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Recent developments in analytical methods for tracing gluten

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ABSTRACT

According to Codex only foods not exceeding a level of 20 mg gluten/kg may bear a gluten-free label. This also sets the standard for analytical methods for gluten detection. In this paper the currently used methods for gluten analysis are reviewed and new developments are discussed. At the moment, the most commonly used methods are ELISA-based, but also PCR-based methods have been successfully employed. Proteomics-based methods such as reversed-phase (RP-) or gel permeation (GP-) high-performance liquid chromatography (HPLC) have been widely used for characterisation of cereal proteins. Methods combining mass spectrometry and liquid chromatography (LC-MS/MS) are the most promising non-immunological approaches for accurate quantitation of gluten traces. However, due to its requirement of expensive equipment and expertise it is not widely used for routine analysis. New developments include immunosensors, aptamers, microarrays, and multianalyte profiling. Despite the merits and challenges of the different methods, the need for an independent reference method and a generally applicable reference material remain.

1. Introduction

A strict lifelong gluten-free diet is currently the only known effective therapy for coeliac disease (CD). Recovery of the small intestinal mucosa is achieved in the vast majority of patients if the daily intake of gluten is less than 20 mg (Catassi et al., 2007). Maintaining a true gluten-free diet is challenging, because gluten occurs not only in obvious food sources such as bread, pasta, and beer. The so-called hidden sources are problematic, because gluten is used extensively also in modified forms as a flavour enhancer, thickener, emulsifier, filler, and fortification ingredient, not only in food products, but also in nutritional supplements, drugs, and cosmetics. Additionally, inherently gluten-free grains, such as maize, rice, buckwheat, quinoa, and amaranth, may become contaminated with gluten during crop rotation, milling, transportation, or processing. To ensure the safety of products for CD patients, foods bearing a gluten-free label must not exceed the level of 20 mg gluten per kg product (Codex Standard 118-1979, 2008). This requirement sets the standard for analytical methods for gluten detection, because these must be capable of detecting gluten traces well below the threshold of 20 mg gluten/kg. Currently, enzyme-linked immunosorbent assays (ELISA) based on the R5 and G12 antibodies are recommended by legislation (Codex Committee of Methods of Analysis and Sampling, 2015). ELISAs are most commonly used for gluten analysis, not only due to their specificity, sensitivity, and suitability for routine analysis, but also for lack of an independent reference method. Alternatives to ELISA based on immunosensors, proteomics, mass spectrometry, genomics and novel approaches such as aptamers, microarrays, and multianalyte profiling are being developed. The most important developments in the fields of legislation concerning gluten-free products and of

Abbreviations: 1D, 1-dimensional; AACCI, American Association of Cereal Chemists International; AOACI, Association of Official Analytical Chemists International; CD, coeliac disease; CFR, Code of Federal Regulations; EC, European Commission; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionisation; EU, European Union; FALCPA, Food Allergen Labelling and Consumer Protection Act; FDR, Food and Drug Regulations; FSANZ, Food Standards Australia and New Zealand; FSC, Food Standards Code; GP, gel permeation; HPLC, high-performance liquid chromatography; IMB, immunomagnetic beads; IMLN, immunoliposomal nanovesicles; LC-MS/MS, liquid chromatography mass spectrometry/mass spectrometry; LFD, lateral flow device; LOD, limit of detection; LOQ, limit of quantitation; mAb, monoclonal antibody; MALDI, matrix-assisted laser desorption/ionisation; MRM, multiple reaction monitoring; NGS, next-generation sequencing; pAb, polyclonal antibody; PCR, polymerase chain reaction; PE, phycoerythrin; PWG, Prolamin Working Group; RM, reference material; RP, reversed-phase; SDS, sodium dodecyl sulphate; TCEP, tris(2-carboxyethyl)-phosphine; TOF, time-of-flight; UPEX, universal prolamin and glutelin extractant solution.

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gluten analysis will be discussed.

2. Gluten-free labelling of foods

2.1. International legislation – Codex Alimentarius

As one of foods and ingredients known to cause hypersensitivity, cereals containing gluten (i.e., wheat, rye, barley, oats, spelt, or their hybridized strains and products of these) shall always be declared on the label of prepacked foods (Codex Standard 1-1985, 2010). Apart from gluten-containing cereals, this standard covers the major food allergens crustaceans, eggs, fish, peanuts and soybeans, milk, tree nuts, and sulphite in concentrations of 10 mg/kg or more. More specific legislation concerning gluten-free products is laid down in the Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten, which was most recently revised in 2008 (Codex Standard 118-1979, 2008). This standard defines that "gluten-free foods are dietary foods consisting of or made from one or more ingredients which do not contain wheat (i.e., all *Triticum* species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer." Alternatively, foods consisting of gluten-containing cereals may be specially processed to remove gluten, so that the level does not exceed 20 mg/kg. Regarding oats, national regulations may be made, because it is acknowledged that most gluten-intolerant people can tolerate non-contaminated oats. In subsidiary definitions "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 0.5 M NaCl". "Prolamins are defined as the fraction from gluten that can be extracted by 40–70% of ethanol" and the "prolamin content of gluten is generally taken as 50%". The decision on whether or not to use the term "gluten-free" on the label lies with each manufacturer and the product is only subject to the respective regulatory framework if a voluntary gluten-free claim is made.

