

Extraction of gluten from food material

Abstract

Food allergies are creating many problems even though the total population allergic to certain proteins in food is less than 1%. Gluten is one among the major proteins present in almost all kinds of flours, especially in wheat and barley in higher quantities. Food industries are now a day's turning towards producing gluten free foods which can be consumed by all. In order to produce them we must know various concentrations of gluten present in them. The aim of the present study is to extract, quantify and estimate the gluten present in materials like wheat, barley, maida, and oats. By this data the effect of gluten related allergies can be studied.

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Sreenivas Reddy Bathula,^{1,2} Ravishankar BV,³ Pujar Basayya Gullaya³¹Adama Science & Technology University, Ethiopia²Savi Easy Life sro, Czech Republic³Department of Biotechnology, Bapuji Institute of Engineering and Technology, India**Correspondence:** Sreenivas Reddy Bathula, Adama Science & Technology University, PO Box 1888, Adama, Ethiopia, Tel +91-9591-2402 34, Email jaishwa@hotmail.com**Received:** December 13, 2017 | **Published:** June 22, 2018

Introduction

Gluten

Primarily, “gluten” (from Latin for “glue”) has been defined as “the rubber-like proteinaceous mass that remains, when wheat dough is washed with water or salt solution to remove soluble constituents and starch granules”. The procedure of wheat gluten preparation was first described by Beccari in 1745 and is nowadays widely used for the industrial isolation of “vital gluten”, a byproduct of wheat starch production. Gluten is a protein fraction from wheat, maida, barley, oats or their crossbred varieties and derivatives thereof, to which some people are intolerant and that is insoluble in water and 0.5 M NaCl. These proteins that naturally occur in a prohibited grain and that may cause adverse health effects in persons in celiac disease. Some food processing procedures increase gluten's solubility, but do not necessarily diminish the protein fraction's harmful effect.¹⁻⁵

Gluten can be readily prepared by gently washing dough under a stream of running water. This removes the bulk of the soluble and particulate matter to leave a proteinaceous mass that retains its cohesiveness on stretching. Gluten comprises some 75% protein on a dry weight basis, with most of the remainder being starch and lipids. Furthermore, the vast majority of the proteins are of a single type called prolamins. Prolamins are a group of proteins that were initially defined based on their solubility in alcohol–water mixtures, typically 60–70% (v/v) ethanol. This definition has since been extended to include related proteins, which are not soluble in alcohol–water mixtures in the native state, owing to their presence in polymers stabilized by interchain disulphide bonds. In wheat, these groups of monomeric and polymeric prolamins are known as gliadins and glutenins, respectively, and together form gluten.

As a result of the formation of a protein matrix, individual cells of wheat flour contain networks of gluten proteins, which are brought together during dough mixing. The precise changes that occur in the dough during mixing are still not completely understood, but an increase in dough stiffness occurs that is generally considered to result from ‘optimization’ of protein–protein interactions within the gluten network. In molecular terms, this ‘optimization’ may include some exchange of disulphide bonds as mixing in air, oxygen and nitrogen result in different effects on the sulphhydryl and disulphide contents of

dough. The natural fate of the wheat grain is not to provide flour for humankind but to germinate to produce a new plant. The biological role of the gluten protein is, therefore, to provide a store of carbon, nitrogen and sulphur to support seed germination and seedling growth. The gluten proteins have no other known biological role and their viscoelastic properties appear to be a purely fortuitous consequence of their sequences and interactions.

Glutenins and gliadins are the most important proteins in the wheat grain which can be used for assessing genetic diversity of species or varieties and for genotype identification in different wheat species. Glutenin subunits are divided into two major groups according to their electrophoretic mobility in SDS-PAGE, the high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits.

Allergic nature of gluten

Demonstrating intolerance to gluten is a complex endeavour. The toxicity of gluten in celiac disease (CD) stems from an immune response involving both innate and adaptive systems. No model is available to replicate the response, although rhesus macaques were recently proposed. The demonstration of gluten intolerance depends on in vivo challenge studies.

