Tampere, Finland: Finnish Coeliac Society, 1998:116.
9. Lohiniemi S, Mäki M, Hallert C, Collin P. Quality of life in coeliac disease patients treated with gluten-free diet for 10 years. Scand J Gastroenterol 1998; 33 Suppl 227:16.
10. Urbonas V. Oats in the diet of coeliac children. Eighth International Symposium on Coeliac Disease, Poster 055. Naples, Italy April 1999.
11. Withers GD, Driedger L J, Trevenen CL, Scott RB, Butzner JD. Addition of oats to the diet of children with coeliac disease. Eighth International Symposium on Coeliac Disease, PD 4. Naples, Italy April 1999

KOREA, REPUBLIC OF

- It is supposed to take time a long to get in conclusion in preparing test method for gluten determination which is sensitive and reliable.
- wish single level criterion for gluten residue would be settled, and the term of 'gluten-free' be reviewed to such as 'gluten-low' simultaneously allowing gluten-free if not containing gluten in the product.
- if the test method would not have been prepared in near future, it can be a way that available method be authorized temporary.

POLAND

According to the Polish draft food legislation the permitted level of heavy metals should be for the gluten-free products:

- Pb – no more than – 0,1 mg/kg
- Cd – no more than – 0,01 mg/kg
- Hg – no more than – 0,01 mg/kg
- As – no more than – 0,10 mg/kg
- Zn – no more than – 30,0 mg/kg.

SPAIN

Subsection 2. 1. Definition:

We suggest deleting the square brackets from oats as this cereal may affect the health of coeliac patients because of possible sensitivity to gluten.

We do not agree with having two different levels of gluten content which we believe could mislead consumers. A single level should be proposed that will protect consumer health and safety, and be based on the establishment of a sufficiently sensitive method of analysis to detect all the prolamins active in causing the disease. This level could be set in principle at 20 ppm until an appropriate method of analysis is determined.

Paragraph 2.2.1:

We suggest that the square brackets around oats be deleted.

Subsection 3.1:

We suggest that the square brackets around oats be deleted.

The comments made on level of gluten in Subsection 2. 1. also apply here.

Subsection 7.1:

We suggest that the wording: "It is however important to stress that the total daily intake of prolamin for coelaic patients should not exceed 10 mg per day" be placed in square brackets until there is scientific justification for such a statement.

General Comments on Section 6. General Outline of the Method of Analysis and Sampling

In view of the title of this section, we must consider:

1. The Content.

   If this is to be an outline, it should only contain general guidelines setting the minimum requirements regarding:

   - Presentation of the section: to reflect the content of the ISO or CEN standards.
   - Minimum requirements of sensitivity, selectivity, precision in repeatability and replicability.
   - Methodological justification, with a general indication of the most appropriate techniques: ELISA, immunoblotting, mass spectrometry.
   - Bibliography.

2. Development of the Section

   The various items are not uniformly structured; some providing general information while others going into minute detail of a particular method rather than providing a general overview as the title would suggest - for example: plate preparation (only applicable to certain methods), reagents, etc.

   Similarly, apparently descriptive paragraphs lack essential details such as final pH, buffer concentration, interpretation of results, readings, calculations, which would make reliable replication of the method difficult.

   Moreover, one vital component of the method, obtaining the aliquots for analysis, is not mentioned anywhere.

3. Criteria for the Selection of Methods.

   Given rapid technological progress, there is little sense in describing an analytical method in a normative document. It would be more appropriate to refer to or recommend internationally accepted methods such as those of the ISO, CEN or AOAC.

   We do not therefore feel that the method should be described here and in such detail. It would be more appropriate to recommend internationally agreed methods and provide sound scientific support and clear references.

   Nevertheless, the following comments refer to specific shortcomings of the document.

SPECIFIC COMMENTS AND SUGGESTED AMENDMENTS TO SECTION 6. General Outline of the Method of Analysis and Sampling

N.B.: The existing text is given in normal print, while proposed additions and amendments are given in underlined italics.

