EDITING GLUTEN

What is Gluten?

Gluten is a protein found in wheat, barley, and other grains. It is extracted and added to many foods that people eat on a general basis, such as baked goods. It gives structure and shape to foods, and it is also used as a binding and thickening agent.

Background information

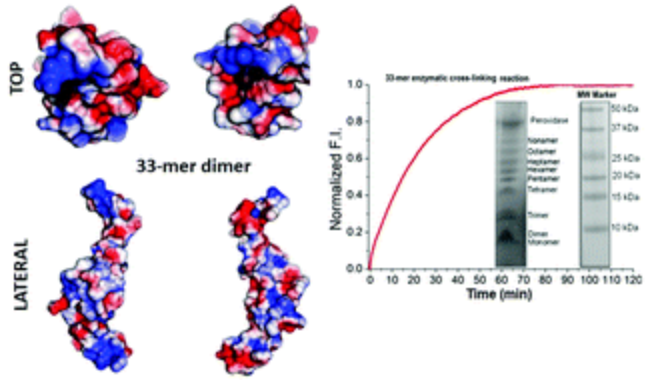
How does celiac disease work?

When Gliadin[1] is consumed, it gets broken down into smaller peptides during digestion. In people without Celiac disease, peptides[2] are usually broken down further by enzymes, however people with Celiac disease are not able to fully break down some peptides especially with specific sequences[3] one of those being 33-mer. 33-mer is a peptide that is resistant to digestive enzymes containing a high number of glutamine, a type of amino acid which is what makes it resistant to those enzymes. It is formed when α-gliadin is broken down. An enzyme called tissue transglutaminase (tTG) modifies gluten peptides by deamidating them, removing the amino group from the glutamine residue[4]. When the gluten peptides are deamidated, they are more likely to bind to receptors on HLA-DQ2 and HLA-DQ8[5]. The receptors present the bound peptides to T-cells which recognize the peptides as an invader triggering an immune response leading to inflammation and damage to villi in the small intestine.

How does Gene editing come into play?

The root of the 33-mer peptide is mainly from α-giladin from gluten, editing out α-gliadin completely to stop the production of 33-mer. In addition to editing out α-gliadin, we would need to modify other gliadin proteins to better compensate for the loss of α-gliadin. It would require additional editing and very careful balancing of the gluten protein network. Alternatively, if the goal is to create wheat that is safe to consume for people with celiac disease, other non-wheat proteins like those from rice, oats, or legumes could be incorporated into the wheat to enhance its gluten-like properties.

PROS	CONS
- Could likely reduce the production of immunogenic 33-mer peptide potentially making it safe for people with celiac to eat and digest	- Gluten loses its elasticity, textures of food become different - Baked products become more dense, crumbly, or chewy



Above is a picture of the 33-mer peptide that comes from alpha(2) gliadin.

Challenges

α-gliadins are the most abundant class of gliadin, constituting around 40-50% of the gliadin fraction.

- When it breaks down it breaks into 33-mer peptide, to delete something that takes up 40-50% of gluten would completely change its molecular composition
- Each molecule of gluten has one type of gliadin, there is not a mix of more than one type

α-gliadins	Present in high amounts 40-50% of the gliadin fraction
γ-gliadins	Present in moderate amounts, making up around 20-30% of the gliadin fraction
β-gliadins	Present in lower amounts typically around 10-20% of the gliadin fraction
ω-gliadins	Present in small amounts, around 5-10% of the gliadin fraction

Overcoming challenges

As α-gliadins take up around half, they play a vital role in gluten, to overcome this challenge there are two options:

- Replacing α-gliadin with something else with a similar composition
- Strengthening β, γ, and ω gliadins, however if we approach it this way we will need to keep in mind that the β, γ, and ω gliadins, also produce the 33-mer peptide when broken down, just in significantly smaller amounts

Strengthening:

Cross linking

What is cross linking?

Crosslinking is a chemical process that joins two or more molecules together

a) Modifying glutenin subunits to increase their ability to cross-link could enhance the strength of the gluten network. Cross-linking is vital for forming an elastic structure in dough, so making glutenin proteins more interactive with each other could lead to a stronger dough
b) Some enzymes, like transglutaminase, can crosslink proteins, helping to create stronger and more elastic networks in non-gluten proteins. By using this method we could potentially enhance the properties of alternative proteins to replace α-gliadin in a gluten free dough system
c) Other polysaccharides, such as cellulose from plants or chitosan from fungi might provide alternative binding and gelling properties to help with the dough's texture.
    - Chitosan is a polysaccharide (complex sugar) composed of randomly distributed double beta linked D-glucosamine and N-acetyl-D-glucosamine (amino sugar)
    - ^ Its source is from treating chitin shells of crustaceans with an alkaline substance eg; sodium hydroxide

Disulfide bonds

What are disulfide bonds?

Disulfide bonds are covalent bonds which are formed between the sulfur atoms of two cysteine amino acids. They are also known as disulfide bridges or S-S bonds.

The functions of disulfide bonds include:

Protein structure: stabilize the structure of proteins, both internally and externally.

Protein signalling: regulating signaling pathways in cells.

a) Could alter the amino acid sequences of β- or γ-gliadins to introduce more disulfide bonds or other cross-linking structures, enhancing their ability to interact with glutenins and strengthen the dough
b) Gene editing could possibly target the proteins involved in folding of the gluten proteins, encouraging the correct folding of glutenins and gliadins to optimize their ability to interact and cross-link; modifying the ratio of disulfide bonds within glutenin and gliadin proteins could strengthen the gluten network, as disulfide bonds are key to maintaining the integrity of the gluten structure.

Replacements

Since α-gliadin itself is a protein, we would need to replace it with something of similar composition

Pea proteins:

- Pea protein isolate, legumin and vicilin (the main storage proteins in peas)

Soy proteins:

- Glycinin and β-conglycinin (major storage proteins in soybeans)

Rice protein:

- Rice proteins are typically composed of glutelins and prolamins similar to wheat, just with less elasticity; they are usually just referred to as rice protein isolate when extracted

Whey proteins:

- β-lactoglobulin, α-lactalbumin (major proteins in whey)

Fava bean proteins:

- Vicilin and legumin (similar to pea proteins)

1. Starting with a pea protein or soy protein for structure and binding
2. Adding a binding agent; incorporating xanthan gum or guar gum to improve texture and help with moisture retention
3. Starches; using a combination of tapioca starch and potato starch for elasticity and structure enhancement
4. Add hpmc (hydroxypropyl methylcellulose); for additional texture if needed, especially helpful for products like bread (baked goods)

Definitions

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[2] a short chain of amino acids linked together by peptide bonds, when two or more amino acids join together through peptide bonds, they form a peptide

[3] Referring to the specific arrangement of amino acids in a protein chain known as peptides

[4] the process of removing the amine (NH2) group from an amino acid residue, from glutamine (amino group -NH2 and carboxyl group -COOH). In celia, the enzyme tissue transglutaminase (tTG) catalyzes this.

[5] Human leukocyte antigen