CD has a highly variable presentation and symptoms are considered unreliable as an indicator of active disease. The defining indicator of gluten-induced damage in CD is histopathology of the mucosa of the small intestine. It only develops in response to ongoing gluten exposure, which means the investigation of gluten intolerance faces design and ethical hurdles. Other factors associated with CD hinder representative studies. They include the heterogeneous presentation of disease, a high rate of under diagnosis and the lifestyle challenges of a truly Gluten Free (GF) diet.⁶⁻⁹

Defining gluten-free foods

In many circles, a zero tolerance approach to gluten in GF foods is considered impractical. With derivatives of wheat, and to a lesser extent barley, used widely in mainstream food channels, GF foods are susceptible to contamination, even when produced in dedicated facilities. Some GF foods in Europe are even based on wheat-starch which, though processed to remove gluten, contains gluten residues. However, many GF foods are entirely free of gluten containing grains

and they are available throughout Europe. The FDA recently proposed draft GF standards for the first time, which are based on final gluten content. Codex is in the final stages of approving similar standards. The draft standards permit trace gluten in GF foods, but aim to keep total dietary gluten well below levels generally accepted as safe for CD patients. They allow ingredients derived from gluten-containing grains in the foods, providing the ingredients have been processed to remove gluten and prescribed limits are met.

In addition, Codex has had standards since 1981, which define GF foods according to the nitrogen content of raw ingredients. These standards are only applicable to GF foods with ingredients derived from wheat, barley or rye. The Codex has endorsed the R5-ELISA as a means of upholding GF standards based on final gluten content. The FDA has tentatively endorsed the method and acknowledges that future methods including other ELISAs, may prove useful in the area. In light of such significant endorsement, it is important to consider the remaining limitations of gluten analysis by ELISA.^{10–15}

The basis for allergicity of gluten's

In vitro, and to a lesser extent, in vivo methods have been useful in dissecting the basis for gluten's immune-toxicity. Activation of CD4 (+) T cells in the small intestinal mucosa by gluten peptides released by digestive enzymes is a key event in CD. A direct effect from other gluten peptides on the intestinal epithelium has a role in inflammation. Auto-antibodies are associated with active CD and their role in pathogenesis is currently an active area of research. Multiple peptides are implicated in T cell stimulation, present in both major fractions of gluten (in wheat these fractions are the gliadins and glutenins). A single peptide located within a region resistant to digestive enzymes is the immune dominant portion of the gluten sub fraction, α -gliadin, at least in adult patients.

The full potency of this peptide is dependent on its modification by a tissue trans glutaminase within the intestinal mucosa. The enzyme introduces a negative charge into the peptide, which enhances class II MHC binding on antigen-presenting cells. The crucial role of T cell activation in disease pathogenesis is evident from the association of CD with class II MHC genotype. Over 90% of patients are positive for the HLA DQ2 heterodimer with the remainder positive for HLA DQ8. The peptides stimulatory to T cells in HLA DQ8 individuals appear to be distinct from those of the HLA DQ2 model.

Celiac disease (CD)

Celiac disease (CD) is an immune mediated inflammatory disease of the upper small intestine in genetically susceptible individuals triggered by the ingestion of the storage proteins (gluten) from wheat, rye, barley, and possibly oats. Intestinal symptoms similar to the clinical picture of CD nowadays were already described in the Roman Empire in the second century AD, and the idea that the disorder may be associated with food ingestion was discovered in the nineteenth century. Yet it was not until the 1950s that gluten was identified as the precipitating factor of CD, and a gluten-free diet was successfully introduced as treatment. For a long time, CD was considered to be a rare childhood enteropathy. Thanks to modern diagnostic techniques and increased awareness, epidemiological studies of the past decade revealed that CD is one of the most frequent food intolerances in many parts of the world, affecting about 1% of the population. While CD is typically characterized by a flat intestinal mucosa with villous atrophy resulting in a generalized malabsorption of nutrients, the spectrum of

clinical manifestations is very complex, including silent and atypical presentations as well as numerous extra intestinal symptoms and nonspecific findings. This is why many cases remain undiagnosed and entail the risk of long-term complications such as osteoporosis, anemia, or malignancy in addition to a substantial burden of illness. Strategies like raising awareness and screening in at risk groups have been successful in identifying previously undiagnosed patients, but the risks and benefits of screening the general population for CD remain controversial.