6. GENERAL OUTLINE OF THE METHOD OF ANALYSIS AND SAMPLING

6. 1. **Introduction** (first paragraph, indents 2 and 4)

- the availability of a standard *for the different prolamins (gliadins, secalins, hordeins and avenins)*.
- the effect of heating of the product on *the configurational stability of the prolamins* and the integrity of *the epitopes*.

6.2. **Determination of gluten in foodstuffs and *food* ingredients**

The determination of gluten in foodstuffs and *food* ingredients shall be based on an immunologic method.

The antibody to be used should react *in a specific manner* with *the allergen proteins from* the cereals that are toxic for persons sensitive to gluten and should not cross-react *with the proteins of* the other cereals or other constituents of the foodstuffs and *food ingredients*.

6.3. **Extraction of prolamins**

6.3.2. Extraction

Studies carried out by the National Food Centre (Centro Nacional de Alimentación - CNA) on the development of an immunoblotting method to gluten in-foods have revealed that a number of factors influence the level of prolamin extraction: nature of the extraction solvent (ethanol, isopropanol, etc.), proportion of extraction solvents, extraction time (15 minutes is insufficient), intensity of agitation (agitation facilitates extraction), temperature of the extraction process and number of times that the extraction process is repeated.

It has also been noted that once the prolamins have been extracted, the supernatant cannot be stored at 4 °C, because there may be precipitation of the allergen proteins. If this precipitate is removed, a significant proportion of the extracted prolamins will most probably also be eliminated.

6.4. **Determination of Gliadin**

6.4.1. *Sensiticity of the microtiter plates*

Fixing the microtiter plates of the monoclonal antibody (or polyclonal, previously obtained against gliadin) is carried out by adding [ ] ml of an appropriate dilution of the antibody (for example, 1:600) in a carbonate-bicarbonate buffer solution [ ] Mm, pH [ ]. The microtiter plates are left to incubate overnight at [ ] °C. They are then washed three times in saline solution with 0.05 % Tween ... (20?, 80?, ...); and subsequently with deionised water containing 0.03 % Na-azide.

The plates prepared in this manner can be then be stored at 4 °C in a sealed plastic bag for ... [ ] ... months without appreciable loss of antibody properties.

6.4.2. *Standard*

It is necessary to use ... *to compare the results obtained in different laboratories and with different analysis techniques*.

6.4.3. Determination

After appropriate dilution of the extract of the samples, these are added to the wells of the plate, together with the appropriate dilutions of the gliadin standard to obtain a standard curve. After incubation of [two] hours at [ ] °C (*the commercial kits currently available on the market recommend 30 minutes at ambient temperature*), the plates are washed three

times (*the commercial kits recommend four washes*) with wash solution (PBS-Tween). The monoclonal or polyclonal antibody against gliadin conjugated with an enzyme is then added to the wells, and after incubation of [*two*] hours at [ ] °C (*the commercial kits currently available on the market recommend 30 minutes at ambient temperature*), the plates are emptied and again washed three times (*the commercial kits recommend four times*) with PBS-Tween solution. The substrate for the enzyme is then added and after an appropriate time (*the commercial kits generally recommend 10 minutes*) the reaction is interrupted with the *retardation solution. The absorption level is measured directly on a microtiter plate reader at the appropriate wavelength which depends on the chromogen used in the reaction (generally at a λ between 405 and 450 nm).*

SPECIFIC COMMENTS ON SECTION 7: REMARKS

7.3. There are at least four types of monomeric gliadin (α, β, γ and ω) which are different in molecular weight and composition. Commercial ELISA kits usually use antibodies against ω-gliadins. However, it has been demonstrated that α-gliadins are also toxic for coeliacs and various toxic fragments have been identified after digestion with trypsin.

Many proteins of the gliadin mixture are made up of identical peptides. Efforts have been made to identify the possible toxic sequences, but ethical problems have arisen in determining their capacity to damage the intestinal cells of coeliac patients. Once the toxic sequence of the different prolamins has been identified, the next step will be to artificially produce very specific antibodies, thus solving one of the major existing problems, namely **false negatives**.

