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Effect of Deoxycholic Acid on Gluten Formation and Formed Gluten

Milan Kanaiyalal Gami[a], **Shanmugam. Sivabalan**[a]
[a] Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

Abstract : Wheat is one of the staple food grains of humankind around the world. However, gluten present in wheat is reported to cause numerous human diseases, which are categorized as gluten-related diseases. Gluten, a glue-like protein, is formed from gliadin and glutenin during the kneading process of wheat flour with water. Bile salts are important molecules for the proper metabolism and absorption of fatty acids, cholesterol, and fat-soluble nutrients. In this study, we planned to analyze the effect of interactions between sodium deoxycholate and constituents of gluten in its formation and on formed gluten. Wheat flour or formed gluten was mixed with various concentrations of deoxycholic acid to study the effect of these molecular interactions. We found that when deoxycholic acid is included in the wheat flour during the kneading process, it disrupts gluten formation in a dose-dependent manner. On the other hand, when deoxycholic acid is added to formed gluten, it disrupts the formed gluten as well. Hence, from this study, we hypothesize that deoxycholic acid and constituents of gluten have strong forces of interaction with each other.

Keywords: Wheat flour; Gluten; Deoxycholic acid;

1. Introduction:

Soft red wheat grains are one of the most widely consumed cereals in the human diet around the world [1]. Gluten is the main storage protein of wheat endosperm [2]. It is a complex mixture of hundreds of related but distinct proteins, mainly gliadin and glutenin [3]. Gluten is a protein formed from its constituents, and various forces of attraction, such as covalent and non-covalent interactions, could play an important role in its formation [4].

As the constituents of gluten are having numerous covalent and non-covalent interactions with its constituents, it could possibly interact with various other biological molecules. For example, ingestion of food containing wheat could interfere with digestive enzymes, bile functions, and nutrient absorption and alter the normal architecture of the gastrointestinal tract. It has been reported previously that gluten is a disease-causing agent and can cause a spectrum of diseases, collectively called gluten-related disorders (GRDs), which includes celiac disease, irritable bowel syndromes, and nonceliac gluten sensitivity [1]. However, the exact mechanism by which these GRDs develop is unknown.

Several previous studies have implied the role of the interaction of gluten with HLA receptors to initiate immunological complications as the mechanism of the development of celiac disease. Others have demonstrated the interference caused by the gluten constituents on protease actions in the gastrointestinal tract [5,6]. However, no studies are available on the interaction of gluten constituents with bile salts in the gastrointestinal tract or its health consequences.

Bile salts are important bio-molecules that play a crucial role in the absorption and transportation of lipids and lipid-soluble nutrients. As per the previous research evidence, malabsorption of bile salt and disregulation of enterohapatic circulation can lead to a variety of metabolic problems, which include nonalcoholic fatty liver disease and gallstones [7]. Malabsorption of vital nutrients could play a role in the development of a myriad of other diseases. However, bile salt's interaction with gluten and its health consequences have not been extensively studied.

In previous work, we have studied the effect of primary bile salt on the formation of gluten.
In this present work, we have studied the effect of deoxycholic acid on various constituents of gluten in its formation and on formed gluten. This study was carried out on raw wheat flour as well as defatted wheat flour. The probable health consequences of interactions of various constituents of gluten with deoxycholic acid are discussed.

2. Materials and methods:
Soft red wheat grains (Triticum aestivum) were purchased from the local market in Puducherry, India. After carefully removing all the impurities manually, the wheat was dried in the sunlight for 48 hours to remove the moisture from the wheat grains and milled. The milled wheat flour particle size was around 100 micrometers, and it was packed in an airtight plastic container and stored in the refrigerator until it was used for further analysis. All of the other chemicals used in this experiment were analytical grade and purchased from Hi-Media, India. The following chemicals were used in this experiment. They are deoxycholic acid, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide, hydrochloride acid, chloroform, and ethanol.

