A Comparative Study on Gluten Percentage and Nutritional Constituents of Various Types of Edible Flours

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Abstract: Gluten is naturally occurring rubber - like protein which forms a network in the dough made out of flour. Gluten is responsible for various characteristics of a dough such as flexibility, elasticity, viscosity, cohesivity etc. As a matter of fact, more the Gluten content of a flour, more will be the elasticity and flexibility of a dough. Gluten belongs to a group of simple proteins known as prolamins (simple proteins) and is found in the endosperm of the grain, especially wheat. Normally the Gluten is digested by some proteolytic enzymes but it has been observed that in some people with Gluten sensitivity or intolerance the its digestion is poor leading to certain Gluten associated disorders like irritable bowel syndrome, coeliac diseases etc. In the present study the Gluten percentage of eight different types of edible flours were determined to suggest replacement of high Gluten containing flours with low Gluten or Gluten free flours in order to avoid Gluten related diseases. The result of the study revealed that the highest percentage of wet as well as dry Gluten was found in all purpose flour (23.58% and 11.04 % respectively) followed by corn flour (12.01 % and 6.03 % respectively) and Wheat flour (11.12 and 5.39 respectively). In the flours such as water chestnut flour (1.56 % wet and 0.72 % dry Gluten), ragi (1.1 % wet and 0.3 % dry Gluten), buckwheat flour (1.23 % wet and 0.42 % dry Gluten) and jackfruit flour (1.47 % wet and 0.664 % dry Gluten) the Gluten was barely present. In the rice flour no Gluten was found at all. The qualitative analysis of various flours confirmed the presence of carbohydrates, proteins, free and aromatic amino acids, fats, unsaturated fatty acids and free fatty acids as well. Saturated fatty acids were absent in all. The present study led to the conclusion that some of the flours in regular use across the world contain a good percentage of Gluten. While some flours are poor in their Gluten content or are Gluten free. In order to keep away from the Gluten or decrease the its consumption, the high Gluten flours can be added with some low Gluten or Gluten free flours and the Gluten related disorders (GRDs) can be escaped.

Keywords: Gluten, Prolamins, cohesivity, viscosity, Proteolytic enzymes, irritable bowel syndrome, coeliac diseases, gluten related disorders (GRDs)

1. Introduction

Since the ancient times different types of cereal crops have been grown all over the world, such as wheat, maize, barley, millets, rice, rye, sorghum etc. and the flours of these cereal grains are consumed as staple food world wide in the form of various items like breads, cakes, cookies, noodles, pasta etc.

Studies have shown that the different types of flours are rich in starch content and hence they are good sources of energy. Flours also consist of proteins, lipids, moisture, fibres, certain minerals and vitamins.

Some flours contain a specific type of protein called Gluten, which is naturally occurring group of proteins found in some cereal grains especially wheat. It is also found in barley, rye and semolina (Hopkins medicine. Org). ^[1] Corn also contains gluten but it is different from wheat gluten in its functions (Patil, 2003; 2004; L. Day et al, 2006) [^{2] [3] [4]}

Gluten is rubber - like elastic mesh work that remains in a dough after the removal of starch and other water soluble components, when the dough is washed with water (Herbert Wieser, 2007).^[5]

Gluten is a major storage protein in grains acting as resource for the amino acids, which plays an important role in seed germination and development of the seedlings (C. W. Wrigley et al, 2006)^[6] Gluten is found in the endosperm of the grain, specially wheat, distributed unequally (P. Shewry, 2019).^[7]

Wheat Gluten has a complex structure. It is made up of two components namely Gliadin and Glutenin which mainly belongs to a class of simple proteins known as prolamins (P. Shewry)^[7]. The Gliadin is present in Gluten as the solvent in which the Glutenins occur (Herbert Wieser, 2007)^[5]. Both these components exhibit different functions but are equally responsible for the deformation and flow properties (rheological properties) of a dough (Herbert Wieser, 2007). ^[5] The Gliadins and Glutenins can be present in Gluten in the form of monomers or oligomers or even as polymers (Wrigleys and Beitz, 1988).^[8] The oligomers or polymers are interlinked by hydrogen bonding or through the disulfide bridges (Wrigleys and Beitz, 1988). [8] The two protein components are rich in aminoacids, especially Proline and Glutamine, while the percentage of charged amino acids and cysteine is low. But in spite of the low cysteine content, it is significant because it leads to the structural organisation of Gluten by forming intra and inter chain disulfide bridges (Grasch and Wieser, 1999; Weiser 2003). ^{[9] [10]}

