



WORLD BREWING CONGRESS

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#ElevateBeer



Production of gluten-free beers using transglutaminase

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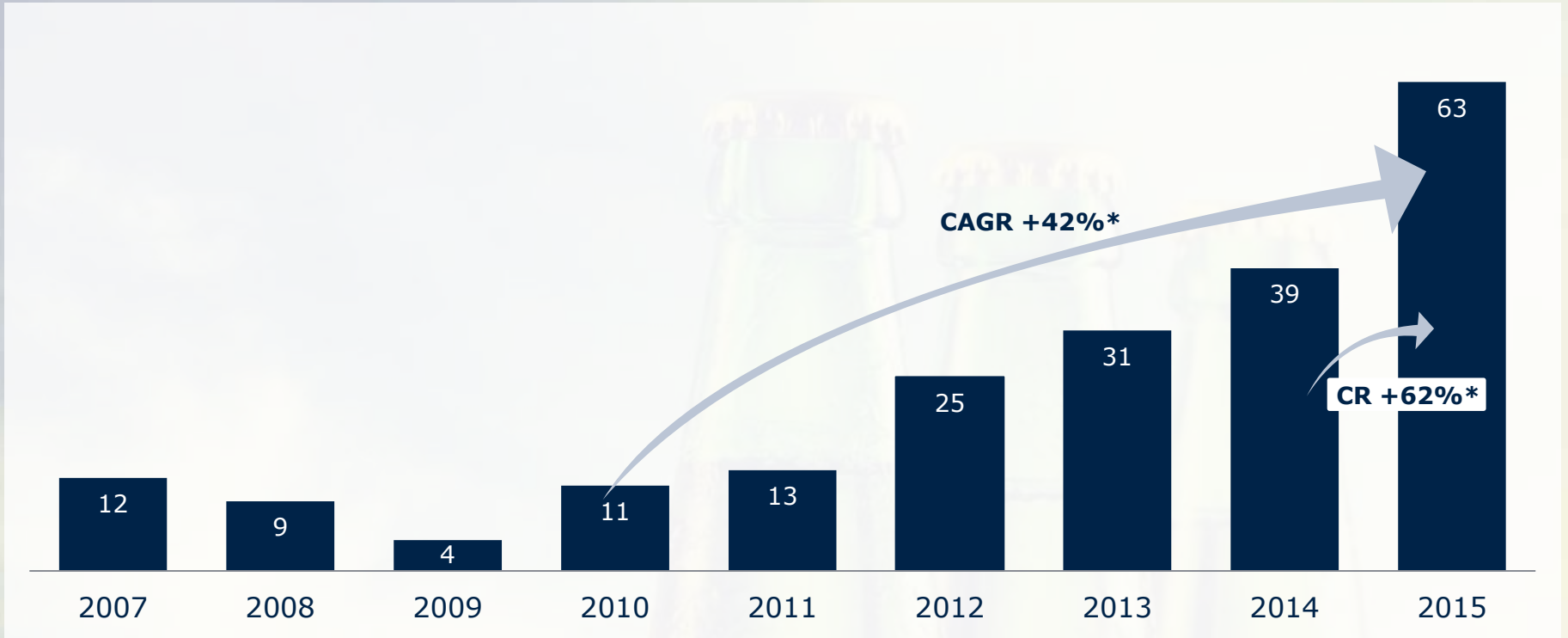
Doehler GmbH, Darmstadt, Germany

August 16, 2016





Gluten-Free Beer – Global New Product Introductions



NUMBER OF NEW INTRODUCTIONS IN BEER WITH A GLUTEN-FREE CLAIM INCREASED SIGNIFICANTLY SINCE 2012. MOST OF THE INTRODUCTIONS WERE LAUNCHED IN EUROPE, FOLLOWED BY NORTH AND LATIN AMERICA.

Source: GNPD / Döhler Market Intelligence

*CAGR = Compound Annual Growth Rate

*CR = Changing Rate



Gluten-Free Beer – Launches in Europe & North America



Source: GNPD / Döhler Market Intelligence



What's So Special About Gluten?

Gluten proteins are **resistant to enzymatic degradation** in the human body.

