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A survey study on safety and microbial quality of "gluten-free" products made in Italian pasta factories

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ABSTRACT

The rising prevalence of celiac disease leads to an increased demand of "gluten-free" products. A survey study on the gluten content and on the microbiological quality of "gluten-free" flour, and processing flour products, was carried out from 2010 to 2015 in Northern Italy. Overall 12,419 samples were analyzed, and 94.7% contained a gluten concentration less than 5 mg kg^{-1} (lower limit of detection). Only 0.1% of samples showed a gluten concentration above 80 mg kg^{-1} (maximum limit of detection). In the remaining 5.2%, the gluten concentration was between 5 and 80 mg kg^{-1}, underlining how a gluten-free diet completely devoid of gluten is unrealistic. The microbiological quality of these products was investigated.

Overall, the majority of samples revealed microbial loads of less than 1 l g CFU g^{-1} (lower limit of detection). High levels of spoilage bacteria were found in egg-containing products. Total mesophilic bacteria were counted in all analyzed food categories with concentrations up to about 6, 8 and 9 l g CFU g^{-1} in dry pasta, flours and egg products respectively. *Listeria monocytogenes* was found only in one sample, whereas *Salmonella* spp. was never found.

Buckwheat flour was the most frequently contaminated product by presumptive *Bacillus cereus*, with a prevalence of 12.5%. Also, a contamination by Coagulase-Positive Staphylococci was found during this investigation, especially in buckwheat dry pasta and flour and in egg dry pasta, with a prevalence of 54.7%.

This study aimed to enhance the knowledge about the "gluten-free" products which are still poorly studied, even if their impact on the food market is increasingly considerable.

1. Introduction

During the recent decades, allergies and certain food intolerances showed a worldwide gradual increase in their prevalence, together with the economic growth, changes in lifestyle and dietary patterns (Gilissen, van der Meer, & Smulders, 2014). They are triggered in individuals with an unbalanced immune system and unregulated intestinal bacterial flora (Sanz 2015), by specific proteins present in many kinds of foods, including cereals. The prevalence of allergies to cereals is low, although no exact frequencies are known (Romano 2014). The most commonly reported are allergies to wheat, maize and rice, and bakers' asthma is the most important as an occupational disease, with the highest economic impact (Ito 2015).

Otherwise, cereal immune-mediated disorders occur at a prevalence of 1–3% of the global population, but it varies between single countries. The causal proteins are gliadins and glutenins from wheat and related proteins from barley and rye, commonly called as 'gluten' (Gilissen et al. 2014). In accordance to the EU Regulation 41/2009, "gluten means a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and which is insoluble in water". The use of wheat flour and gluten in foodstuffs is extremely common because of their heat stability, and useful effects on texture, moisture retention and flavour (EU, 2009).

In Italy, the prevalence of celiac people was about 0.25%

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(148,662 individuals) as reported by the census made by the Italian Ministry of Health, updated on December 2012 (Bioletti et al., 2016). However, the continuous increase of celiac population leads to a higher demand of gluten-free products, without (or low containing) wheat, rye, barley or spelt wheat proteins (EU Regulation, 2014). Most of the typical Western cereals products, like bread and pasta, are based on wheat or rye, and have a long tradition in the daily diet (Davis, Bryan, Hodgson, & Murphy, 2015). Also, the importance of their market and the relative economic impact is highly considerable (Akineden, Murata, Gross, & Usleber, 2015).

Pasta is one of the most consumed foods in the world and it is a traditional product obtained from semolina. According to the Italian legislation, 'Pasta' is defined as "the product obtained by extrusion or lamination, and successive drying (up to 12.5% maximum water content), of a dough made of durum wheat semolina and water" (DPR, 2001; Mastromatteo, Chillo, Iannetti, Civica, & Nobile, 2011).

There are many aspects related to the food safety such as contamination by biological, physical or chemical substances. All these factors may pose significant health risks to the consumers, including celiac subjects who undergo to alterations in their intestinal microbiota (Verdu, Galipeau, & Jabri, 2015) and are particularly affected by foodborne microbiological infections (Sarno, Discepolo, Troncone, & Auricchio, 2015). Generally, the shelf life of dry pasta can persist up to 2 years. Although the growth of pathogenic bacteria may not be supported in dry pasta, pathogens can contaminate flour, cereals or bakery products and may survive for long periods.

According to the Rapid Alert System for Food and Feed portal (*http://ec.europa.eu/food/safety/rasff/index_en.htm*), *Salmonella* is the most frequently notified microorganism in cereals and bakery products: in 2016 *Salmonella* spp. was found in wheat flour in Germany and in 2015 it was found in cookies from Hungary. Also, coagulase-positive *Staphylococcus* was found in egg pasta in 2015 in Germany (EU Commission, 2010).