Subsequently, the aminoacid sequence of gluten in the germplasm of allergen cereals could be biotechnologically manipulated with the development of transgenic crops containing non toxic prolamin.

7.4. There is a very close genetic relationship between wheat and rye, less so among wheat, barley and oat, and still less between rice and maize. The prolamins of the most closely related cereals are sufficiently similar to provoke immunological responses in human beings, showing considerable cross-sensitivity.

Troncone et al already demonstrated in 1987 that manifested cross-sensitivities suggest that antibodies obtained by immunizing rabbits with gliadin or α-gliadin also produce high antibody properties against prolamins in barley, oat and ***maize***. Coombs et al had already observed a similar phenomenon in 1983 from the immunization of guinea pigs.

The antibody could react directly to a common sequence for these compounds, but this is unlikely to be the toxic sequence, as it has been seen to form part of the non-toxic proteins. In other words, evidence to date indicates a dissociation between the immunogenetic properties of cereals and their toxicity to coeliac patients.

The immunological reaction that exists in different cereals is the greatest problem when employing immunological methods to detect gluten in gluten-free foods for coeliacs, because of the chances of obtaining ***false positive results***.

This has been shown in studies by the CNA using an immunoblotting method to determine gluten in foods: the electrophoretic separation of the proteins extracted from different cereals (both allergenic and non-allergenic for coeliacs), followed by transfer onto nitro-cellulose membrane and incubation with conventional rabbit polyclonal antisera to gliadin, has demonstrated the existence of such cross-reactions basically with non-allergenic proteins

present above all in maize. These non-allergenic proteins, which are also capable of reacting with anti-gliadin antibodies, have been differentiated and identified thanks to their different electrophoretic mobility from their lower molecular weight.

For this reason, the ELISA method needs to be supplemented by one or more other methods involving some form of separation of the different cereal proteins (immunoblotting, HPLC, mass spectrometry) which will reveal the false positive.

SWEDEN

Background

The current standard (1981) regulates cereal products rendered substantially gluten-free. An upper limit for residual proteins is defined as a maximal nitrogen content in the specific cereal product (e.g. wheat starch). For products naturally free from gluten it is stated that these products shall contain no gluten. That is gluten not detectable.

Since the eighties it has been proposed that the standard should be revised so that "gluten-free" means that the total content of prolamins from cereals shall not exceed a specific limit. In addition, the determination should be performed by an antibody-based immunological method for the specific determination of gliadin (gluten). In November 1998 CCMAS stated that it would have no objection to the use of proprietary analytical methods, provided that similar methods or materials supplying similar results were available (para 8, Alinorm 99/23 CCMAS November 1998).

Swedish position

Sweden is in favour of advancing the proposed draft revised standard in CX/NFSDU 98-4 with two different maximum levels, 20 ppm for products naturally free from gluten and 200 ppm gluten for products rendered gluten-free. Both levels shall refer to products ready for consumption. A single general limit of 200 ppm cannot be accepted, since data suggest (Enclosure 1) this gluten level inappropriate for persons who are especially sensitive to gluten. Highly sensitive consumers suffer adverse reactions to products containing as little as 70 ppm gluten. To satisfy the particular dietary requirements for such sensitive individuals, the gluten content of naturally gluten free products should be undetectable with currently available methods i.e. below 20 ppm gluten.

For less sensitive celiac patients gluten-free products containing wheat starch have been accepted as food (mainly as flour-mixes) in Sweden since 1966. These mixes contain small amounts of gluten. Analyses at the National Food Administration show ranges from below 20 ppm to about 400 ppm gluten. The use of wheat starch in gluten-free mixes gives a much better gluten-free bread than other kinds of starch which can help these patients to adhere to their diet.

