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**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON NUTRITION AND FOODS FOR SPECIAL DIETARY USES
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DRAFT REVISED STANDARD FOR GLUTEN-FREE FOODS [1]

1. SCOPE

1.1 This standard applies to those foodstuffs and ingredients which have been especially processed or prepared to meet the dietary needs of persons intolerant to gluten.

1.2 The standard refers only to the special dietary purpose for which these foodstuffs and ingredients are intended.

2. DESCRIPTION

2.1 DEFINITION

"Gluten-free" foods are foodstuffs so described:

a) consisting of or made only from ingredients which do not contain any prolamins from wheat or all *Triticum* species such as spelt (*Triticum spelta* L.), kamut (*Triticum polonicum* L.) or durum wheat, rye, barley, [oats] or their crossbred varieties with a gluten level not exceeding [20 ppm]; or

b) consisting of ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties, which have been rendered "gluten-free"; with a gluten level not exceeding [200 ppm]; or

c) any mixture of the two ingredients as in a) and b) with a gluten level not exceeding [200 ppm]

2.2 SUBSIDIARY DEFINITIONS

2.2.1 Gluten

For the purpose of this standard "gluten" is defined as a protein fraction from wheat, rye, barley, [oats] or their crossbred varieties and derivatives thereof, to which some persons are intolerant and that is insoluble in water and 0.5M NaCl.

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1 This text was previously published as CX/NFSDU 98/4.

2.2.2 Prolamins

Prolamins are defined as the fraction from gluten that can be extracted by 40 - 70% of ethanol. The prolamin from wheat is gliadin, from rye is secalin, from barley hordein and from oats avenin.
It is however an established custom to speak of glutensensitivity. The prolamin content of gluten is generally taken as 50%.

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 GLUTEN-FREE

For the purpose of this standard "gluten-free" means that the total content of gluten in products defined in 2.1a) shall not exceed [20 ppm], that the total content of gluten from wheat, rye, barley, [oats] or crossbred varieties of these does not exceed [200 ppm] in these foodstuffs or ingredients defined in 2.1 b) and c) on a dry matter basis. The prolamin content of liquid food products is in the same way expressed in ppm of the original product.

3.2 "Gluten-free" foodstuffs, substituting important basic foodstuffs should supply approximately the same amount of vitamins and minerals as the original foodstuffs they replace.

3.3 The product shall be prepared with special care under Good Manufacturing Practice (GMP) to avoid contamination with prolamins.

4. LABELLING

The term "gluten-free" shall be given in the immediate proximity of the name of the product.

5. CLAIMS

5.1 A foodstuff or ingredient that meets the requirement set out in Section 3.1 may be labelled "gluten-free".

6. GENERAL OUTLINE OF THE METHOD OF ANALYSIS AND SAMPLING

6.1 INTRODUCTION

To enforce the compliance to the limits for gluten-free products set in the preceding paragraphs an analytical method is needed which has a high level of accuracy. Up till now it has not been possible to design such a method in detail, as several factors impair its performance:

- the extend of compositional mismatch between contaminating or residual proteins and a gluten standard,
- the availability of a gluten standard,
- the selectivity of the antiserum,
- the effect of heating of the product on the extractability and epitope integrity.

As the proposed limit is near to the level which might be toxic for coeliacs, a more comprehensive investigation to address these questions has to be carried out. The general outline of the method of analysis and sampling presented below will constitute the framework for such an investigation.
When a standard is generally accepted as a point of reference, the general outline can be used as a basis for the determination of gliadin until all reagents and equipment necessary for the determination has been standardized and evaluated.

6.2 DETERMINATION OF GLUTEN IN FOODSTUFFS AND INGREDIENTS

The determination of gluten in foodstuffs and ingredients shall be based on an immunologic method. The antibody to be used should react with the cereals that are toxic for persons sensitive to gluten and should not cross-react with the other cereals or other constituents of the foodstuffs and ingredients.