In addition to medical sciences like pediatrics, pathology, immunology, gastroenterology, neurology, and dermatology, other sciences like pharmaceuticals, analytical and food chemistry, food technology, cereal breeding, genetic engineering, and law are involved in CD and gluten research. Over the past few decades' tremendous progress has been made in elucidating the complex pathomechanism of CD, which involves an intricate interplay of gluten, other environmental factors, genetics, and immunity. The strong association between HLA DQ2/8 alleles and CD development has been proven conclusively, but the relative contributions of other unknown genetic risk factors and environmental factors such as infections, timing of gluten introduction into the infants' diet, and standards of hygiene have yet to be evaluated. Beside CD, other related disorders like dermatitis herpetiformis, gluten ataxia, wheat allergy, irritable bowel syndrome, and non-celiac gluten sensitivity are included as well as associated genetic and autoimmune diseases. The onset of CD may occur at any age, and the majority of patients nowadays present with predominantly extra intestinal complaints. This diagnostic challenge has been met with improved serological tests and HLA-DQ genotyping, but small intestinal biopsy is still considered the gold standard. Recent advances have been made in understanding the steps in the pathomechanism of CD, beginning with gluten intake and digestion followed by epithelial passage and induction of the adaptive and innate immune responses with each advance in understanding within the single steps, new questions have arisen that will need to be investigated. As probably the only autoimmune disease in which the triggering environmental factor (gluten) is known, CD may serve as a unique model of autoimmunity that may be transferred to other immune-mediated diseases.^{16–19}

This literature gives an overview on cereals and provides an in-depth description of cereal proteins. The storage proteins of all cereals have a very intricate composition with hundreds of single proteins. When comparing the amino acid sequences, there are distinct differences between the CD toxic storage proteins from wheat, rye, barley, and oats and the CD safe storage proteins from corn, rice, sorghum, and millet. Advances in DNA and RNA sequencing have contributed to an increased knowledge of protein structures, which helps identify CD-toxic epitopes. Testing for CD toxicity may be done in vivo or in vitro using cereal flours or extracts, protein fractions, protein types, single proteins, or peptides. Whereas prolamins from wheat with an emphasis on α -gliadins and avenins have been studied most thoroughly regarding their CD toxicity, little is known about prolamins from rye and barley and all glutelins. CD epitopes are derived from all protein types from wheat, rye, barley, and oats and further research on these proteins will help identify more relevant structures and allow an assessment of toxicity levels.

The treatment of CD includes both conventional and alternative therapies. The conventional treatment, a strict, lifelong gluten-free diet, is currently the only safe option available. Patients adhering to a gluten-

free diet need to be followed up for an assessment of improvement of symptoms and recovery of normal intestinal architecture, not least because of the risk of refractory CD. Compliance to the gluten-free diet is essential not only to prevent a recurrence of symptoms, but also to reduce the risk of complications. An assessment of nutritional status is recommended at regular intervals, and monitoring the health related quality of life of CD patients may help identify factors for its improvement. Alternative therapeutic options such as oral enzyme therapy, permeability inhibitors, inhibition of trans glutaminase 2, HLA-DQ blocking, modulation of inflammation and vaccination are in various stages of development and may become promising strategies. As more and more steps in the pathomechanism of CD are unraveled, further interesting approaches for alternative treatments will arise.^{20–28}

The main objective of this work is to extract the gluten from different food feedstocks, to estimate the amount of gluten present in different food feedstocks. It could make people aware of what is gluten, where is gluten found in food, beverages, cosmetics, and medications, who needs to avoid it, and why?

Materials and methods

Extraction of gluten from the wheat flour, maida, barley and oats was done by entrapping the gluten proteins in a cheese cloth and the amount of gluten proteins was estimated from the sample.

Weigh 50g powder of each food material was mixed with 50ml of distilled water in bowl and keep it for one hour. Food materials transferred into cheese cloth and washed it using running tap water until white turbidity goes out. Gluten is present inside the cheese

cloth. Weighed empty petridish and collect gluten from cheese cloth. Collected gluten from cheese cloth is placed in petridish and takes weight of petridish with gluten. Keep petridish in hot air oven for drying at 105 °C for two hours. Make gluten into small pieces and Keep it for another 30min. After drying make dry gluten into powder using pestle and mortar.

Weigh 20mg of all those glutes in powdered form using weighing balance. Take 4 centrifuge tubes and add weighed wheat, maida, barley and oats gluten powder to each tube. Add 10ml phosphate buffer to each tube. Set table centrifuge at 4000rpm for 10min for centrifugation. Repeat the same procedure to get the clear supernatant. Collect 20ml of supernatant from each tube.

