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EDITORIAL

The global use of oats for food products has increased by 10 percent over the last 30 years. About one quarter of the world`s oats production is used in food. They have a high nutritional value, as they are high in protein and oil, but low in starch. Oats are not only processed to oat meal, flour, muesli or granola bars, but also serve as stabilizers, emulsifiers and food extenders in industrial food processing.

People suffering from celiac disease need follow a life-long, strict, gluten-free diet, where they need to avoid gluten containing cereals like wheat, rye, barley and their crossbread varieties. Whether celiacs can also eat oats is an ongoing debate.

Michael Prinster

The oat mystery – Are they gluten free?

Production of gluten free oats

The main problem with oat production is cross contamination with other gluten containing grains, as they are planted in the same fields, harvested with the same equipment, are stored and transported together. In the case of regular oats, contamination of 1% with other grains is allowed and common. If gluten free oats are to be produced, special care has to be taken. One example is the production of a certain type of proprietary, gluten-free oats. The requirements are that these oats need to be planted on ground that did not have gluten containing cereals for the last four years. Near harvest time, the grower walks through the fields and pulls out any stray of gluten grains. This is feasible because of the short stature of this proprietary oat variety. Wheat, rye and barley are much taller and easy to see. Afterwards, a gluten-free inspector walks around the field and certifies that it is clean. Harvesting is done with certified gluten-free combines, which are only used for gluten-free oat production. These oats are stored in new bags or certified clean bins to avoid another source of cross contamination. Production of gluten-free oats can only take place in fields and not by post-harvest cleaning.

Further processing of gluten-free oats has to follow strict gluten-free management to avoid any sort of cross contamination.

Clinical aspects

On the one hand, there are several studies demonstrating that oats are safe to be consumed by celiacs [1 – 5], but on the other, certain studies show that oat sensitivity in celiacs does exist [6 – 11]. One problem is a high drop-out rate for oat studies but still show that about 5 % of patients show celiac symptons when gluten-free oats are consumed. Up to now, there have been no clinical consensus if gluten-free oats are safe for celiacs to eat.

Labelling regulations for gluten-free oats

In Canada, it is not allowed to label oats as gluten-free as the Canadian Labelling Regulation for Food Allergen and Gluten Sources states that gluten means any gluten protein from the grain of barley, oats, rye, triticale and wheat [12]. Oats can only be labelled as "pure and uncontaminated". Additionally, there is Health Canada´s position on oat safety for celiacs [13] which says that moderate amounts of oats (50 – 70 g/day and 20 – 25 g/day for children) can be well tolerated by the majority of celiacs. It is also requested to have a further definition of the terms "pure and uncontaminated," in terms of production, sampling and testing of oats. According to Health Canada, the fact that about 5% of celiacs cannot tolerate even pure oats needs further investigation.

In 2013, the US FDA published the Gluten-Free Rule [14], which states that any grain other than gluten containing wheat, rye, barley or their cross-bread hybrids like triticale can be labeled gluten-free if the presence of any unavoidable gluten due to cross contact situation is less than 20 mg/kg. Therefore, oats that are labeled gluten-free must contain less than 20 mg/kg gluten.

In addition to the FDA Rule, several local certification bodies give their approval for gluten-free products that fulfill specific requirements. To meet criteria for Celiac Sprue Association (CSA), the product needs to contain less than 5 mg/kg gluten. Gluten Interance Group (GIG) with its Gluten Free Certification Organization (GFCO) sets their limit at 10 mg/kg gluten.

European Regulation EC 41/2009 [15] states that gluten means a protein fraction from wheat, rye, barley and oats or their crossbred varieties. But there are further definitions for oats, saying that oats contained in foodstuffs for people intolerant to gluten must have been specially produced, prepared and/or processed in a way to avoid contamination by wheat, rye, barley, or their crossbred varieties and the gluten content of such oats must not exceed 20 mg/kg.

Association of European Celiac Society (AOECS) certifies products containing oats to be gluten-free when their gluten content is below 20 mg/kg. These products containing oats must clearly be labeled with the capital letters "OATS" followed by the certification number of these products given by the AOECS.

Analytical aspects of gluten detection in oats

Oats contain one family of prolamins, so-called avenins. They make up to 10 – 15 % of total seed protein compared to up to 80 % total seed content of prolamins in other gluten-containing grains. Avenins show high proline and glutamin content, low lysine and are insoluble in water.

Two celiac disease relevant T-cell epitopes have been defined in oats (*see Figure 1*) [8,16], but the structure of prolamins from oats differs from other gluten-containing cereals.

DQ2.5-ave-1a PYPEQ**E**EPF (Av-α9A)
DQ2.5-ave-1b PYPEQ**E**QPF (Av-α9B)

E …Glutamate residues formed by TG2-mediated deamidation, which are important for recognition by T-cells are shown in bold

Figure 1. *Celiac Disease (CD) releveant T-cell epitopes*

The two epitopes shown in *Fig.1* have been found in each of 13 oat species studied by Londono *et al.* [17].