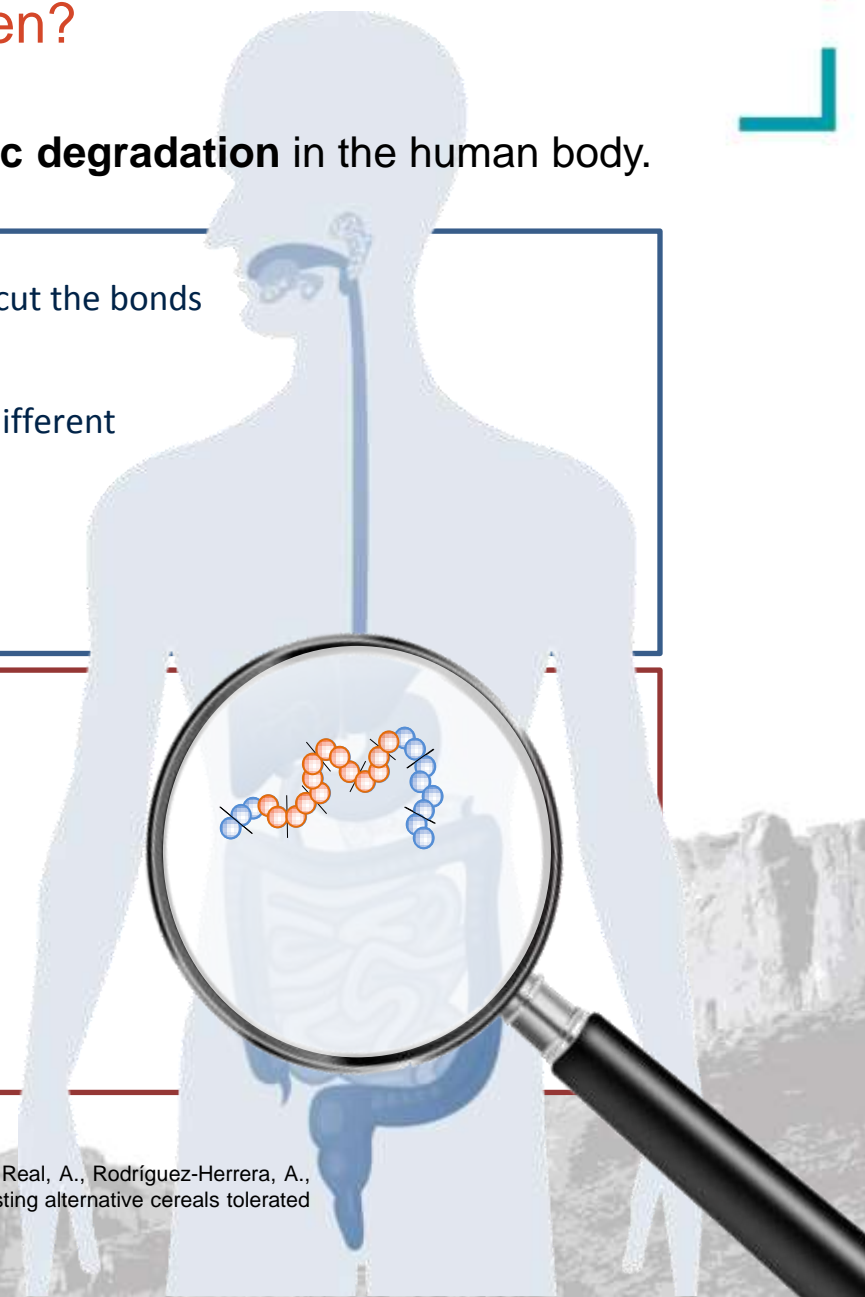
Normal protein

- When eating proteins, digestive enzymes cut the bonds between amino acids.
- Normal proteins are largely degraded by different proteolytic enzymes.
- Single amino acids and small peptides are absorbed by the small intestine.

Gluten protein

- Gluten and gluten-like proteins are rich in proline and glutamine residues.
- The human digestive system is lacking prolyl endopeptidases, resulting in an incomplete proteolysis.
- Long toxic oligopeptides are accumulating in the small intestine.

