

# Protein cross-linking – a method for improving the quality of rye baked goods

THE BAKING PROPERTIES OF RYE ARE RATHER LIMITED. WITH PROF. DR. THOMAS BECKER OF THE UNIVERSITY OF MUNICH IN THE CHAIR, SCIENTISTS EXAMINED AN ENZYMATICALLY INDUCED PROTEIN CROSS-LINKING METHOD FOR THE IMPROVEMENT OF THE BAKING PROPERTIES



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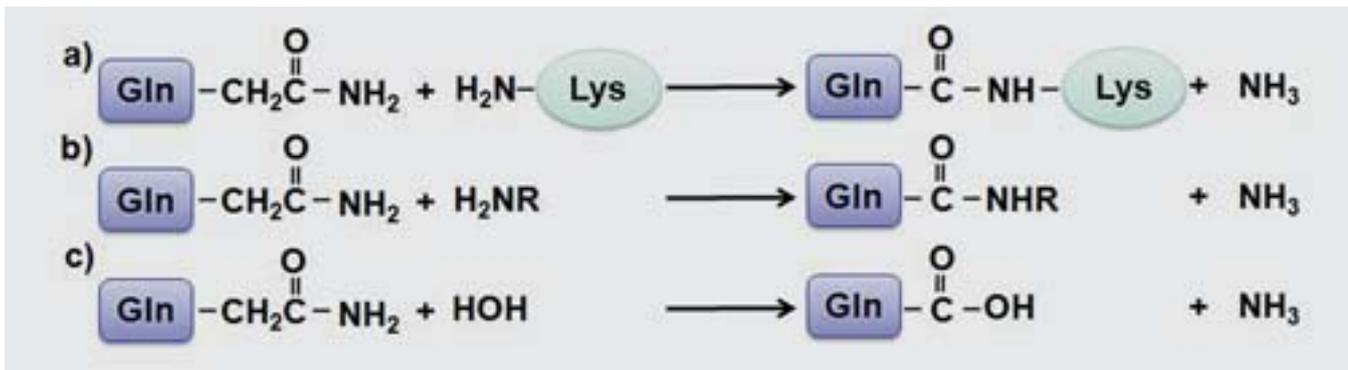
✚ Baked goods made with milled rye products (*Secale cereale L.*) are becoming more and more popular in Germany. This is underlined by a representative survey conducted by CMA/ZMP Market Research according to which even young consumers view rye and mixed rye bread as having an overall positive and cutting edge image. Consumers prefer dark, hearty rye baked goods due to their outstanding nutritional profile including high amounts of minerals, trace elements, B-vitamins and digestion-promoting dietary fibers (pentosan content in rye flour: 6-8%, in wheat flour: 2-3%). However, despite the beneficial nutritional profile, the production of rye baked goods is declining slightly. One of the reasons is the poor baking property of rye. The overall baking performance of rye is among other things due to the functionalities of the pentosans. At low pH, in particular, they are able to bind water and to increase the viscosity of the dough and with that its flow properties so that the dough will retain its shape during proofing and baking. However, the rye proteins (secaline) are not able to form three dimensional structures comparable to the ones created by wheat proteins. Firstly, the pentosans impede the cross-linking of the proteins and secondly, the protein structures have a different design than wheat proteins. The result: rye dough has a comparably higher plasticity, the surface is moist and smudgy and it is hard to process by machines or manually.

Water binding capacity and firmness of rye dough could be improved by an increased cross-linking of the rye proteins which does not or only to a limited extent take place during the traditional processing of milled rye products. The result

would first of all be drier dough with more stability. During baking the additionally bound water would be available for starch gelatinization which in turn yields a moister crumb and improved freshness. A comparably new enzymatic method which promotes the linkage of proteins has opened the opportunity of building a protein network in rye dough and improving the structure. The enzyme transglutaminase (TG) is used in this process. It catalyzes the formation of stable linkages between the rye proteins and the development of large, possibly three dimensional polymer units similar to the gluten network formed by wheat proteins. While transglutaminase in wheat dough, where the protein networks originate all by themselves, can provoke firmer dough, the enzyme can be used in rye dough for optimizing the protein structure in a completely new fashion.