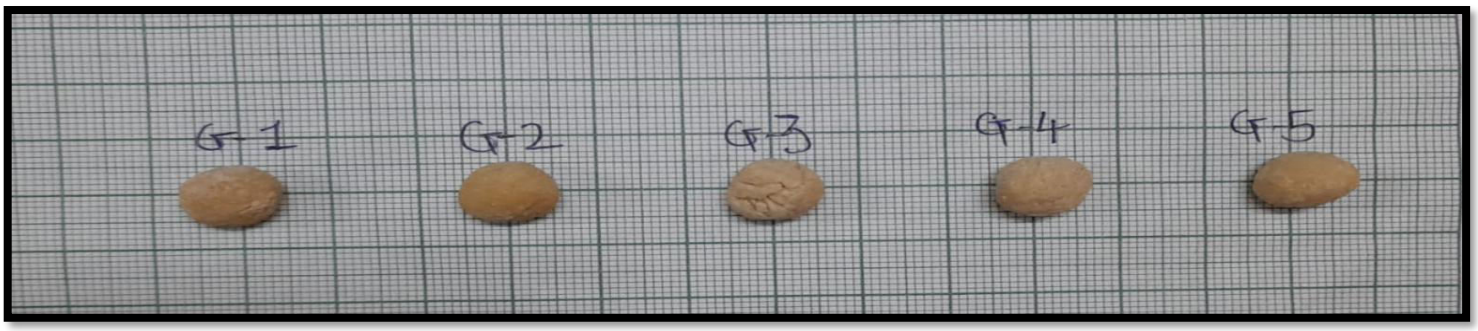
Removal of fat-soluble substances from the wheat flour:
To remove fat-soluble compounds from wheat flour, it was washed with Chloroform at 27 ℃ and air-dried for 30 min. The process was performed twice as indicated[8].

Removal of albumin and globulin from wheat flour:

Albumin and globulin proteins present in the wheat flour were removed by adding 0.04 M Nacl in the NaH2Po4 buffer (200ml) to the wheat flour and the suspension was shaken for 10 min and then it was centrifuged to isolate the supernatant which would contain the albumin and globulin. This procedure was repeated two times [9]. After removing the soluble proteins from the flour, the flour was dried in a hot air oven at 37 ℃ for 24 hours.

Extraction and determination of wet gluten from dough:

Wet gluten was extracted by AACC(2005) method with modest modification. 500 mg of raw wheat flour and defatted wheat flour were mixed with 350µl distill



water and the mixture was kneaded thoroughly to make a dough ball so that the gluten to form completely. The kneaded dough was immersed into the phosphate buffer (Ph-7) for 60 minutes which is shown in Figure no.1. After sixty minutes of shocking in the phosphate buffer, the dough was washed in a stream of tap water over a laboratory sieve size of 32μm, until all the starch were removed. The complete removal of starch from the formed gluten content was determined using the iodine test. The procedure for starch detection is mentioned below. The gluten that remained after starch[10].

$$\text{Wet gluten yield} = \frac{\text{Weight of wet gluten obtained}}{\textbf{W}\text{eight of flour}} \times 100$$

Fig.1: Dough of flour mixed with Deoxycholic acid.

Measurement of dry gluten content:

The dry gluten weight was measured after drying the wet gluten at 100℃ hot air oven for 24 hours (AACC 2005). And dry gluten was measured by the formula given below[10].

$$\text{Dry gluten yield} = \frac{\text{Weight of dry gluten obtained}}{\textbf{W}\text{eight of wheat flour}} \times 100$$

Detection of starch in the gluten with Iodine test:

We used the iodine test to detect the presence of starch in the isolated gluten. Iodine solution was prepared by mixing 2 g of iodine and 4 g of potassium iodide in 200 ml of distill water. And the solution was used to check the presence of starch in the isolated gluten. Gluten was immersed in the water, and the presence of starch was checked by the addition of solution in it [11].

Measurement of moisture content of the gluten:

The moisture content of the gluten was calculated by the AACC(2005) method. Moisture content of gluten was measured by drying wet gluten in an oven at 100°C for 24 hours. And moisture content was measured by the formula given below [10].

$$\text{Moisture content} = \frac{\text{Weight of wet gluten} - \text{weight of dry gluten}}{\textbf{W}\text{eight of wet gluten}} \times 100$$

Effect of deoxycholic acid on gluten formation:

To investigate the effect of deoxycholic acid on gluten formation, five different concentrations of deoxycholic acid, such as concentrations 1) 100 mg, 2) 200 mg, 3) 300 mg, and 4) 400 mg, were separately mixed with a fixed amount (500 mg) of wheat flour and 350 ul of distilled water and knead thoroughly for 5 minutes for the gluten formation to occur. After removing the starch by water washing, the amount of gluten formed was measured by weighing. This procedure was conducted for raw wheat flour as well as defatted wheat flour.