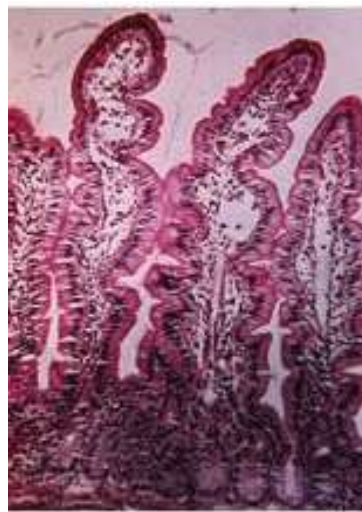
Reference: Comino, I., Moreno, M. de Lourdes, Real, A., Rodríguez-Herrera, A., Barro, F., and Sousa, C. The gluten-free diet: testing alternative cereals tolerated by celiac patients. *Nutrients* 5:4250-4268, 2013.





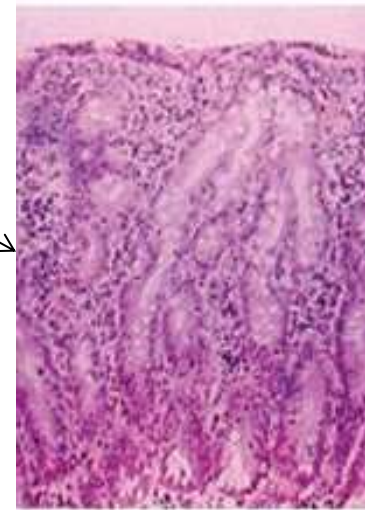
Why is Gluten Considered Bad?

Gluten and gluten-like proteins are the causative agent for **Celiac Disease**: an immune-mediated enteropathy triggered by the ingestion of gluten-containing cereals or products in genetically susceptible individuals.



Normal villi

Mucosa of the small intestine



Damaged villi

Effects:

- Chronic inflammation of intestinal mucosa
- Atrophy of intestinal villi
- Malabsorption of important nutrients including vitamins and minerals
- Abdominal pain, diarrhea, weight loss, fatigue, anemia, osteoporosis, ...



Production of Gluten-Free Beer – Three Innovative Solutions

How can the Gluten content be effectively reduced in traditionally produced beer?

| Application of Tannic Acid or Silica Gel | Application of Prolyl Endopeptidase (PEP) | Application of Transglutaminase (TG) |
|---|--|--|
| Reduction of Gluten by protein precipitation or adsorption in combination with special filtration technology . | Reduction of Gluten by enzymatic modification of the respective toxic epitopes due to extensive protein degradation . | Reduction of Gluten by enzymatic modification of the respective molecules due to protein crosslinking and separation. |

According to the Codex Alimentarius (CODEX STAN 118-1979), food products containing ≤ 20 ppm (mg/kg) **Gluten** can be labeled as “gluten-free”.





Characteristics of Microbial Transglutaminase

- Naturally occurring enzymes obtained from microorganisms, e.g. *Streptomyces mobaraensis* (former *Streptoverticillium mobaraense*)
- Commonly used to improve textural properties of food products
- Approved for food applications in the U.S.A, Canada, Europe (except for Denmark), Brazil, China, Japan, and Korea amongst others
- Monomeric proteins with molecular weights of approximately 38 kDa
- Optimum temperature 40-50°C
- Optimum pH 5-8
- Permits the claims “GMO-free”, “organic”, and “vegan”





Function and Advantages of Transglutaminase

Transglutaminase ...

... is an enzyme that catalyzes the formation of isopeptide linkages between proteins.

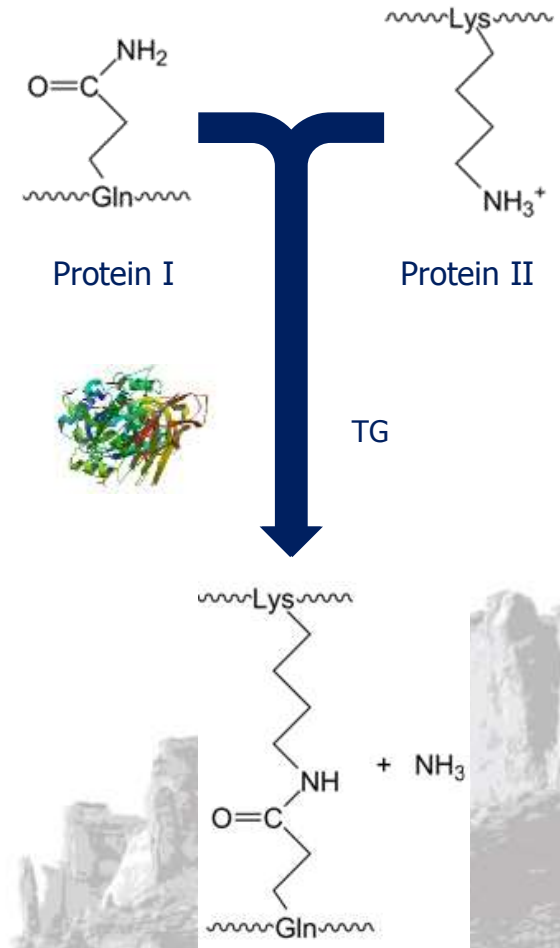
... is crosslinking glutamyl and lysyl residues of proteins or peptides.