Comino *et al.* [18] showed a correlation of the reactivity of the monoclonal G12 antibody with the immunogenicity of prolamin extracts from different oat varieties.

Romer Labs conducted a preliminary study on its AgraQuant® Gluten G12 Sandwich ELISA and different oat varieties to clarify the situation on the detection of gluten in oats using the G12 antibody. The objectives of the study were to find out if the AgraQuant® Gluten G12 ELISA test kit can detect gluten in pure oats and if there is a difference in the gluten level of different varieties. The results of the AgraQuant® Gluten G12 ELISA have also been compared to the R5 Sandwich ELISA.

More than 80 pure, uncontaminated oat varieties from the U.S., Canada and Europe were collected. Most samples were from seed banks and, therefore, proven to be pure and uncontaminated. Samples which were not obtained from seed banks were hand selected to prove their pureness. The lab mill was cleaned extensively between the milling of each variety. The oat varieties were extracted according to AOAC Official Method 2012.01 [19], with reducing agents and analyzed with the AgraQuant® Gluten G12 Sandwich ELISA test kit (LOD 4 ppm gluten). Several varieties were also analyzed with the R5 Sandwich ELISA test kit (LOD 5 ppm gluten) AOAC Official Method 2012.01 [19]

Detailed results are shown in *Table 1 – 5* - varieties analysed by AgraQuant® Gluten G12 Sandwich ELISA and R5 Sandwich ELISA.

Table 1

Oat Variety	AgraQuant® Gluten G12 Sandwich ELISA	R5 Gluten Sandwich ELISA
Bastion	<5 ppm	<5 ppm
04-704-Cn 7/2	<5 ppm	<5 ppm
Chris	<5 ppm	<5 ppm
Gerald	<5 ppm	<5 ppm
Brachan	<5 ppm	<5 ppm
Tardis	<5 ppm	<5 ppm
Dalguise	<5 ppm	<5 ppm
Balado	<5 ppm	<5 ppm

Table 2

Oat Variety	AgraQuant® Gluten G12 Sandwich ELISA
Classic	<5 ppm
Dallas	<5 ppm
Jim	<5 ppm
AC Juniper	<5 ppm
Aarre	<5 ppm
Roope	<5 ppm
Riser	<5 ppm
Sisko	<5 ppm
Solva	<5 ppm
Lustre	<5 ppm
AC Mustang	<5 ppm
Chairman	<5 ppm
Florida 502	<5 ppm
Prairie	<5 ppm
INO9201	<5 ppm
SSH 423	<5 ppm
Brawn	<5 ppm
Brawn	<5 ppm
Unregistered Hulless ID #02 ANS 68	<5 ppm
Gehl	<5 ppm
AC Aylmer	<5 ppm
Furlong	<5 ppm
Shadow	<5 ppm
Turcotte	<5 ppm
Alcyon	<5 ppm
Oscar	<5 ppm
Leggett	<5 ppm
Proprietary Oat 1	<5 ppm
Veli	<5 ppm
Hakea	<5 ppm
Pallinup	<5 ppm
Burton	<5 ppm
Cevamex	<5 ppm
NC Hulless	<5 ppm
AC Morgan	<5 ppm
Glider	<5 ppm
Quoll	<5 ppm
Excel	<5 ppm

Table 3

Oat Variety	AgraQuant® Gluten G12 Sandwich ELISA	R5 Gluten Sandwich ELISA
Buffalo	6 – 10 ppm	<5 ppm
Husky	6 – 10 ppm	<5 ppm
Mascani	6 – 10 ppm	<5 ppm
Mason	6 – 10 ppm	<5 ppm
Becon	6 – 10 ppm	<5 ppm
Hendon	6 – 10 ppm	<5 ppm
Canyon	6 – 10 ppm	<5 ppm
Zuton	6 – 10 ppm	<5 ppm
03-36Cn	6 – 10 ppm	<5 ppm
05-82ACn19	6 – 10 ppm	<5 ppm
Rhapsody	6 – 10 ppm	<5 ppm
03AW24	6 – 10 ppm	<5 ppm
03-37Cn	6 – 10 ppm	<5 ppm
Unknown - from Sunburst, MT	6 – 10 ppm	<5 ppm
Summit	6 – 10 ppm	<5 ppm
00-61 Cn	6 – 10 ppm	<5 ppm
Lenon	6 – 10 ppm	<5 ppm
04-66Cn7	6 – 10 ppm	<5 ppm

Table 4

Oat Variety	AgraQuant® Gluten G12 Sandwich ELISA
Karma	6 – 10 ppm
Possum	6 – 10 ppm
Proprietary oat 2	6 – 10 ppm
Wintaroo	6 – 10 ppm
Katri	6 – 10 ppm
Lisbeth	6 – 10 ppm
Riel	6 – 10 ppm
Euro	6 – 10 ppm
Numbat	6 – 10 ppm
Bulwark	6 – 10 ppm