Effect of deoxycholic acid on the formed gluten:

In order to know whether deoxycholic acid disrupts and solubilizes formed gluten, varying concentrations of deoxycholic acid, such as a) 100 mg, b) 200 mg, c) 300 mg, and d) 400 mg, were mixed with a fixed amount of 150 mg of wet gluten. After mixing with the spatula, 5 ml of dihydrogen phosphate buffer (pH 7) was added and allowed for 10 min. After that, the stability of the gluten integrity was observed visually and photographed, and it was compared with raw gluten.

Statistical Analysis:

Each experiment was conducted three times to ensure the reproducibility of the values. For each experiment, the mean value and standard deviations of experiments were calculated. The P value was calculated at significant difference (0.05) using One Way Anova test by SPSS software.

3. Result and discussion:

Effect of deoxycholic acid on gluten formation:

Table No. 1 presents the remaining extracted wet gluten and dry gluten yield after washing out the starch and other non-aggregated gluten parts. After washing the dough, the observation indicated that increasing the concentration of deoxycholic acid in flour affected gluten formation. In the control group, the gluten formed fully, while in concentration-3, it decreased from 35% to 2.4% and did not form in concentration-4. Dry gluten shows the dry matter of wheat flour. Dry gluten was formed from 14% to 0.5% in concentration 4, as followed by wet gluten. The moisture content of gluten was significantly increased.

D.C.A. Concentration	Weight of dough(Mg)	Wet gluten(%)	Dry gluten(%)	Moisture content(%)
G-1 Control	773.33 ± 0.011	30.40 ± 0.114	14.13 ±0.30	53.48 ± 0.80
G-2 (100mg)	973.33 ± 0.015[a]	26.13 ± 1.646[a]	11.13 ±0.50[a]	57.05 ± 0.52[a]
G-3 (200mg)	1183.33 ± 0.011[a,b]	7.332 ± 0.114[a,b]	2.4 ±0.20[a,b]	63.66 ± 2.42[a,b]
G-4 (300mg)	1333 ± 0.064[a,b]	2.490 ± 0.09[a,b]	0.55 ±0.02[a,b]	77.91 ± 3.98[a,b]
G-5 (400mg)	1473± 0.040[a,b]	-	-	

Table 1: Effects of on Deoxycholic acidgluten formation

Value expressed as Mean ± STD of three independent experiment

[a] Significant as compared to the control group (P value measured by One-way Anova)

[b]Significant as compared to the control group (P value measured by One-way Anova)

Effect of deoxycholic acid on gluten formation of defatted and other soluble protein extracted flour:

To study the effect of deoxycholic acid solely on gluten peptides, fat and other soluble proteins were removed from the flour. The wet gluten yield and dry gluten were decreased with increasing concentrations of deoxycholic acid in flour (Table No. 2). In the control group, after the washing steps, gluten peptides were aggregated and formed gluten. Whereas in concentrations 2 to 4, the p-value was significantly lowered ($p<0.05$) while compared with control. Deoxycholic acid interacted with gluten peptides, and as its concentration increased, the aggregation of gluten peptides decreased. The moisture content of the extracted gluten was increased as the size of the formed gluten decreased.

D.C.A. Concentration	Weight of dough (Mg)	Wet gluten (%)	Dry gluten (%)	Moisture content(%)
G-1 Control	736.66± 50.33	21.332 ±1.15	11.86 ±0.23	44.60 ± 0.74
G-2 (100mg)	860.0 ± 17.32[a]	12.33 ± 0.57[a]	5.60 ±0.20[a]	54.58 ± 0.04[a]

G-3 (200mg)	1010 ± 1.00[a,b]	3.93 ± 0.11[a,b]	1.74 ±0.03[a,b]	62.56 ± 0.09[a,b]
G-4 (300mg)	1260 ± 30.00[a,b]	0.72 ± 0.28[a,b]	0.30 ±0.05[a,b]	58.33 ± 0.06[a,b]
G-5 (400mg)	1360± 65.57[a,b]	-	-	-