Analytical method

Sweden proposes the method described in AOAC Official Methods of Analysis; Supplement March 1995: Chapter 32:13.32.1.24.AOAC Official Method 991.19 Gliadin as a measure of gluten in foods. Colorimetric monoclonal antibody enzyme immunoassay method (or similar) for CCMAS approval. A collaborative study has been carried out and was presented at the last CCNFSDU-meeting september 1998. The report "Gluten determination in foods by an enzyme immuno assay - collaborative study" by Ingrid Malinheden Yman can be obtained from the National Food Administration.

AOECS - ASSOCIATION OF EUROPEAN COELIAC SOCIETIES

2.1 Definition

The square bracket should be deleted from "Oats". A common consensus of the medical consultants of AOECS members has not been reached till now, whether oats can be consumed by coeliacs or not. Further studies about the tolerance of oats for coeliacs are requested by scientists. This point of view is expressed by 17 countries, one country, Finland, did not support.

Regarding the Definition 2.1 a) the figure of 20 ppm should be replaced by the **lowest detectable limit of the analytical method**. It is very essential for coeliacs to exclude contamination with gluten in these kinds of products.

Regarding the Definition 2.1 b) and 2.1 c) and the mentioned figure of 200 ppm **AOECS asks the manufacturers to continue to work to produce wheatstarch at the safest possible level.**

However, the figures of "gluten-free" according 2.1 a), b) and c) should refer to the end product and not to dry matter basis, because this is more informative for gluten intolerant consumers. Additionally, the analysis always refers to the endproduct.

4. LABELLING

After the sentence "the term 'gluten-free' shall be given in the immediate proximity of the name of the product" we ask to add:

"The type and amount of starch used in accordance with 2.1b) must be specified".

5. CLAIMS

We ask the Committee to accept a new point

"5.2. A food which is naturally free of gluten (2.1 a) can be labelled 'gluten-free by nature' only in accordance to 3.1 and 3.3 to claim it is suitable for use in a gluten-free diet.

There are products on the market with the claim "gluten-free by nature", which are not special dietary products. We feel it is necessary to protect the claim "gluten-free by nature", it should only be used if analytical certification has proved that the product has not been contaminated during transport and/or production procedures.

ISDI - INTERNATIONAL SPECIAL DIETARY FOODS INDUSTRIES

INTRODUCTION

- Taking into account available information on clinical tolerance, analytical methodology and current practice, the International Special Dietary Foods Industries (ISDI) conclude that a maximum level of 200ppm gluten should be allowed for all foods presented for coeliacs. This position paper should serve as a justification of this conclusion discussed with and endorsed by several parties. These parties are indicated in the Annex.

  This paper should be read in conjunction with the separate paper submitted to the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) presenting results of a recent discussion between representatives of ISDI, the Association of European Coeliac Societies (AOECS) and the scientific expert group on Prolamin Analysis and Toxicity.

BACKGROUND

- The current Codex Standard for Gluten-free Foods (CODEX STAN 118-1981) "applies to those processed foods which have been specially prepared to meet the dietary needs of persons intolerant to gluten".

  Currently, it describes a "gluten-free food" as:

  - consisting of or containing as ingredients such cereals as wheat, triticale, rye, barley or oats or their constituents, which have been rendered "gluten-free"; or
  - in which any ingredients normally present containing "gluten" have been substituted by other ingredients not containing "gluten".

- The 22nd Session of the Codex Alimentarius Commission in June 1997 adopted the proposed draft standard for gluten-free foods at Step 5 of the Procedure. However, it was recommended that comments on methods of analysis and on amounts of gluten in gluten- free foods should be taken into account when finalising the standard.

  The adopted proposed draft defines three groups according to their gluten contents in the end products:

  - foods from naturally gluten-free ingredients: max. level of [20ppm] gluten
  - foods from ingredients which have been rendered "gluten-free": max. [200ppm] gluten
  - foods from any mixture of the above two categories: max. [200ppm] gluten

  The values were retained in square brackets, as further discussions were required to ascertain whether such levels were possible and to allow further debate.

