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Letter

Addition of transglutaminase to cereal products may generate the epitope responsible for coeliac disease

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Transglutaminase is a crosslinking enzyme that is being used more extensively in foods and has been widely accepted as a processing aid. We, and others, have reported the use of transglutaminase in the baking industry to improve the functional properties of bread, pastry and croissant dough. Early work suggested that transglutaminase may reduce the allergenicity of wheat flour. However, recent research into the molecular mechanism of coeliac disease suggests the disturbing possibility that transglutaminase in baked products may act upon gliadin proteins in dough to generate the epitope associated with the coeliac response. Further work is urgently required to assess this possibility. In the meantime, we do not recommend the use of transglutaminase in baked products.

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Introduction—transglutaminase

The transglutaminase enzymes have attracted much attention over the last few years, both as food ingredients (Yokoyama, Nio, & Kikuchi, 2004) and for their role in the body (Esposito, & Caputo, 2005). Transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) catalyses acyl-transfer reactions, introducing covalent crosslinks between protein molecules (Nonaka *et al.*, 1989). Crosslinks are formed between lysine residues and glutamine residues producing an ε-(γ-Glu)-Lys bond. If this takes place in food, the nutritional value of the lysine residue is not reduced (Seguro, Kumazawa, Kuraishi, Sakamoto, & Motoki, 1996). Transglutaminase may also catalyse the introduction of free amine groups into proteins via the amide moiety of a glutamine residue and, in the absence of available amines, hydrolyse a glutamine residue to a glutamate residue (Nielson, 1995) (Fig. 1). A glutamate residue may also result from the hydrolysis of the crosslink in the gut.

Transglutaminase in foods

The use of transglutaminase to enable higher product quality in a wide variety of foods is increasing, particularly since large quantities of microbial transglutaminase have become commercially available (Yokoyama *et al.*, 2004). It is generally assumed that the effects of transglutaminase in foods are due to its crosslinking activity. However, the direct experimental evidence for this supposition is limited (Christensen, Sorenson, Hojrup, Petersen, & Rasmussen, 1996; Babiker, Khan, Matsudomi, & Kato, 1996; Yildirim, & Hettiarachychy, 1997; Yasunaga, Abe, Nishioka, & Arai, 1998) and the possible effect of non-crosslinking activity has not been widely considered. Food treated with transglutaminase potentially contains deamidated protein residues and proteins conjugated to any free amines in the food matrix. The exact product mix is likely to depend on the particular processing conditions.

We and others have reported the beneficial effects of transglutaminase in cereal products (Alexandre *et al.*, 1993; Gerrard *et al.*, 1998; Gerrard *et al.*, 2000; Gerrard *et al.*, 2001; Larre *et al.*, 2000a,b; Bauer, Koehler, Wieser, & Schieberle, 2003a,b; Collar & Bollain, 2004; Rasiah, Sutton, Low, Lin, & Gerrard, 2005). Transglutaminase has been shown to crosslink specific gluten proteins, with consequent