... is produced by natural microorganisms (non-GMO product).

... completely removes Gluten from conventionally produced beers.

... does not affect the sensory properties of the gluten-free beer.

TG crosslinking reaction

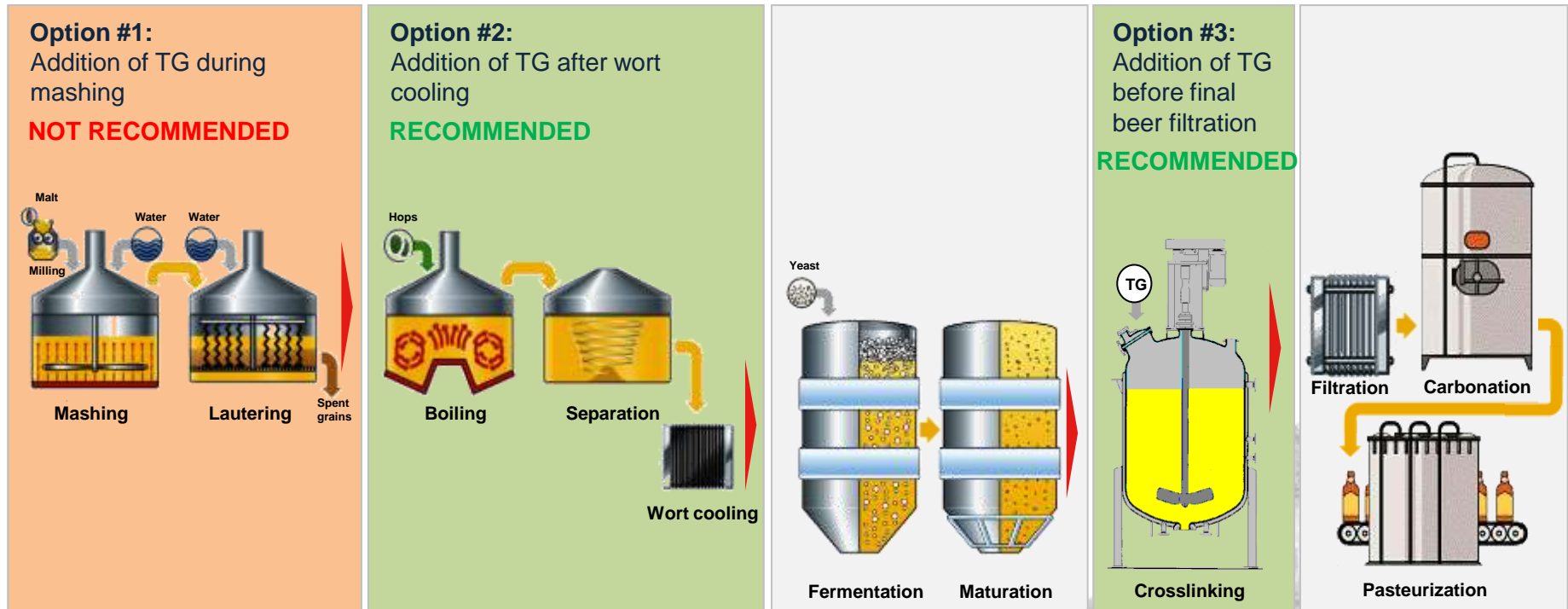




Implementation of Transglutaminase into the Brewing Process



Activities

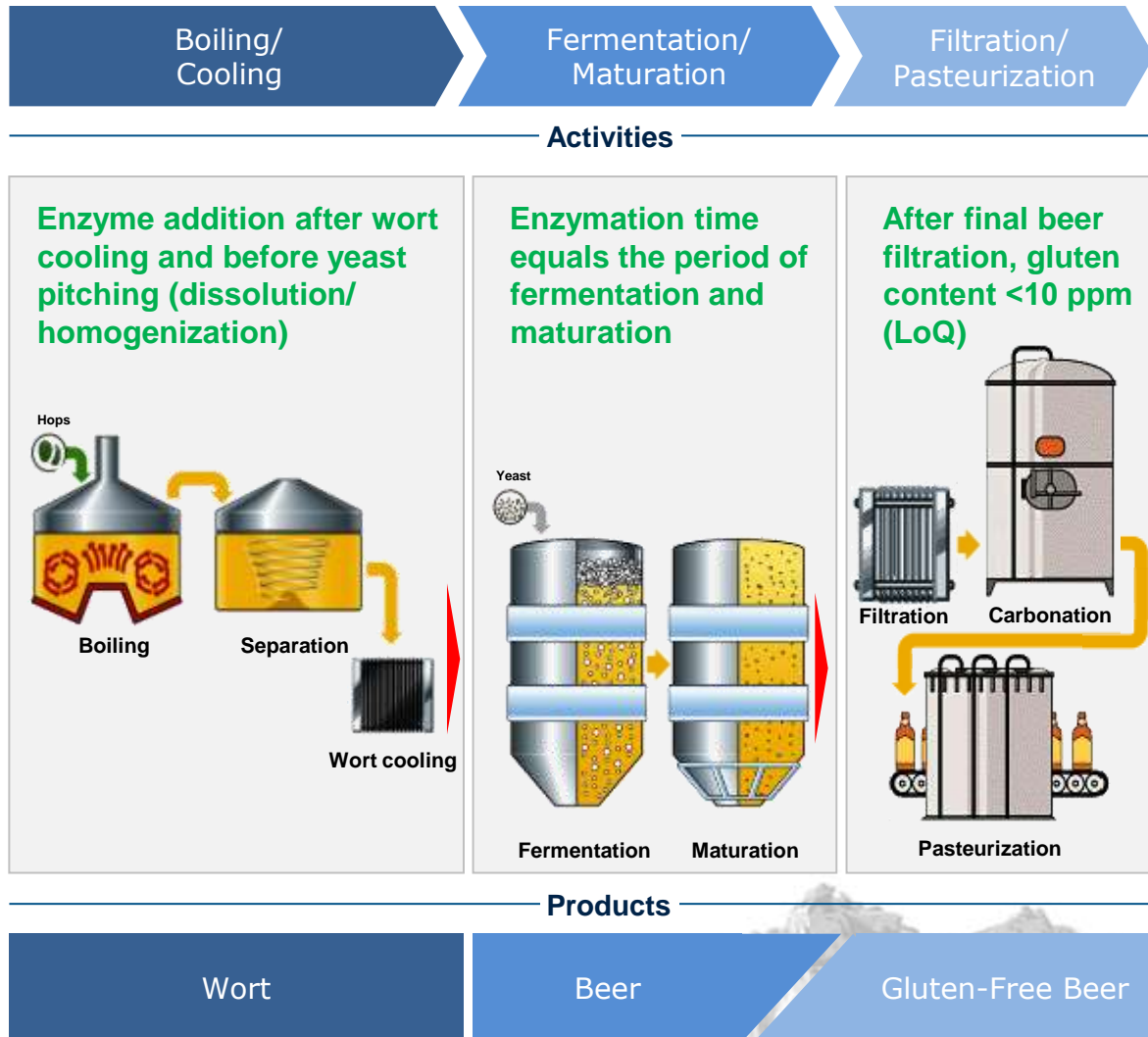


Products





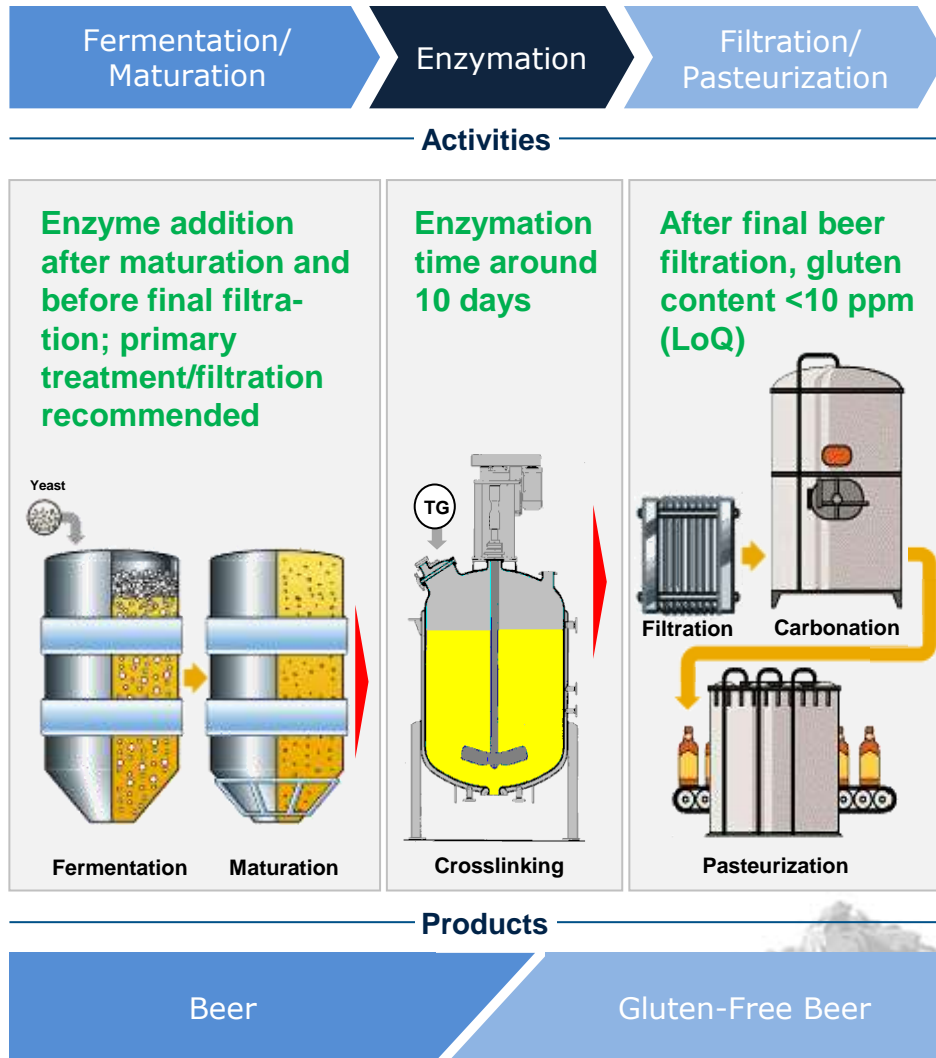
Option #2: Addition of Transglutaminase to Cold Wort



- Easy integration into the conventional brewing process
- Application of TG in powder or liquid form
- Recommended dosage for 100% barley malt worts (12 °Plato): 40-50 units/L ≈ 4-5 g/hL (TG powder)
- Total removal of cross-linked gluten protein complexes by standard beer filtration
- Lowest possible gluten content, analyzed by RIDASCREEN® Gliadin competitive ELISA method
- No impact on taste or foam of the final gluten-free beer



Option #3: Addition of Transglutaminase to Pre-Filtered Beer



- Use of liquid TG formulation
- TG maintains its total enzyme activity at temperatures close to 0°C
- No adverse effect on filtration performance (e.g. kieselguhr filtration)
- No impact on the flavor or foam stability of the final gluten-free beer
- Gluten is not a physiological substrate of TG; it has, however, a high affinity to this enzyme due to the polyproline type II helices.