CURRENT POSITION

- At the 21st Session of the CCNFSDU held in Berlin in September 1998, the revised Standard on Gluten-free Foods discussed at Step 7 of the Procedure did not advance. Instead, it was returned to Step 6 of the Procedure for further consideration. The Committee also agreed that the question regarding proprietary techniques should be raised to the CCMAS as a general matter.

  This was following much debate and difference of opinion on:

  - gluten detection limits
  - the lack of analytical methodology to measure accurately low levels of gluten
  - clinical levels of significance
  - labelling statements for gluten-free products

ISSUES OF CONCERN

<u>Analytical methodology</u>

A crucial issue is to establish a validated method in terms of accuracy, precision and reproducibility, allowing proper quantification of gluten, to which coeliacs are intolerant, while remaining low cost to permit wide usage. In the absence of such a method, any other consideration is meaningless, as verification of levels cannot be proven. The Working Group on Prolamin Analysis and Toxicity[1] is a body of experts, which has been meeting on an annual basis since 1985, with the aim of assessing toxicity of foods for coeliac patients, and co-ordinates the research of its members in this field. To

[1] Working Group on Prolamin Analysis and Toxicity: Chairman- Prof. Dr. M.Stern, Universitäts-Kinderklinik, Tübingen

date, there has been no definitive test method agreed but progress has been made and this work is steadily continuing. The signatories to this text urge speed to resolve these issues and support the Prolamin Working Group, and other scientific organisations, in all their efforts.

Limit of determination

Limits of 20ppm and 200ppm are proposed for foods prepared from naturally gluten-free ingredients, and ingredients which have been rendered gluten-free, respectively. Questions have been raised with each of these levels. The scientific literature does not determine a limit of clinical significance that would help in understanding the consequences of the limit of determination. It shows studies that may be criticised in a variety of aspects and thus do not provide a positive scientific basis on which a clinically significant level can be established. Tests performed may have been in artificial conditions[2], are not always double blind[3] or cannot be deemed as conclusive as a reliable means of measuring gluten is not yet available.[4]

Naturally gluten-free (20ppm)- this level seems illogical and was arbitrarily chosen following selected test results from analyses of products presented by the Swedish delegation, but not on the basis of medical requirement.[5] Several experts believe that a level of 20ppm for 'naturally gluten-free' foods seems unnecessarily low. There have been no known adverse effects to gluten in trace amounts. Any drive towards very low gluten levels is likely to have unnecessarily adverse effects on the foods (cost, availability, sensory quality) which would ultimately be to the detriment of those individuals with Coeliac Disease.

It is assumed that foods prepared from naturally gluten-free ingredients will be absolutely free of gluten, so long as adequate manufacturing procedures have been followed, in order to avoid contamination and carry over. The reality of the situation[6] is that a significant level of carry over from wheat in naturally gluten-free ingredients is common at any early stage in the supply chain. A small level of carry over is accepted within the cereal industry and existing Codex Standards currently determine a level of contamination of cereals by other edible grains.[7] A contamination of rice with only 1% wheat would result in a gluten content of 850ppm. In order to remain within the limit of 20ppm the contamination of the grain would have to be below 0.023%.

The Coeliac Society of the UK has expressed concern[8] that some naturally gluten-free products have been shown, in analysis in both Sweden and UK, to contain levels in excess of 40ppm gluten thus proving that control to such a low level of 20ppm is currently virtually impossible. There is concern that manufacturers may feel it is no longer commercially viable to fund expensive analysis of products to guarantee such low levels, which are difficult to achieve. This would particularly affect products that are currently included in food lists of products deemed to be 'gluten-free' and made available to Coeliacs. The effect of such measures could lead to the supply of only a few specialist

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[2] Lavo B (1990). *Gastroenterology* 99(3): 703-7; Greco, L (1991). *Arch.Dis.Child.* 66: 83-5

[3] Chartrand LJ (1997). *J.Am.Diet.Assoc* 6: 612-618

[4] Chartrand LJ (1997). *J.Am.Diet.Assoc* 6: 612-618; Ellis HJ (1998). *Gut* 43: 190–195