Protein estimation by lowry's method

Protein solution(Stock standard) – weigh accurately 50mg of bovine serum albumin and dissolve in distilled water and make up to 50ml in a standard flask. Working standard – dilute 10ml of stock solution to 50ml with distilled water in a standard flask 1ml of this solution contains 200mg of protein. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard in to a series of test tubes. Make up the volume to 1ml in all the test tubes and a test with 1ml of distilled water serves as blank. Add 5ml of reagent ACS (Alkaline Copper Solution) to each tube including the blank. Mix well and allow standing for 10min. Then add 0.5ml of reagent FCR (Follin ciocalteau Reagent). Mix well and incubate at room temperature in the dark for 30min. Blue color is developed. The readings were taken at 660nm. Draw a standard graph and calculate amount of protein in the sample (Appendix).

Table 1 Explains estimation of protein concentration in wheat, barley, maida and oats flour

S.N	Volume of working solution (ml)	Concentration in µg	Volume of distilled water (ml)	Alkaline Copper Solution	FCR	Wheat OD at 660 nm	Barley OD at 660 nm	Maida OD at 660 nm	Oats OD at 660 nm
1	0	0	1	5	0.5	0	0	0	0
2	0.2	40	0.8	5	0.5	0.04	0.1	0.11	0.01
3	0.4	80	0.6	5	0.5	0.06	0.12	0.16	0.04
4	0.6	120	0.4	5	0.5	0.08	0.15	0.18	0.05
5	0.8	160	0.2	5	0.5	0.1	0.17	0.2	0.07
6	1	200	0	5	0.5	0.11	0.18	0.22	0.09
7	Wheat flour-0.5		1.5	5	0.5	0.04	0.14	0.17	0.07

Allow it to stand for 10 minutes

Incubate for 30 minutes at room temperature in dark

Table 2 Estimation of protein concentration in wheat, barley, maida and oats gluten

S.N	Volume Of Working Solution (ml)	Concentration in µg	Volume of distilled water (ml)	Alkaline Copper Solution	FCR	Wheat OD at 660 nm	Barley OD at 660 nm	Maida OD at 660 nm	Oats OD at 660 nm
1	0	0	1	5	0.5	0	0	0	0
2	0.2	40	0.8	5	0.5	0.01	0.1	0.1	0.01
3	0.4	80	0.6	5	0.5	0.04	0.15	0.15	0.04
4	0.6	120	0.4	5	0.5	0.05	0.17	0.17	0.05
5	0.8	160	0.2	5	0.5	0.07	0.19	0.19	0.07
6	1	200	0	5	0.5	0.09	0.21	0.21	0.09
7	Wheat gluten(0.5)		1.5	5	0.5	0.08	0.14	0.17	0.08

Allow it to stand for 10 minutes
Incubate for 30 minutes at room temperature in dark

Table 3 The estimated concentration of protein in 0.5ml is as given below (appendix I)

Concentration of protein in 0.5ml	In micro grams
Wheat flour	52
Wheat gluten	180
Barley flour	78
Barley gluten	117
Maida flour	97
Maida gluten	160
Oats flour	120
Oats gluten	137

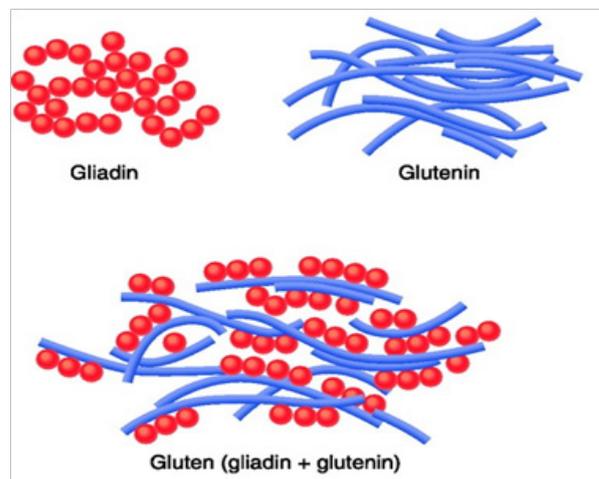


Figure 1 Formation of gluten.

Results and discussion

Estimation of moisture present in wheat, barley and maida flour

$$\text{Moisture percent} = (W_1 - W_2) \times 100 / W_1 - W$$

W_1 = Weight in gm of the dish with the material before drying.

W_2 = Weight in gm of the dish with the material after drying.

