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Quantifying the Concentration of 33-mer in Wheat Flour

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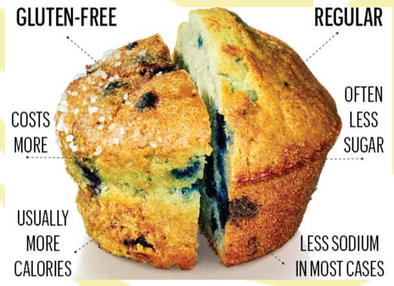
Abstract

Celiac Disease is a type IV hypersensitive response to gluten caused by HLA-DQ2 or HLA-DQ8 T-cell presentation, initiating destruction of intestinal epithelial cells. Currently, the only remedy for those suffering from celiac disease is the complete elimination of all gluten from the diet. Studies indicate that an indigestible fragment of the gluten molecule, alpha-gliadin subcomponent 33-mer, rich in proline and glutamine, is responsible for the hypersensitivity response. Determination of 33-mer concentration in wheat lines could be beneficial to the future development of wheat lines with reduced 33-mer concentration. In this study, gliadin protein from wheat flour was extracted and separated from other proteins of wheat flour. The extract of gliadin was then subjected to enzyme-linked immunosorbent assay (ELISA) technique in order to quantify the concentration of the 33-mer fragment. This is the critical next-step in allowing us to identify and develop wheat lines with reduced concentrations of 33-mer. It is possible that wheat with reduced 33-mer may be suitable for consumption by individuals with celiac disease.



Introduction

Celiac Disease (CD) is a gluten hypersensitivity characterized by severe physical symptoms caused by genetic and environmental factors. Genetically, 90-95% of CD patient's T-cells respond to the gluten derived peptides presented by HLA-DQ2 or HLA-DQ8 cell surface receptors that trigger the destruction of intestinal epithelial cells (Tian, et.al, 2015). Environmentally, CD is triggered by the ingestion of gluten, a general term referring to to the aggregation of the storage proteins gliadin and glutenin (Van den Broeck, et al, 2009), which are found in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.). Gliadin is further subclassified into alpha, beta, and gamma subcomponents. Studies indicate that an indigestible alpha gliadin subcomponent, rich in proline and glutamine, is responsible for the hypersensitivity response. The presence of such amino acids make this alpha-9 gliadin subcomponent resistant to digestion by proteases, which is unaffected by normal digestion (Hollon, et al, 2015). Studies show that this particular epitope of gliadin, alpha-9, disrupts digestive processes most dramatically (van de Broek, et. al., 2009). Of particular interest is the peptide sequence, 33-mer, that binds to the HLA-DQ complex and then to T-cell receptors, triggering an adaptive immune response resulting in inflammation, and ultimately death of intestinal cells, giving rise to the characteristic symptoms of CD (Barone, et.al, 2015). Development of wheat lines with reduced 33-mer may be valuable to individuals suffering from CD. Such a wheat variety may not trigger an autoimmune response, permitting CD patients to eat gluten-containing foods without experiencing adverse side effects.

Material and Methods

Previously, the protein concentration in the wheat flour extraction samples was confirmed through a serial dilution of the protein extract with 4 sample concentrations at 1mg/mL, 100 µg/mL, 10µg/mL, and 1µg/mL. Enzyme-Linked Immunosorbent Assay (ELISA) was used to specially tag the protein of interest, 33-mer, and confirm the concentration gradient. In the first stage of this process a sample of wheat flour was compared to a gliadin standard. Using 1 mL extracted gliadin, a series of dilution were performed using coding buffer. Following the dilutions, the samples were incubated in a dark refrigerator overnight. The wells were then aspirated and washed with PBS/Tween-20. 0.5% blocking buffer and allowed to rest overnight. Primary antibody (anti-gliadin) was diluted with 0.5% blocking buffer at a ratio of 1µL:10mL, and after repeated dilutions into ELISA wells, the plates were incubated for two hours at room temperature (37°C). The following day, the plates were aspirated and washed with PBS/Tween, diluted with the secondary antibody (Anti-Mouse-HRP) in blocking buffer at 1µL:5mL. The secondary antibody was then added to the ELISA wells for one hour at room temperature. After removing the liquid, the substrate was added (50mL:50mL:1µL) to each well. The ELISA plate was then visualize on the Glomax Microliter Plate

Results and Discussion

The ELISA results were not highly correlated with the expected linear range of concentrations within the seven serial dilutions. Decreasing ELISA signal would be expected to be associated with decreasing protein concentration. The unexpected data may be due to low levels of analyte concentrations established in the wells. The next samples are proposed to have a higher analyte concentration in an effort to correct the problem.

Table 1: ELISA 4 Test Data

	2	4	6	8	10	11
B	1231.74	1075.1	988.525	1057.91	983.248	957.831
C	1134.97	1011.35	982.656	969.287	987.928	1001.32
E	1044.38	936.372	554.142	491.626	502	502.169
F	570.359	72.4502	73.291	73.3184	73.309	73.2863

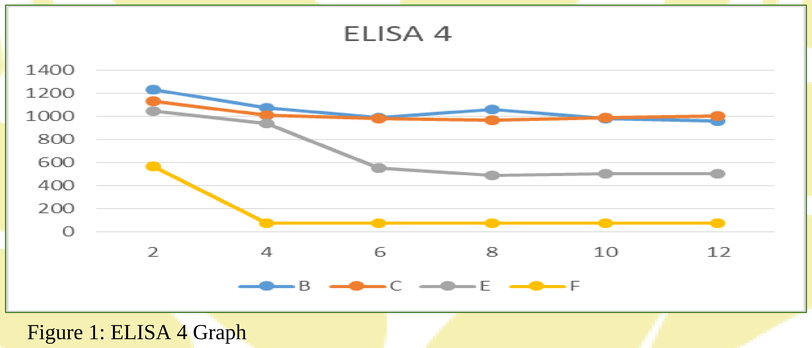


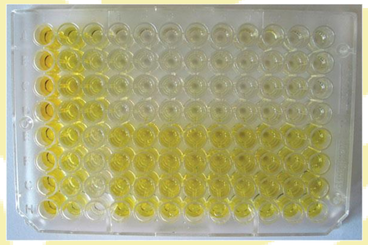
Figure 1: ELISA 4 Graph

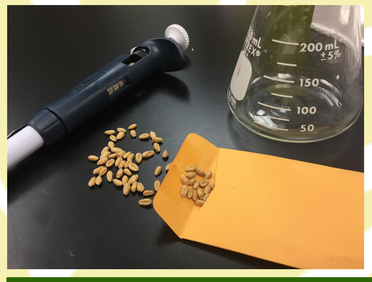
Conclusion

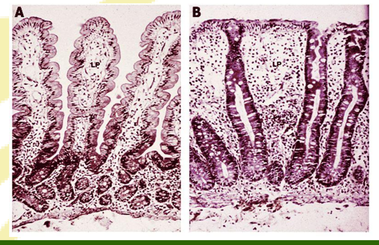
The gliadin protein was successfully isolated and used to create a serial dilution series. ELISA was then used to identify the 33- mer fragment of interest. Refinement of this procedure is required to successfully quantify the concentration 33-mer in each sample.

Future Research

Further research needs to be conducted in order to refine the protocol for detection of the presence of the 33-mer concentration in wheat flour. This will allow screening of multiple wheat lines, and determination of genetic diversity for breeding and selection. This could lead to the development of a wheat line containing reduced 33-mer concentrations, which may be safe for individuals with CD to consume without the adverse health effects of traditional gluten products.







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