The goal of this study was to evaluate the safety of flour and processing flour products labelled as "gluten-free", testing the gluten presence according to the EU Regulation 1169/2011, Annex II (EU, 2011). Moreover, to evaluate their microbiological quality, a portion of them were analyzed together with other food samples to establish the nature, the distribution and the levels of spoilage and pathogenic bacteria.

2. Materials and methods

2.1. Samples

From 2010 to 2015, a survey study about the gluten content and microbiological quality of cereal products was carried out in Northern Italy.

For gluten tests, a total of 12,239 food samples labelled as "gluten-free" (dry pasta: 12,072; flours: 167) derived from maize, rice, quinoa, and buckwheat, and 180 environmental swabs collected in different pasta factories, were analyzed.

To evaluate the microbiological quality of processing flour products, a total 1250 samples were analyzed: 628 among the previously analyzed samples for gluten content, 448 egg products (dry egg pasta: 223; egg dough: 213; liquid egg: 12) and 174 samples of wheat dry pasta.

Sampling techniques for environmental surfaces, according to ISO 18593, included direct contact plates, swabs, sterile pre-moistened towel, and sponges (ISO 2004a).

2.2. Immunoassay tests

2.2.1. Gluten detection

To quantify gluten, a commercially available enzyme-linked immunosorbent assay (ELISA) kit was used (Ridascreen® Gliadin R7001, r-biopharm, Darmstadt, Germany) based on the R5 antibody-Mendez method (Type I), as stated by the Codex Alimentarius (Codex Alimentarius, 2008). Briefly, proteins were extracted by mincing pasta samples and using Mendez Solution (containing β-mercaptoethanol) at 50 °C and 80% ethanol. An antigen-antibody ELISA sandwich reaction was used, with specific antibodies (R5) against gliadins on extracted specimens. The result was an antibody-antigen-complex, and components not bound to the antibodies were removed in washing steps. For each analysis session, a specific set of standards, provided by the kit, was tested together with samples. Absorbance was measured at 450 nm. Finally, the gliadin concentration was read from the RIDA® SOFT Win calibration curve (r-biopharm, Darmstadt, Germany) and multiplied by 2, in order to obtain the gluten concentration (gliadin usually represents 50% of gluten proteins).

2.2.2. Staphylococcal Enterotoxins (SE) detection

For the detection of Staphylococcal Enterotoxins the AOAC Official Method (AOAC, 2007) was used. The VIDAS Staph Enterotoxin Test (bioMe'rieux, Marcy-l'Etoile, France) was carried out by using theVIDAS automated system. The test used an enzyme-linked fluorescent assay for semi-quantitative detection of SE. The results were indicated as Test Values (TV) corresponding to the ratio between the Relative Fluorescent Value (RFV) of the samples compared with RFV of the standard, and when the value of TV was less than 0.13 values, samples were considered to be negative according to the manufacturer's instructions.

2.3. Microbiological analyses

Twenty-five (25) grams of product were homogenized 1:10 (w:v) in sterile peptone water (PW) (CONDA, Madrid, Spain) for 3 min using a Stomacher 400 blender (Seward Medical, London, UK). Decimal dilutions in sterile PW were prepared for the bacterial enumeration.

2.3.1. Total Mesophilic Bacteria (TMB)

Total Mesophilic Bacteria were enumerated in agreement with ISO 4833 (ISO, 2003) by pour plating 1 ml of appropriate dilution in Plate Count Agar (PCA; Oxoid, Basingstoke, UK) and incubated at 30 °C for 48–72 h.

2.3.2. Enterobacteriaceae (ENT)

For the enumeration of *Enterobacteriaceae* the appropriate dilutions were pour plated (1 ml) in violet red bile glucose agar (VRBGA; BioRad, Marnes la Coquette, France) and incubated at 37 °C for 24 h in agreement with ISO 21,528-2 (ISO, 2004b).

2.3.3. Yeasts and moulds (Y&M)

The colonies number of yeasts and moulds was evaluated by surface plating on Oxytetracycline Glucose Yeast Extract Agar (OGYEA, Oxoid) plates count incubated at 20 °C for 5 days.

2.3.4. Coagulase positive Staphylococci (CPS)

Coagulase-positive Staphylococci were determined following method ISO 6888- 1:1999/Amd. 1:2003 by surface plating on Baird Parker agar supplemented with Rabbit Plasma Fibrinogen (Microbiol Diagnostici, Cagliari, Italy) and incubating plates at 37 °C for 30–48 h.