[5] 20th Session CCNFSDU, Bonn 1995**.** Alinorm 95/26

[6] ISDI comments to CCNFSDU on Alinorm 97/26: ISDI ref. 98/135, 15 April 1998

[7] maize(corn) CODEX STAN 153-1985 (Rev.1–1995); wheat CODEX STAN 199-1995; oats CODEX STAN 201-1995

[8] Position paper prepared by R.Ward, Coeliac Society of UK, February 1999

items, of poorer sensory quality, with limited availability. The British Nutrition Foundation (BNF)[9] has acknowledged the better taste of wheat starch-containing products, noting a residual quantity of gluten is present, tolerated by most coeliacs; they recommend that those few individuals who present with symptoms while consuming a gluten-free diet should resort to wheat-free products.

Rendered gluten-free (200ppm) - Justification for the choice of this level needs to be supplied as some delegations are challenging this level. This level is in fact a factor of about two lower than the amount of gluten observed in products prepared with ingredients at the current Codex limit of 0.05% nitrogen in the cereal grain.[10] Opinion[11] suggests that, from a patient intolerance viewpoint, and the safety of products, a level of 200ppm is reasonable. Products containing gluten levels at this level are currently marketed in several EU countries e.g. UK, France, The Netherlands, Spain, Portugal, Sweden, with no known problems or adverse effects reported. In the opinion of renowned experts,[12] support is given for a level of 200ppm since the proposal to support a reduction of the maximum level of gluten in gluten-free foods to 20ppm has no scientific grounding.

It is reported[13] that there was no evidence of small intestinal mucosal damage in patients with coeliac disease who have been on wheat-containing gluten-free products for a mean of ten years. There is no clinical evidence to suggest that a drastic lowering of the permitted gluten level would help avoid potential complications of malignant transformation in Coeliac Disease. Hekkens[10] reports that the risk of malignancy in coeliac patients taking a gluten-free diet is the same as in the normal population. He concludes that since a gluten-free diet contains some gliadin then the intestine can handle small amounts of prolamins.

A recent paper published in 1999 in Finland[14] details an investigation of wheat-starch based gluten-free products to determine their safety in the light of the Codex proposals. The study, involving 52 patients over an average of 8 years, showed the effects of wheat-starch based gluten-free products, containing a mean daily intake of 34mg gluten (equivalent to 340ppm)[15] per day. It was concluded that the wheat starch based products are well tolerated among patients with coeliac disease and dermatitis herpetiformis, and they do not cause small-bowel mucosal damage even during long-term ingestion.

Selby *et al.*[16] in 1999, reported a study where persistent mucosal gut abnormalities in coeliac disease were investigated, identified as necessary work as the Codex Alimentarius Commission was

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[9] British Nutrition Foundation. Adverse Reactions to Food - Factfile 2. Wheat Intolerance: What's the Real Story, 1999

[10] Paper presented in CCNFSDU, Bonn 1995 by W. Hekkens. Available from ISDI, on request.
Hekkens WT (1991). 'The Evolution In Research in Prolamin Toxicity: From Bread to Peptide' in Food Allergy and Food Intolerance. Nutritional Aspects and Developments, Eds Somogyi JC, Muller HR & Ockhuizen Th. Bibl. Nutr. Dieta, Basel, Karger, 48, pp 90–104

[11] Prof. P. Ciclitira, Coeliac Societies of the UK

[12] Prof. P. Ciclitira, CRD 39, Berlin, September 1998

[13] Ejderhamn J, Veress B, Strandvik B (1988). The long-term effect of continual ingestion of wheat starch containing gluten-free products in coeliac patients. In ' Coeliac Disease: 100 years', Eds Kumar PJ & Walker-Smith JA. University Printing Centre, University of Leeds, pp 294-7

[14] Kaukinen K et al. (1999). *Scand.J.Gastroenterol*. 34: 163-9

[15] Equivalent to 340ppm, assuming that 100g gluten-free wheat starch-based flours is ingested per day