TG (protein-glutamine- $\gamma$ -glutamyltransferase, EC 2.3.2.13) is an enzyme belonging to the group of transferases. It catalyzes three reactions. The first reaction initiates the cross-linking between protein-bound glutamine and lysine residues and with this the set up of protein chains via isopeptide bonds. This reaction is considered to be the prevailing one (figure 1a). If primary amines are present, they are linked with glutamine (figure 1b). In the absence of primary amines, TG catalyzes the deamidation of glutamine to glutamic acid (figure 1c).

Since 1993, the enzyme TG has been commercially available in Japan. It is used on an industrial scale mainly in the meat processing and fish industries for the production of restructured meat as well as for dairy and tofu products. TG for industrial use is obtained from the microorganism *Streptover-*



++ figure 1  
Mechanisms transglutaminase-catalyzed reactions:

- a) Cross-linking between glutamine and lysine  
b) Formation of links between glutamine and primary amines  
c) Deamidation of glutamine to glutamic acid

*ticillium mobaraense* by fermentation. For a few years now, TG has sometimes been also used in the cereal field (wheat and pseudocereals) where the transglutaminase displays a strengthening effect on the dough. It increases the resistance to extension, lowers the extendibility of wheat dough and solidifies the crumb structure in bread. The enzyme can also contribute to an increase in volume. It is worth mentioning that nutritional studies with TG-treated wheat products revealed a reduced allergenic potential for people suffering from coeliac disease.

The treatment of 100% rye dough with TG has so far hardly been the subject of examination. This is the reason why this enzyme is now being investigated. Other than the viscoelastic structure of the wheat gluten which is stabilized predominantly by disulphide bonds, the enzymatic transformation of rye proteins yields covalent isopeptide bonds. It is expected that this will lead to a stable network. Added to this, it is possible that the pentosans will no longer disturb the network formation but rather can be integrated into the rye protein matrix because of the protein side chains.

Current measurements have shown that the properties of rye dough and rye bread can be beneficially influenced with transglutaminase. Rheological and analytical examinations of the dough showed more elastic, firmer and less sticky properties. This is mainly due to the linking of individual small rye proteins via intramolecular cross-links between lysine and glutamine side chains to form larger, structuring protein elements which are the basis for uniform and well leavened baked goods. Regarding the rheological properties, elasticity and firmness increase up to a TG addition of 1000 U (U = Unit). A higher amount will modify the elasticity and the firmness of the rye only marginally. 1000 U TG corresponds in this case to 0.9 g TG added to 1 kg medium rye flour German type 1150.

The baked goods produced during baking trials showed a slightly increased volume (+6%) and more stability. The increase in dough elasticity determined in rheological trials was confirmed by measurements of the product. The addition of transglutaminase (0-1000 U) results in a higher crumb stability (not depicted here) and a linear increase in elasticity of the rye bread crumb (figure 2, see next page). This effect is due to the formation of a protein structure in rye dough

and rye baked goods which changes the crumb structure into a more uniform pore pattern. At the same time, the cohesion of the crumb and the sliceability are improved. Following the positive results obtained by linkages in rye dough and rye baked goods, microscopic images were used to clarify the linking reactions of rye proteins. The protein structures were depicted using Confocal Laser Scanning Microscopy (CLSM) (figure 3) with the white structure being the rye proteins stained with the dye Rhodamine B. Different amounts of TG were added (300 U and 900 U, resp.) ▶