Table 2: Effects of on Deoxycholic acidon defatted flour containinggluten formation

Value expressed as Mean ± STD of three independent experiment

[a] Significant as compared to the control group (P value measured by One-way Anova)

[b]Significant as compared to the control group (P value measured by One-way Anova)

Effect of deoxycholic acid on the formed gluten:

The effect of deoxycholic acid on defatted formed gluten was checked by its mechanical mixing with the formed gluten. As illustrated in Figure 1, an increase in deoxycholic acid led to a greater disruption of the formed gluten. G-1 was used as a control, which is intact gluten without mixing with the deoxycholic acid. As deoxycholic acid is partially soluble in buffer, it was shown as another control to compare with the other groups. As the concentration of deoxycholic acid increased, the disruption of formed gluten increased in all four groups. This result showed that deoxycholic acid disrupted the insoluble and aggregated gluten.

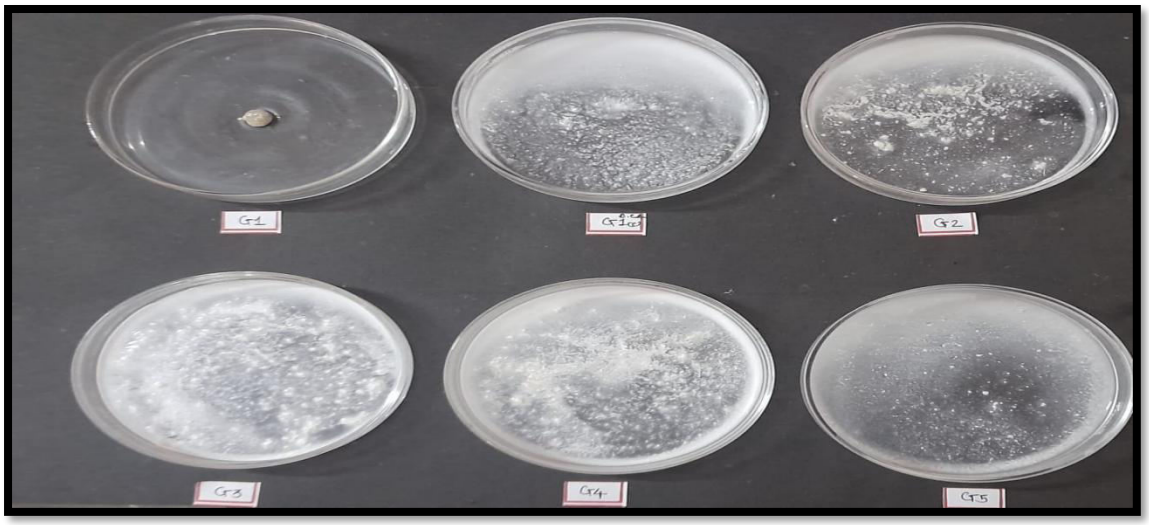


Fig. 2: Effect of Sodium deoxycholate on formed gluten.

4. Discussion:

People worldwide are prone to wheat-related disorders. As per the report, up to 10% of westerners are affected by gluten-related disorders [11]. The inability of the intestinal system to digest gluten peptides leaves them available for interaction with intestinal molecules. Surprisingly, no extensive study has been done on how gluten is causing serious problems. Therefore, we have studied the interaction of wheat gluten with sodium deoxycholate hydrates.

If gluten interacts with any of the intestinal molecules, it could alter its function. Previous research has demonstrated that the interaction of gluten with various GI enzymes inhibits lipase, protease, and amylase, resulting in impaired protein digestion.[12].

Our results indicated that the increased concentration of deoxycholic acid in wheat flour leads to impaired gluten formation. While comparing the extracted gluten of the normal flour with defatted flour, defatted flour showed slightly lower extraction of gluten. This demonstrated the interaction between deoxycholic acid and the flour's fat.

Previous research has demonstrated the interaction of bile salt with corn proteins, revealing that the hydrophobic amino acid of the defatted corn protein forms a hydrophobic bond with the deoxycholate [113]. Another study showed the interactions of bile salt with intact whey protein, bovine serum albumin, and beta lactoglobulin [14]. They concluded that hydrophobic interaction plays an important role in bile salt's interaction with all of these proteins.