6.3 THE EXTRACTION OF PROLAMINS

6.3.1 Pretreatment of solid foodstuffs and ingredients

Depending on the fat-content of the product, either of two pretreatments is used:

a) Products with a fat-content higher than 10%: Five grams of the product are homogenized with a blender in 50 ml hexane. The suspension is centrifuged for 30 min at 1500xg; the supernatant discarded and the extraction step is repeated until the sample is fat-free. The pellet is dried at 60°C, weighed, milled and an aliquot is used for analysis.
b) In products with a fat-content lower than 10% an extraction is generally not necessary. Five grams of the product are dried at 60°C, milled and an aliquot is used for analysis.

6.3.2 Extraction

a) solid foodstuffs and ingredients

An aliquot of the dried sample is homogenized with 60% aqueous ethanol in a volume 10 times its weight; homogenized for 2 minutes and after 15 minutes centrifuged for 10 min at 1500xg. The supernatant is taken off and stored if necessary at 4°C before determination. When a precipitate is formed this is spun down and discarded.

b) liquid foodstuffs and ingredients

An aliquot of a liquid product is diluted with ethanol; the added volume of ethanol being calculated to yield 60% ethanol in the resulting mixture. The mixture is homogenized and further treated like solid food extracts.

6.4 DETERMINATION OF GLIADIN

6.4.1 Plate preparation

Microtiter plates are coated overnight with an antibody against gliadin in an appropriate dilution (e.g. 1 in 600) in a sodium carbonate buffer. The plate is washed three times with phosphate-buffered saline with 0.05% Tween (PBS-T) and once more with deionised water containing 0.03% Na-azide. Plates can be stored at 4°C in a sealed plastic bag.

6.4.2 Standard

It is necessary to use a gliadin standard in order to minimize interassay variation. Apart from that a "golden standard" should be used to make comparison of the results from different laboratories with different ELISA-techniques and with different antisera possible. This "golden standard" should be prepared by one laboratory under strictly standardized conditions.

6.4.3 Determination

After appropriate dilution of the extract the samples and the necessary standard dilutions to obtain a standard curve are brought into the wells of the plate. After incubation for 2 hours, the plates are washed three times with cold PBS-T. To the wells the monoclonal or polyclonal antibody against gliadin conjugated with an enzyme is added and after incubation for 2 hours the plates are emptied and washed three times with PBS-T. Then a substrate for the enzyme is added. After an appropriate time the reaction is stopped. The absorption is measured directly in the microtiter plates.

6.4.4 The gliadin concentration is determined from the standardcurve obtained. The result is multiplied by 2 to obtain the gluten content and expressed in ppm of the original product.

7. REMARKS

7.1 The method determines the amount of prolamin in a product. It is however important to stress that the total daily intake of prolamin for coeliac patients should not exceed 10 mg per day.

7.2 The method is sensitive for native prolamins. The sensitivity for heated products is - depending on the temperature and time of heating - lower. It may be reduced to 10% of the original sensitivity. The reduction in sensitivity is related to the amount of α-gliadin in the sample and the sensitivity of the antibody for the different subfractions in the gliadin.

7.3 Depending on the specificity of the antibody, the method determines also the prolamins from rye, barley and oats as gliadin equivalents. The response in the assay however can be different from that for gliadin and must in that case be determined separately with an appropriate standard.

7.4 If the method gives a positive result and there is some doubt about the specificity, a blot after electrophoretic separation of the sample can be performed.

7.5 Products from partial hydrolysis of prolamins can, depending on the degree of hydrolysis, not always been determined by the method described.

7.6 Polyphenols such as those from tea, hops or cocoa decrease the yield of the extraction of prolamins by binding to the latter. Addition of casein as a competing protein as well as urea is necessary in that case.

7.7 The detection limit of the method should be at least 10 ppm in the product on a dry matter basis.