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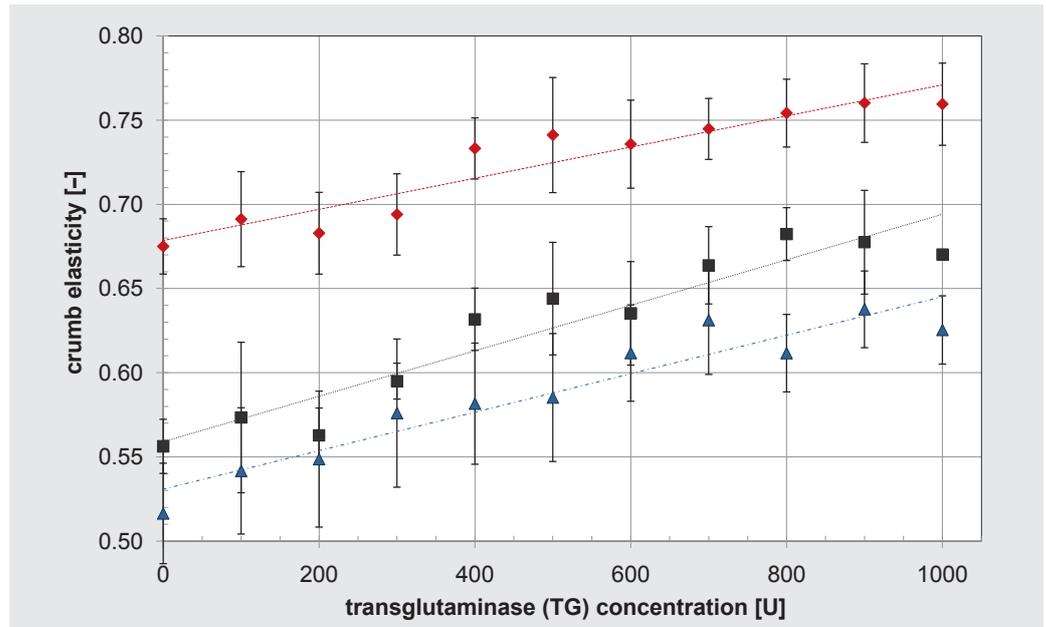
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**++ figure 2**

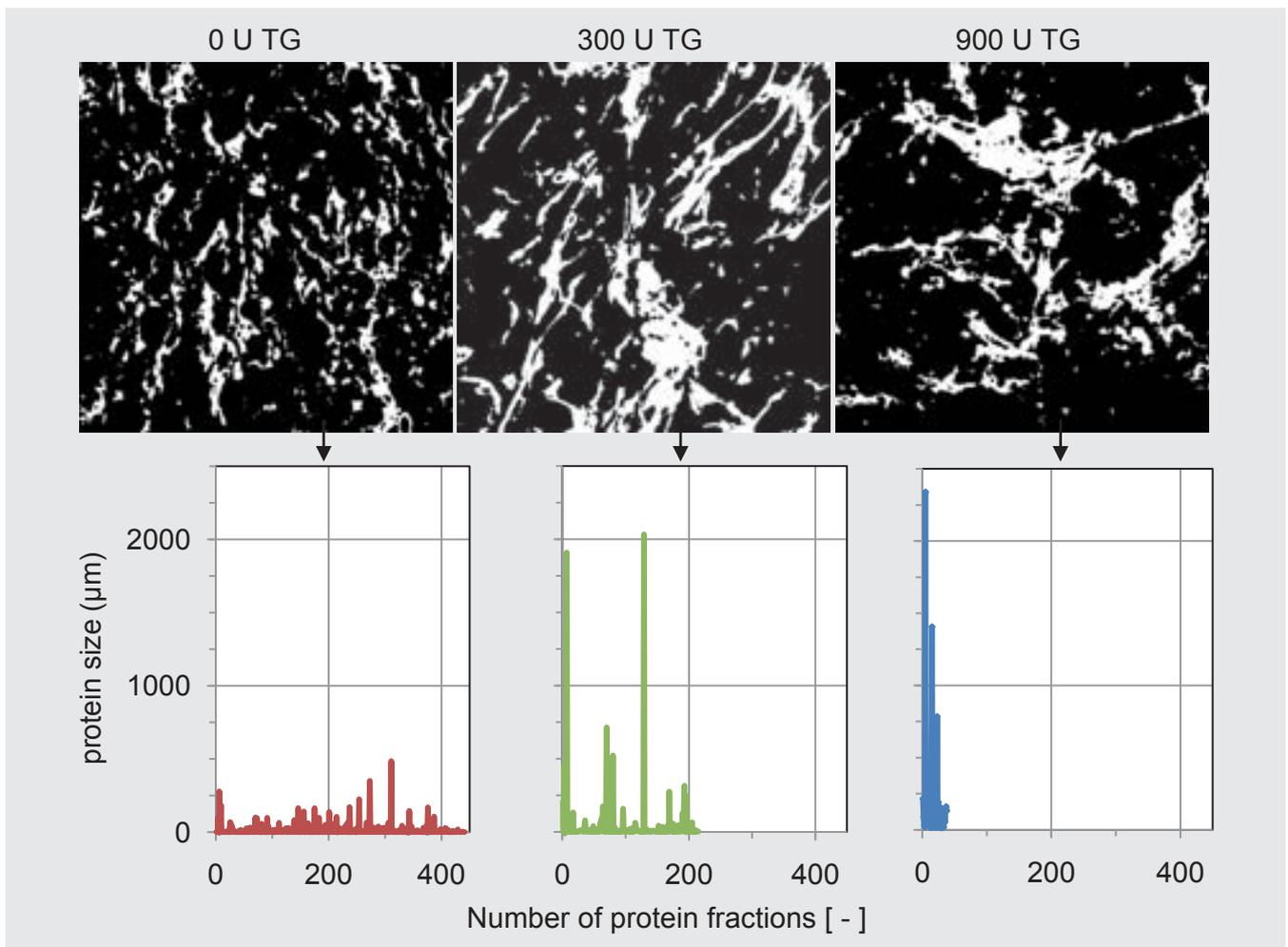
Effect of increasing transglutaminase (TG) concentrations (U = unit) on crumb elasticity of 100% rye bread on dependency of the storage period. Results show the mean value from three measurements, confidence interval  $\pm 95\%$ .