Here, we also proposed that gluten contains 35% hydrophobic amino acids, and due to the high hydrophobic binding capacity of bile salt, deoxycholic acid might have hydrophobic interactions with gluten peptides[15].

Bile salt is involved in the transportation and absorption of lipids. The interaction of bile salt with other proteins has been reviewed previously; some investigators have observed the effect of soy protein on decreased lipolysis when it is used to stabilize the oil in water emulsion under gastrointestinal conditions compared with beta-lactoglobulin. It also reduces lipid digestion [14]. If gluten interacts with sodium deoxycholate, it can potentially hinder its function. And can lead to the malabsorption of lipid and lipid-soluble vitamins.

5. Conclusion:

Gluten constituents have a higher binding affinity with sodium deoxycholate than their own constituents. The deoxycholic acid also affects formed gluten in a dose-dependent manner. Further studies are required to understand the interactions at a molecular level.

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6. References:

1) Sabença C, Ribeiro M, Sousa Tde, Poeta P, Bagulho AS, & Igrejas G.(2021)Wheat/Gluten-Related Disorders and Gluten-Free Diet Misconceptions: A Review. Foods 10(8): 1765.
2) Shewry PR, Halford NG, BeltonPS, & Tatham AS (2002). The structure and properties of gluten: An elastic protein from wheat grain.Philos Trans. R. Soc. Lond. B357:(1418) 133–142.
3) BiesiekierskiJR(2017)What is gluten?J Gastroenterol Hepatol(Aust) 32:78–81.
4) WieserH(2007). Chemistry of gluten proteins. Food Microbio24: 115–119. Sharma N, Bhatia S, Chunduri V, Kaur S, Sharma S, Kapoor P, Kumari A, Garg M (2020). Pathogenesis of Celiac Disease and Other Gluten Related Disorders in Wheat and Strategies for Mitigating Them.Front Nutr.7: 6.
5) Freitas D, Gómez-Mascaraque LG, Brodkorb A (2022) Digestion of protein and toxic gluten peptides in wheat bread, pasta and cereal and the effect of a supplemental enzyme mix. Front Nutr9:986272.
6) Ticho AL,MalhotraP,DudejaPK,GillRK,AlrefaiWA.(2019)Intestinal Absorption of Bile Acids in Health and Disease.Compr Physiol 10(1): 21–56.
7) Barak, S., Mudgil, D., & Khatkar, B. S. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties.LWT - Food Sci Technol51(1), 211–217.
8) Ndayishimiye JB, Huang W ning, Wang F, Chen Y zheng, Letsididi R, Rayas-duarte P, et al. (2016). Rheological and functional properties of composite sweet potato – wheat dough as affected by transglutaminase and ascorbic acid. J Food Sci Technol. 53:1178–88.
9) AACC (2005). in: Approved method of analysis, 11th ed.Method 38-12A St Paul, MN American Association of Cereal Chemist.
10) Elzagheid MI, (2018). Laboratory Activities to Introduce Carbohydrate Qualitative Analysis to College Students.World J. Chem. Educ. 6(2), 82–86.

11) Pathogenesis of Celiac Disease and Other Gluten Related Disorders in Wheat and Strategies for Mitigating Them - Scientific Figure on ResearchGate. Available from: www.researchgate.net [accessed 12 Aug 2024]
12) Cuccioloni M, Mozzicafreddo M, Ali I, Bonfili L, Cecarini V, Eleuteri AM, Angeletti M. Interaction between wheat alpha-amylase/trypsin bi-functional inhibitor and mammalian digestive enzymes: Kinetic, equilibrium and structural characterization of binding. Food Chem. 2016 Dec 15;213:571-578. Epub 2016 Jul 6. PMID: 27451220.
13) Kongo-Dia-Moukala JU, Zhang H, Claver Irakoze P (2011)In Vitro Binding Capacity of Bile Acids by Defatted Corn Protein Hydrolysate. Int J Mol Sci 12(2): 1066–1080.
14) Bellesi FA, Pilosof AMR, (2021)Potential implications of food proteins-bile salts interactions. Food Hydrocoll118:106766.
15) IwakiS, Hayakawa K, Fu BX,Otobe C (2021)Changes in Hydrophobic Interactions among Gluten Proteins during Dough Formation.Processes9(7): 1244.