2.2. European Union

Definitions, thresholds, and labelling currently specified in the European Commission Regulation (EC) No 41/2009 of 21 January 2009 are equivalent to those in Codex Standard 118-1979 (2008). The new Regulation of the European Parliament and of the Council (EU) (2013), No 609/2013 'on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control' to be implemented from 20 July 2016 will repeal EC No 41/2009. As stated in article 41 of No 609/2013, the rules on the use of the statement "gluten-free" are to be regulated under Regulation of the European Parliament and of the Council (EU) (2011), No 1169/2011 'on the provision of food information to consumers'. Having taken effect on 13 December 2014, EU No 1169/2011 aims to provide a high level of consumer protection by establishing requirements for food information and labelling of prepacked and non-prepacked foods. Under this regulation, indication of any ingredient or processing aid listed in Annex II causing allergies or intolerances used in the preparation of a food and still present in the finished product, even if in altered form, shall be mandatory. Among the substances listed in Annex II are cereals containing gluten, namely wheat, rye, barley, oats, spelt, kamut or their hybridized strains, and products thereof. The rules on the use of "gluten-free" as contained in EC No 41/2009 will be transferred into EU No 1169/2011 to ensure at least the same level of protection for gluten-intolerant people as currently provided. It should also be ascertained that CD patients are adequately informed of the difference between a food that is specially treated to reduce the gluten content and other food that is made exclusively from ingredients naturally free of gluten.

2.3. United States of America and Canada

The 'Food Allergen Labelling and Consumer Protection Act of 2004' (FALCPA) amended Section 343 of the 'Federal Food, Drug, and Cosmetic Act'. Under FALCPA, the labelling of eight major food allergens including wheat is required either as a 'Contains' statement or in the list of ingredients. The final rule on 'Gluten-free labelling of food' (Food Labeling, 2013) became effective on 4 September 2013 and was issued under the FALCPA as amendment to part 101 of Title 21 of the Code of Federal Regulations (21 CFR). The definitions of "gluten-containing grains" (grains of the genera *Triticum*, *Secale* or *Hordeum*) and "gluten" are followed by the requirements for "gluten-free" claims. These requirements include that any unavoidable presence of gluten in the food bearing a gluten-free claim in its labelling shall be below 20 mg gluten/kg.

In Canada the food regulatory framework consists of the 'Canadian Food and Drugs Act' and the 'Canadian Food and Drug Regulations' (FDR) (Government of Canada, 2013). The FDR were amended as of 4 August 2012 to enhance the labelling of food allergens and gluten sources in prepackaged foods. Subsection B.01.010.1 states that if "a food allergen or gluten is present in a prepackaged product, the source of the food allergen or gluten, as the case may be, must be shown on the label of the product" either in the list of ingredients or in a 'Contains' statement. Gluten means any (modified) gluten protein from the grain of any of the following cereals or hybrids thereof: barley, oats, rye, triticale and wheat. Division 24 of the FDR that applies to 'Foods for Special Dietary Use' specifies the rules for making a "gluten-free" claim on foods, but no specific threshold is defined. However, based on the available scientific evidence, Health Canada takes the view that gluten-free foods, which contain levels of gluten not exceeding 20 mg/kg as a result of cross-contamination, meet the health and safety intent of B.24.018 (Canada, 2012). Regarding oats, Health Canada registered a marketing authorization on 19 May 2015 which permits the use of gluten-free claims for gluten-free oats. These gluten-free oats must have been specially produced and processed, must not contain more than 20 mg/kg of gluten from wheat, rye, barley or their hybridized strains and be clearly identified as "gluten-free oats". An update of the FDR is currently being developed.

2.4. Australia and New Zealand

The bi-national 'Food Standards Australia and New Zealand' (FSANZ) government agency develops and administers the 'Australia New Zealand Food Standards Code', 2014 (FSC). Regulations related to gluten are laid out in Standards 1.2.7 (Nutrition, Health and Related Claims of 30 October 2014) and 2.9.5 (Food for Special Medical Purposes of 30 October 2014). Both standards will be revoked on 1 March 2016 by the new Standard 5.1.1. According to the current Standard 1.2.7, a "gluten-free" food must not contain detectable gluten, or oats or their products, or cereals containing gluten that have been malted, or their products. Food with a "low gluten" content may not contain more than 20 mg gluten per 100 g of the food. Standard 2.9.5 prohibits the use of a claim in relation to the gluten content of a food for special medical purposes unless expressly permitted. A "gluten-free" or "low gluten" claim may be made only if the above requirements are met. If a claim is made, the label on the package must include the average quantity of the gluten in the food. In Australia and New Zealand, gluten-free means that gluten is not detectable by the most appropriate techniques currently available (<3 mg/kg). This strict interpretation has been

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