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Among the two components of Gluten, the Gliadin is low molecular weight protein and soluble in water - ethanol mixture due to its simple structure. Electrophoretic separation studies have revealed that the Gliadin fraction of Gluten can be divided into four groups - alpha, beta, gamma and omega. All four of them differ slightly in their amino acid composition (P. Shewry).^[7] According to Weiser and Keiffer, 2001^[11] the alpha and beta content are more than the other ones. The Gliadin fraction is responsible for the viscosity and cohesivity of Gluten (Jingyuan Xu et al 2007).^[12]

Another fraction, Glutenin has a comparatively complex structure hence it has higher molecular weight ranging between 50, 000 to 10 millions (Weiser et al 2006)^[13] and is insoluble in the water - ethanol mixture. The Glutenins are made up of two types of subunits linked by interchain disulfide bridges. First one is low molecular weight glutenin subunit (LMW - GS) forming about 20% of total gluten and another one is high molecular weight Glutenin subunit (HMW - GS) forming about 10% of the total gluten. (Weiser and Keiffer, 2001).^[11]

According to a review article by Jessica R Biesiekierski^[14], the Gluten consists of almost equal percentage of Gliadin and Glutenin and P. Shewry^[7] says that the structure, properties and the functions of Gluten depends completely on the percentage of Gliadins and Glutenins in it. The percentage of Gluten, in turn, affects the viscosity and elasticity of a flour. The quality of flour depends upon its Gluten content (Dobraszczyk et al, 2001).^[15] The amount of Gluten in a grain is affected by certain environmental factors such as temperature during the grain development and the availability of nitrogen and sulphur.

When a dough is prepared by adding water to the flour, Gluten provides cohesivity, that is, it binds and holds the food molecules together. Gluten makes a dough flexible and extensive. The water absorption and water holding capacity of a dough depends on its gluten content (C. W. Wrigley et al, 1999)^[16]. According to Dendy and B. J. Dobraszczyk, 2001 ^[17]. Gluten forms a network due to formation of disulfide bridges which traps the air and makes the dough fluffy. A number of studies have shown that Gluten has various advantages. It is prebiotic, that is, it adds good bacteria to the gut (Ramedani N. et al, 2020). ^[18] When added to the processed food it enhances the texture and flavour. Gluten increases the moisture retention in a dough. Due to the properties of Gluten such as viscosity, flexibility, elasticity, cohesivity and extensibility, Gluten containing flours are widely used to prepare different types of food items like breads, pastas, noodles, cookies, biscuits, cakes etc.

Normally the Gluten is digested in the body by specific proteolytic enzymes of the gastrointestinal tract (Silvia Fernández - Pérez et al 2020)^[19] though its digestion is a complex process due to the presence of high proline content (Anastasia V. Balakireva et al, 2016).^[20] The Gliadin of Gluten is first digested to specific, small 18 and 33 amino acid peptides which are further digested in the lower part of gastrointestinal tract (Holmes et al 2000)^[21] by some other proteases. In some of the individuals, the small specific

peptides formed by incomplete Gluten digestion triggers the T - lymphocytes to activate the immune system and produce response in the gastrointestinal tract (Holmes et al 2000)^[21] leading to coeliac disease. Some other gluten associated disorders are also known like Gluten intolerance, Gluten sensitivity, Gluten ataxia (Taraghikhah, Net al, 2020).^[22] People suffering from any of these disorders are suggested to decrease or avoid dietary Gluten.

The present study aims at determining the percentage of Gluten in various edible flours and suggests the replacement of Gluten rich flours with low Gluten or Gluten free flours, in cases of Gluten associated disorders.

The study also aimed to analyse the flours qualitatively for the presence of various nutrients such as carbohydrate, protein, free amino acids, aromatic amino acids, total fats, free fatty acids, saturated and unsaturated fatty acids.

2. Materials and Methodology

Samples (Materials) -

Eight different types of commercially available edible flours (figure 1) - wheat flour, All purpose flour (maida), corn flour, water chestnut flour (Singhada Atta), Buckwheat flour (kuttu ka Atta), ragi flour (nachni flour), rice flour and jackfruit flour were bought from the local market of Indore, Madhya Pradesh, India.