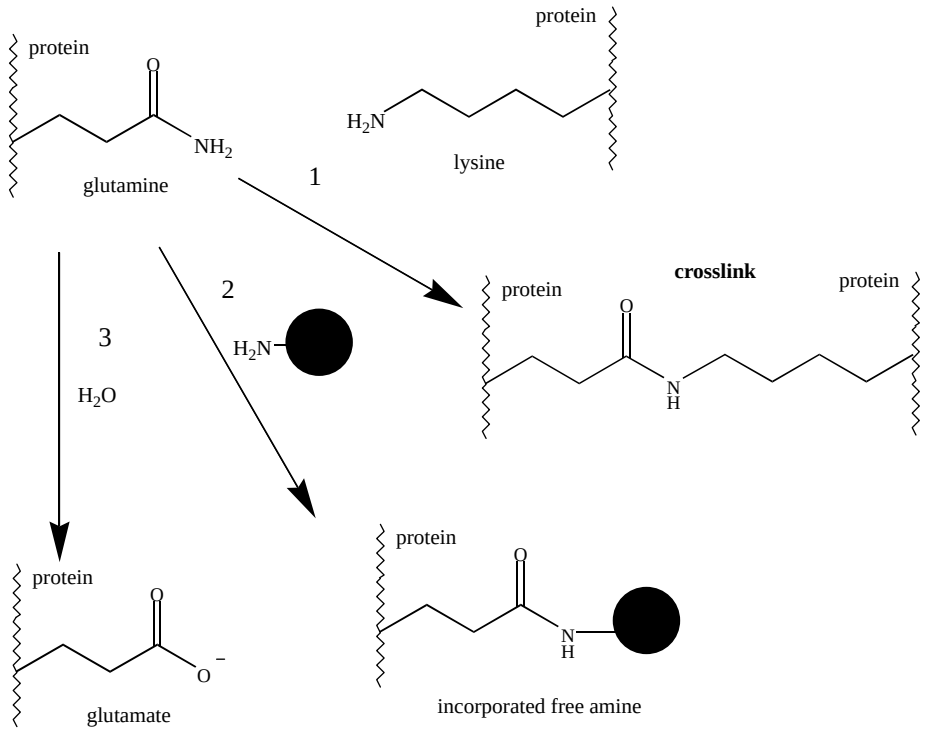

Fig. 1. The three reactions catalysed by microbial transglutaminase: (1) protein crosslinking, (2) incorporation of a free amine, and (3) deamidation of a glutamine residue to form glutamate.

changes in the properties of the final product. In general, transglutaminase strengthens dough, increasing the strength of the final product. It has proved particularly beneficial in protecting frozen doughs from damage, leading to a higher quality product on thawing and baking. Additionally, an early report (Watanabe, Susiki, Ikezawa, & Arou, 1994) suggested that treatment of gluten with transglutaminase reduces its allergenicity. Thus, transglutaminase has emerged as an ideal processing aid to manufacturers of cereal products (Poza, 2002).

Transglutaminase and the coeliac response

Coeliac disease is a chronic inflammatory disorder of the intestines, induced by ingestion of gluten-containing grains (Reif, & Lerner, 2004). Early reports in the medical literature implicated transglutaminase found in the gut tissue in the reaction of coeliac sufferers on exposure to wheat protein (Dieterich *et al*., 1997; Marsh, 1997). Since then, our understanding of the key role of transglutaminase in the disease has increased (Anderson, Degano, Godkin, Jewell, & Hill, 2000; Arentz-Hansen *et al*., 2000; Reif, & Lerner, 2004).

Several epitopes that activate T-cells have been isolated from the intestinal mucosa of coeliac patients; these tend to occur mainly in the gliadins. One recent study has revealed that a crucial sequence in gliadin proteins, QPFPQP*Q*LPYPQPQ, is deamidated by tissue transglutaminase to form QPFPQP*E*LPYPQPQ (see reaction 3), Fig. 1). The latter sequence is an active epitope, and activates the T-cells thought to be involved in the autoimmune response in coeliac suffers (Skovbjerg, Koch, Anthonsen, & Sjostrom, 2004). The authors did not test microbial transglutaminase, but raise the issue that this enzyme may also catalyse the crucial deamidation step.

A risk associated with the use of transglutaminase in cereal foods?

If microbial transglutaminase does catalyse the deamidation reaction, and conditions during processing are favourable, then any product containing both gluten and transglutaminase may contain the epitope that activates T-cells in the coeliac response. This would be highly undesirable. Transglutaminase is normally expressed in the human intestine and it may be that pre-treatment of cereal products with bacterial transglutaminase renders them no more toxic to coeliacs; however, this should be established before use of this enzyme becomes widespread. Information is also required to establish whether elevated coeliac-active peptides in food may trigger the coeliac condition in

genetically-predisposed individuals for whom the response is dose dependent.

Conclusions

Although there is, to date, no experimental evidence to suggest that goods baked in the presence of transglutaminase may exacerbate or trigger symptoms of coeliac disease, the research presented in the recent literature is sufficient for us to recommend that its use should be discontinued in cereal products containing wheat, barley, rye or oats, until safety checks have been carried out.

Since the coeliac response is specific to certain cereal grains, there is no reason to suppose that the use of transglutaminase in other food systems presents any risk to consumers. However, this paper highlights the fact that the general assumption that enzymes in food are safe, and more acceptable to consumers than chemical additives, may in certain circumstances be incorrect.

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