▲ after one day of storage,  
 ■ after four days of storage,  
 ◆ after seven days of storage.



and compared to a standard rye dough without TG (0 U). Compared to the non-TG reference sample, distinct linkages between the proteins and with this elongated protein structures were found in dough with 300 U added TG. Using

special software for the evaluation of the structures, the changes were quantified using image processing analysis and converted into figures. Here, the size of the proteins in  $\mu\text{m}$  was plotted against the number of proteins. Straight,



**++ figure 3**

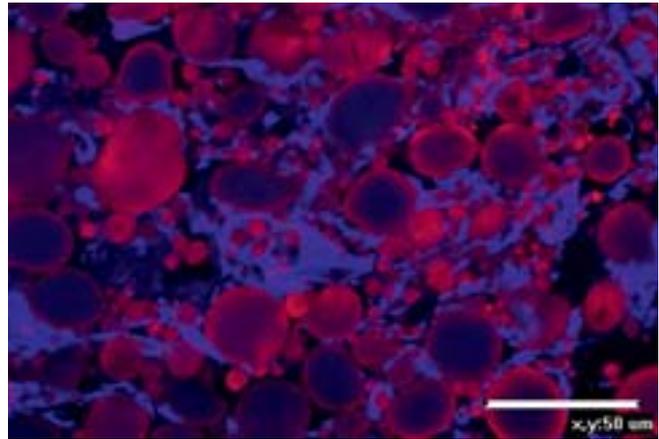
Top: Binary images obtained by Confocal Laser Scanning Microscopy (CLSM). The white structures are dyed rye proteins. Pictures show an image section of  $212 \mu\text{m} \times 212 \mu\text{m}$ . Pictures show the comparison of rye protein without TG (0 U TG), 300 U TG

and 900 U TG (from left to right) after one hour proofing time ( $30^\circ\text{C}$ , 80% RH). Below: The respective protein sizes were plotted against the number of protein fraction.

non-acidified rye dough (0 U TG) displayed many small protein fragments which do not form coherent structures other than the proteins in wheat dough. The comparison of the reference dough (0 U TG) with a rye dough with 300 U TG showed a reduction in the number of protein fractions by about 50% with a simultaneous increase in the length of the individual protein chains. As the study showed, the further addition of TG (900 U) changed the protein structure from elongated protein strands (300 U) to aggregated proteins. Baked goods with more than about 900 U TG have lower bread volumes than the reference bread (0 U TG). The aggregation of the rye proteins which occur at too higher amounts of TG may be the reason for the reduction in baked goods volume.