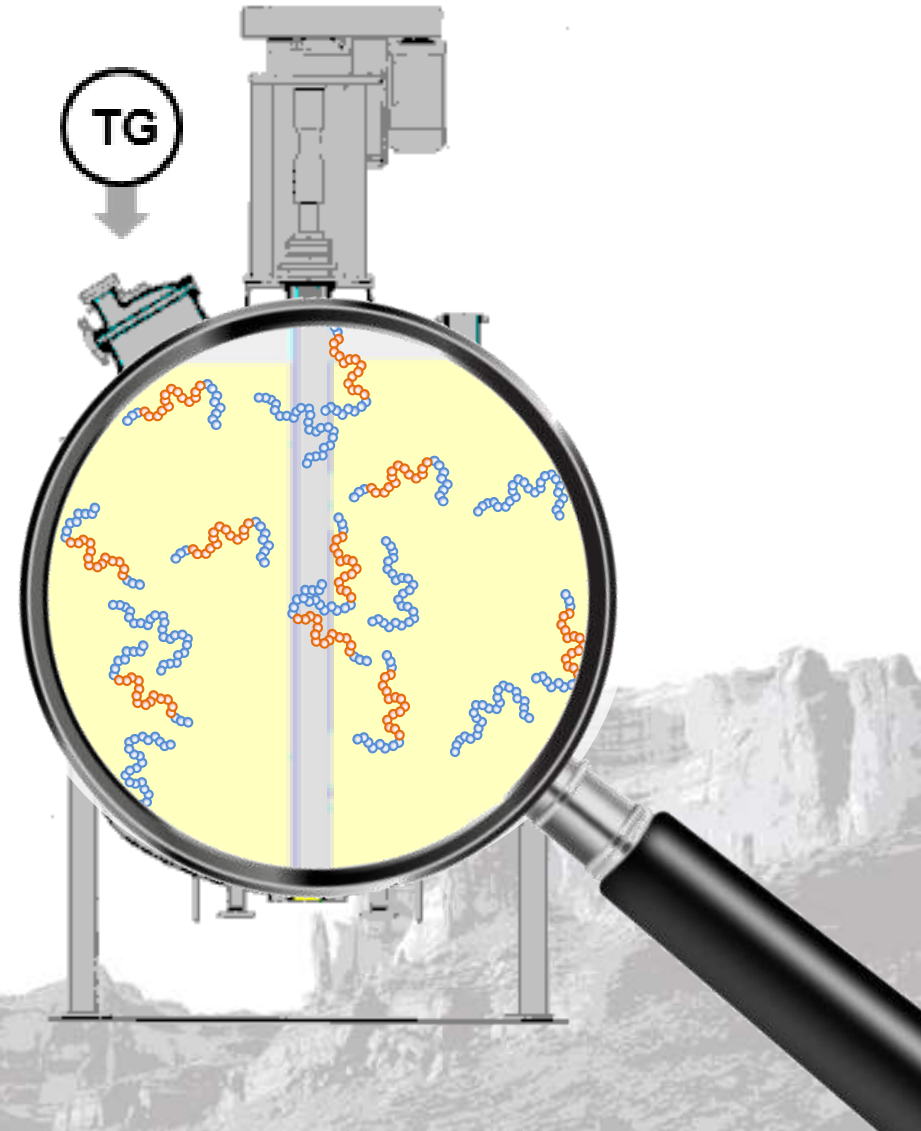
Enzymatic Modification of Proteins by Transglutaminase



Before enzyme treatment



After enzyme treatment





Effect of Transglutaminase on the Quality of Gluten-Free Beer

| Quality parameters | Foam | Color | Taste | GMO-free | Total Gluten removal from final product |
|--------------------|------|-------|-------|----------|---|
| TG | ● | ● | ● | ● | ● |

Foam:

Use of TG has no effect on beer foam stability, even if the enzyme is over-dosed; i.e. removal of Gluten proteins is not affecting foam-positive proteins

Color:

Use of TG may have a slight impact on beer color, which increases with increasing enzyme dosages; therefore over-dosing should be avoided

Taste:

Use of TG has no effect on the flavor, taste, and smell of gluten-free beer

Reference: Taylor, J.P., Jacob, F., and Arendt, E.K. Fundamental study on the impact of transglutaminase on hordein levels in beer. *Journal of the American Society of Brewing Chemists* 73(3):253-260, 2015.



Conclusion – Brewing Gluten-Free Beer with Transglutaminase

Easy process integration & **reliable** process stability

The only enzymatic **GMO-free** solution

Total Gluten removal from the beer

Lowest possible Gluten content

Permits the claims "**GMO-free**", "**organic**", and "**vegan**"

No impact on flavor, taste, smell, and foam of the gluten-free beer

Technology is patented; however, the enzyme TG can be sourced from multiple suppliers in powder or liquid form



Any Questions?



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... THANK YOU for your attention.