W = Empty dish. Wheat moisture percent = $(57.5 - 51) \times 100 / 57.5 - 45 = 52\%$
 Barley moisture percent = $(60 - 52) \times 100 / 60 - 48 = 66.66\%$
 Maida moisture percent = $(63.5 - 52.5) \times 100 / 63.5 - 47 = 66.66\%$

Estimation of moisture present in wheat, barley and maida Gluten
 Gluten on dry weight basis = $\text{Weight of gluten} \times 100 \times 100 / \text{Exact weight of sample} \times (100 - \text{moisture content})$
 Wheat gluten on dry weight basis = $6 \times 100 \times 100 / 50 \times (100 - 52) = 25\%$
 Barley gluten on dry weight basis = $4 \times 100 \times 100 / 50 \times (100 - 66.66) = 23.995\%$
 Maida gluten on dry weight basis = $5.5 \times 100 \times 100 / 50 \times (100 - 66.66) = 32.99\%$

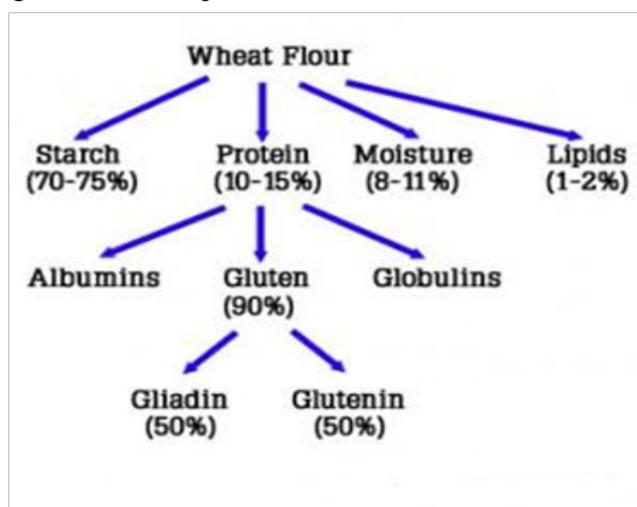


Figure 2 Different components present in a wheat flour.

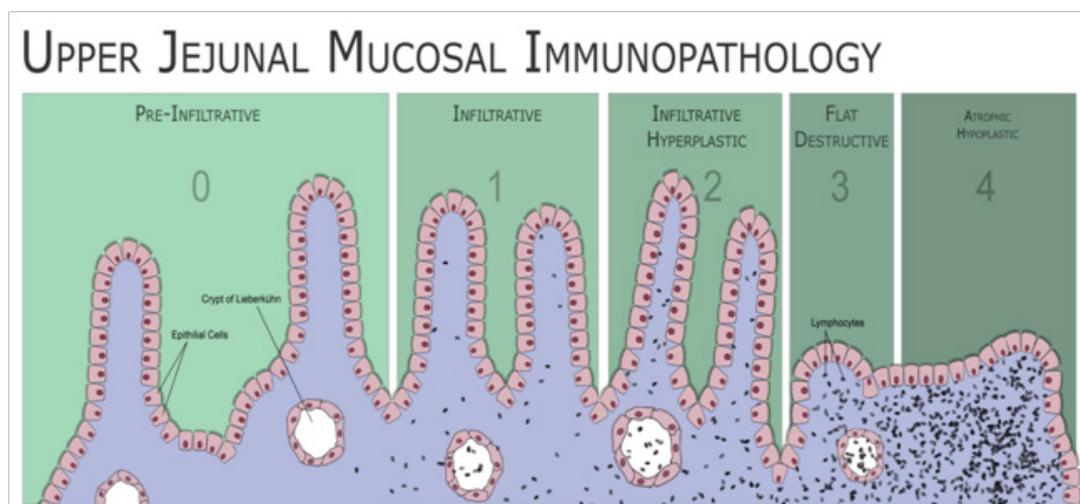


Figure 3 Diagram to show the different stages of celiac disease.

Conclusion

The present study to extract the gluteins from the seed and to estimate the amount of gluteins present in the seeds, common wheat, maida, barley, oats flours were collected from the local regions of Davangere, India. Gluten protein was quantified by Lowry's method which gave consistent, repeatable and reliable estimates with high sensitivity.

Gluten content was found to be more in wheat, then in maida, barley and oats respectively. The tolerable level of gluten for CD patient was found to be <10mg. As long as the value of food ingredients is based on protein content, the incentive to adulterate these materials by measures designed to inflate protein measurement will exist. The use of 19th-century crude protein measurements that do not detect many types of adulteration can be detrimental to public health, as recently demonstrated by melamine incidents. Development and implementation of more suitable protein measurement methods ones that cannot be as easily falsely manipulated have the potential not only to reduce the risk to public health but also to advance protein measurement science beyond total nitrogen based methods.

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None.

Conflict of interest

The author declares that there is no conflict of interest.

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