### Conclusion

Current works show the optimization opportunities for rye dough and rye baked goods via rye protein linkage induced by TG. The rye dough treated with TG yielded more elastic, firmer and less sticky dough pieces than rye dough without TG. Added to this, the crumb elasticity and stability increased clearly with increasing addition of transglutaminase which improved the coherence of the crumb and with that the sliceability. Regarding the rye bread volume, an increase of up to about 6% was determined. Thus the application of



**++ figure 4**  
Images obtained by Confocal Laser Scanning Microscopy (CLSM): Phase distribution of starch granules (red) and proteins (blue) in 100% rye dough.

TG in the production of rye products allows a targeted manipulation of rheological and technological dough properties.

### Acknowledgments

We wish to acknowledge the support received from the AIF via FEI (Research Association of the German Food Industry, Bonn) and the Ministry of Economics (Project No. 15457 N). **+++**

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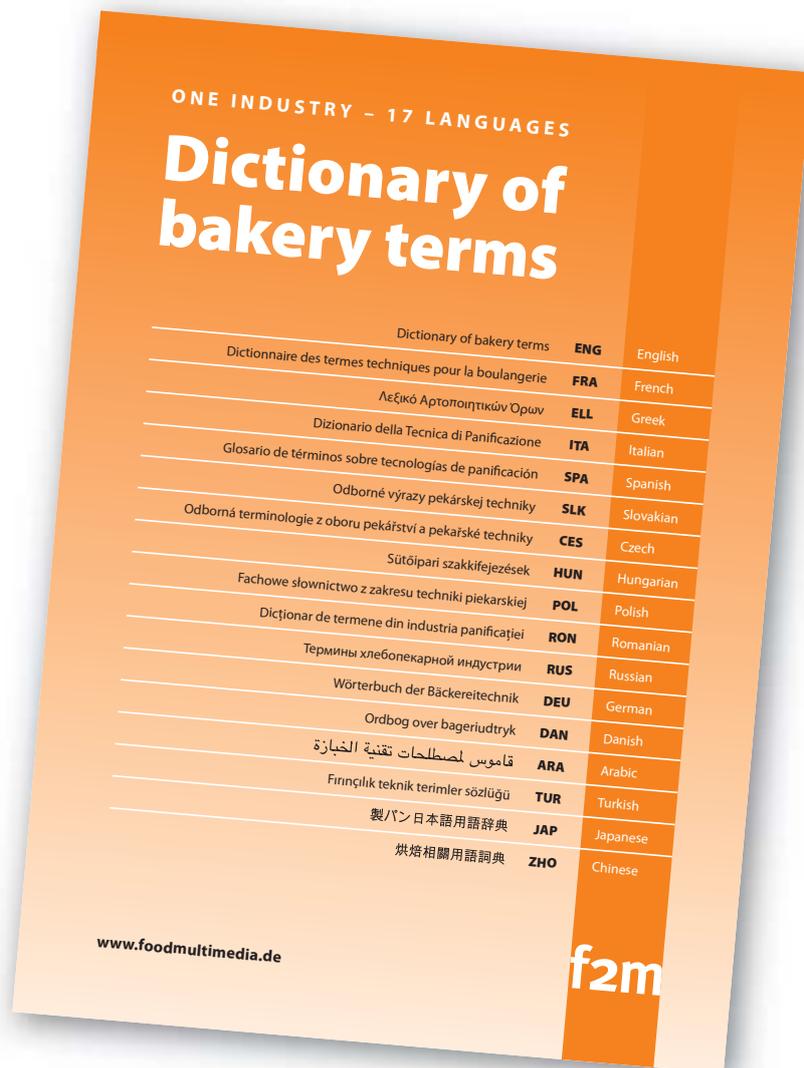
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