Determination of Gluten percentage

The crude gluten content was determined by AACC (American Association for Clinical Chemistry) International Method 38 - 10.01 (Gluten—Hand Washing Method), (Approval April 13, 1961. AACC International: St. Paul, MN).^[23] The wet and dry Gluten percentage was determined by AACC Method 38 - 12.02 (Perten. H, 1990).^[24]

Carbohydrate content

Presence of total carbohydrates was checked by Molisch's test and that of reducing sugars among the carbohydrates was done by Benedict's test.

Protein content

The presence of proteins in various flours was confirmed by Biuret test, all aromatic amino acids by Xanthoproteic test, tyrosine by Millon's test, free amino acids by Ninhydrin test,

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Lipid content

Total Lipids in all the eight types of flours were detected by Sudan III test, presence of unsaturated fatty acids were checked by Bromine water test and iodine test as well. Saturated fatty acids too were checked by an iodine test. Free fatty acids were detected by using phenolphthalein and sodium hydroxide. Standard reagents of the analytical grade were used for all the experimental work.

Statistical Analysis

The gluten content in all the eight flour samples was determined in triplicates and their mean were calculated to express the results of wet and dry gluten percentage. Other qualitative procedures were also performed in triplicates to ensure accuracy.

3. Result and Discussion

The percentage of wet and dry Gluten (figure 2a & 2b) were found to be different in all the eight flour samples (Figure 3). The maximum percentage of wet and dry Gluten was found in the all purpose flour (23.58% and 11.04 % respectively). In the wheat flour the % of dry and wet Gluten was found to be 11.12 and 5.39 respectively. The Gluten content in corn flour was 12.01 % (wet Gluten) and 6.03 % (dry Gluten). Negligible content of Gluten was present in water chestnut flour (1.56 % wet and 0.72 % dry Gluten), ragi flour (1.1 % wet Gluten and 0.3 % dry Gluten), Buckwheat flour (1.23 % wet and 0.42 % dry Gluten) and jackfruit flour (1.47 % wet and 0.664 % dry Gluten). While no Gluten at all was found in the rice flour.



Figure 2 (a): Wet Gluten



Figure 2 (b): dry Gluten

All the flours were found to be consisting of carbohydrates, proteins, free amino acids, lipids, unsaturated fatty acids and free fatty acids in them. The satura fatty acids were absent in all the eight flour samples.

The violet colored ring of different intensities obtained on applying Molisch's test to the flour samples indicated that the carbohydrates are more in the rice flour, all purpose flour, wheat flour, lesser in corn flour and water chestnut flour and least in the flours of jackfruit, ragi and buckwheat.

The presence of different intensities of violet colour in all the eight samples obtained after performing Biuret test inferred that the protein content was highest in ragi flour, lesser in other flours and least in the rice flour. Free and aromatic amino acids were found in all the eight flours.

On applying the test for total fats, darker colour was found in the test tube with jackfruit flour sample and lesser color was obtained in other test tubes. Positive test results were obtained for free and unsaturated fatty acids while the test results for saturated fatty acids was negative in all the flours.



Figure 3

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4. Conclusion

Gluten is a storage protein which is mainly found in the wheat endosperm. Reviews of a number of literature have indicated that Gluten is not always harmful for the health of an individual. Specific Gluten digesting proteolytic enzymes are present in the body since birth which efficiently digests gluten, though their efficiency decreases with age. But some people who have Gluten intolerance or are sensitive to it or are suffering from irritable bowel syndrome may not digest the Gluten appropriately. For such people avoiding Gluten is the only remedy to overcome the related hazards. To avoid the Gluten in diet, high Gluten containing food items should be replaced by low gluten or gluten free diet.

The present study have revealed that the wheat flour and maida, which are almost daily consumed worldwide, especially in India, contains high percentage of Gluten while some of the flours such as water chestnut flour, buckwheat flour, ragi flour, jackfruit flour or rice flour have insignificant Gluten percentage, hence can be added to the wheat flour or else the wheat flour can be completely replaced by either of these flours. Use of these substitute flours will also fulfil the carbohydrate, protein and fat demands of the body and will also help avoiding Gluten.

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