[16] Selby W A. et al (1999). *Scand. J. Gastroenterol*. 9: 909-914

reviewing the current standard. 89 patients with long-standing coeliac disease were examined. The results showed no relationship between villous atrophy (an effect on the lining of the gut) and ingestion of either a gluten-free diet, as defined by Codex, or a diet containing no detectable gluten. The results of the study indicate that continuing abnormalities do not appear to be related to the small amounts of gluten found in the gluten-free diet, to the level proposed by Codex i.e. 200ppm. It is concluded that the risk of malignancy is not increased in patients adhering to such a diet, and further gluten restriction for this indication would seem unnecessary.

On the basis of the above, the view of ISDI and the signatories listed in the Annex is:

- for a single level for both 'naturally gluten-free foods' and those foods which have been 'rendered gluten-free' to reduce the possibility of misleading or confusing the consumer. One level would permit the continued supply of many of the most popular and palatable products, which have been specially manufactured.
- a level of 200ppm for all foods, based on the current state of the art; to be reconsidered in the future based on any new evidence from clinical challenge studies or developments in test methodology.

Labelling statements

The 21st Session of the CNFSDU noted that the proposed term 'gluten-free' might mislead the consumer and suggested that the term 'low or reduced in gluten' should be considered[17] for products rendered gluten-free. The UK Coeliac Society prepared a brief report on these issues.[8] They consider that the suggestion that this terminology, as a possibility for foods which contain ingredients that have been rendered gluten-free could have, in practice, disastrous consequences for individuals with Coeliac Disease. The current gluten-free diet, successfully followed by thousands of Coeliacs e.g. in UK for almost 50 years, could no longer be termed gluten-free. The result would be that the most popular and palatable, specially manufactured products (over 80% of bread and flour based on wheat starch) would have to be re-named 'gluten reduced' or 'low gluten'. It is likely that the Coeliac, particularly the newly diagnosed, would choose not to take such products, when a choice of a 'gluten free' product was still available. The majority of currently used products would be seen as inferior quality and would eventually phase themselves out, as occurred in Australia where this type of terminology has been used in recent years. With a more limited, less acceptable diet, compliance is jeopardised, with subsequent long-term effects on health and well being. The consequence in Australia has lead to a call for such a labelling system to be re-considered.

Nutrition claims are already established by Codex[18] where a threshold level is in force e.g. sodium-free = not more than 5mg per 100g; fat-free = not more than 0.5g per 100g. A definition of zero gluten to support a gluten-free claim is thus only theoretical as the concept of threshold limits is already accepted. It is in the interest of Coeliac individuals to have a variety of products labelled to meet their needs, to provide choice, and that these products are affordable. If it is felt that differing label statements are required, to differentiate products, which are 'naturally gluten-free' against products which have been 'rendered gluten-free', the terminology 'gluten-free by nature' and 'gluten-free' respectively should be adopted. Although it is acknowledged that such distinctions could create misleading and unjustified discrimination between products which are currently considered 'gluten-free' and are harmless to coeliac patients.

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[17] Report of the 21st Session of CCNFSDU, Berlin, September 1998

[18] Guidelines adopted at 22nd Session of the Codex Alimentarius Commission (ALINORM 97/40)

CONCLUSION

It is concluded that:

- A suitable test method, reliable in precision and accuracy, must be defined and made available.
- The work of the Prolamin Working Group, and any other scientific organisations on analytical methodology or clinical research on prolamin intolerance, should be fully supported.
- A single limit for the maximum permitted gluten content should be adopted for all foods presented for coeliacs.
- Current information justifies a limit of 200ppm gluten for all foods presented for coeliacs.
- New clinical evidence or analytical methodology may require reconsideration of this limit in the future.
- If differing label statements are required to differentiate between ingredients in foods 'naturally gluten-free' and 'rendered gluten-free' the terms used should be 'gluten-free by nature' and 'gluten-free'.