Table 5

Oat Variety	AgraQuant® Gluten G12 Sandwich ELISA	R5 Gluten Sandwich ELISA
Nusso	>10 ppm <20 ppm	<5 ppm
Maverick	>10 ppm <20 ppm	<5 ppm
Monico	>10 ppm <20 ppm	<5 ppm
Unknown - from Lakewood, NJ	>10 ppm <20 ppm	<5 ppm
Rhiannon	>10 ppm <20 ppm	
Lamont	>10 ppm <20 ppm	
Powell	>10 ppm <20 ppm	
SSH 421	>10 ppm <20 ppm	
Miku	>10 ppm <20 ppm	
Bates 89	>10 ppm <20 ppm	
Provena	>10 ppm <20 ppm	

About half of the oat varieties analyzed showed gluten levels below 5 mg/kg when analyzed with G12 and R5 Sandwich ELISA test kits. About one third of the oat varieties gave low positive results, between 6 and 10 mg/kg of gluten analyzed with the monocolonal G12 antibody, but below 5 mg/kg gluten analyzed with R5 Sandwich ELISA test kit. Slightly more than ten percent of the collected oat varieties showed clear positive results of between 10 and 20 mg/kg gluten when tested with the AgraQuant Gluten G12 Sandwich ELISA, but still below the limit of detection when analysed with the R5 ELISA.

Conclusion

The positive results from the monoclonal G12 antibody appear to be a specific reaction of the antibody to the toxic fragment, rather than a non-specific response. All pure oat varieties analyzed gave results below 20 mg/kg gluten and, thus, are below the legal threshold of 20 mg/kg for gluten-free labelling in Europe and US.

According to Comino *et al.* [18], the cross reactivity of the monoclonal G12 antibody for certain oat varieties shows higher results than confirmed in our study. This is due to the fact that a competitive assay was used for determination of cross reactivities in Comino *et al.* [18] compared to Sandwich assays in our study.

It can be said that CD relevant T-cell epitopes are found in several oats [17] but the prolamin (avenin) content in oats is much lower (about 10 – 15 %) than compared to other gluten containing grains (80 %). Our study gave further indications that there is a difference in oat varieties in terms of the gluten content. Due to the different structure and sequence of avenins, a competitive ELISA assay would probably be more suitable for the determination of the gluten content in pure oats.

In general, more research on peptide sequences of avenins is necessary. Furthermore, an independent external study on pure oats will be conducted with AgraQuant® Gluten G12 and other Gluten ELISA test kits.

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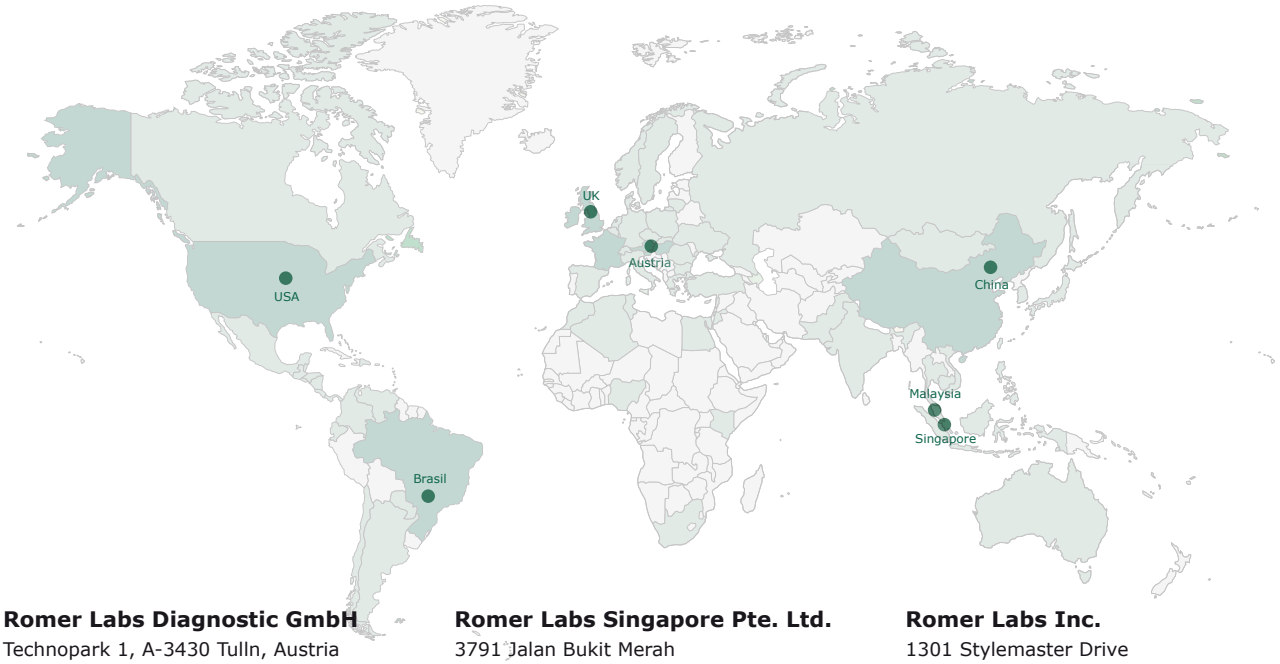

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