ANNEX

SUPPORTERS OF THE ISDI POSITION PAPER

- UK Coeliac Society
- UK Health Food Manufacturers' Association (HFMA)
- European Federation of Associations of Health Product Manufacturers (EHPM)
- Prof. Paul Ciclitira (St.Thomas' Hospital, London)
- Dr. Wim Hekkens, founder of Prolamin Working Group (Leiden, The Netherlands)
- Prof. Dr. Henk K.A. Visser (Paediatrician, Erasmus University Rotterdam, The Netherlands)
- Prof. Dr. Martin Stern, (Paediatrician, University Children's Hospital, Tübingen, Germany)
- Association des Amidonneries de Cereales de l'Union Européenne (AAC)

PWG - WORKING GROUP ON PROLAMIN ANALYSIS AND TOXICITY

Gluten effects, analysis, and limits in coeliac disease

Coeliac disease is an autoimmune disorder triggered by the cereal protein gluten. Most patients respond positively to the livelong institution of a gluten-free diet. The international working group on prolamin analysis and toxicity founded in 1985 by Wim Hekkens (Leiden) has focused its work to investigate methods of assessing the toxicity of food for coeliac patients. This implies a dual approach: laboratory analysis of foods for their prolamin (gluten) content and clinical evaluation of patients' sensitivity to prolamins and derivatives. The group has issued annual reports and, in 1999, has obtained status of an observer non-governmental organisation at the Codex Alimentarius Commission and its subsidiary bodies. The following position paper briefly summarises current knowledge on chemical analysis and clinical effects of gluten in coeliac disease. It has medical, nutritional and food legislative implications.

Studies on coeliac toxicity of gluten, particularly of its ethanol-soluble fraction, gliadin, indicate that all gliadin types and probably most gliadin components contain the precipitating factor. According to gliadins, those sequence regions rich in repetitive units and with high contents of glutamine, proline and phenylalanine/tyrosine appear to be most important. These regions are unique for coeliac-toxic cereal proteins and do not occur in non-toxic cereals and other food proteins. According to recent in-vivo and in-vitro studies on synthetic peptides, sequences like QQQPFPPQQPY and QPQPFPSQQPY could be of key importance. However, toxicity and immunogenicity of gluten peptides are not identical.

Wheat gluten/gliadin and related prolamins cause damage to the small intestine of coeliac patients producing intestinal and extra-intestinal symptoms. Gluten-free diet is the decisive therapy. Any effort should be made to obtain patients' compliance. There is no conclusive indication about any dose of gluten which could be tolerated by all coeliac patients. It will be difficult to establish such a figure in consideration of the clinical heterogeneity of coeliac disease and of reports on very high sensitivity. Further microchallenge studies are necessary and reinforced by the prolamin group to establish if doses less than 100 mg/day gliadin are still sufficient to cause inflammatory and morphologic changes in coeliac patients. At the same time evidence is also needed to establish long-term health risks of coeliac patients exposed to small doses of gliadin. In the meantime, patients and their associations, doctors, industries, governments and international institutions should make any effort to keep the diet of coeliac patients as much gluten-free as possible.

Given the uncertainty with methods published on gluten analysis in food (including Skerritt's monoclonal antibody ELISA), the prolamin group does not favour inclusion of a final analytical method in the Codex Alimentarius Standard for gluten-free foods. The development of better ELISA methods accompanied by a non-immunological chemical standard method (HPLC, MALDI-TOF, capillary electrophoresis) as presently undertaken by the prolamin group, including collaborative trials and quality assurance, will solve current problems of standardisation, precision and accuracy in gluten analysis particularly in the relevant low level range. The prolamin group recommends to keep to one single limit for gluten-free foods. As long as no further data are available, the current limit of [200] ppm gluten remains questionable and should remain in brackets. New clinical or analytical information may require reconsideration in the future.

Codex Alimentarius efforts to find more effective ways establishing relevant gluten limits and reliable analytical methods have come to an important stage. The prolamin group offers co-operation to concentrate research activities in the field of gluten effects in coeliac patients and analysis in food.