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Effect of Sodium Taurocholate Hydrate and Sodium Glycocholate Hydrate on Gluten Formation and Formed Gluten

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Abstract: Many people across the world are affected by gluten-related disorders. However, the exact mechanism of how it is causing the various disorders has yet to be extensively studied. Therefore, in this study, we aimed to examine the effect of primary bile salt on gluten formation and formed gluten in vitro conditions. Gluten was extracted by hand wash method after mixing with the various concentrations of sodium deoxycholate hydrate, and sodium taurocholate hydrate separately with flour. The result showed that adding STH reduced gluten formation from (44.94%) to no gluten formation. With the augmentation of the concentration of SGH, gluten formation decreased from (45.88%) to no gluten formation. Additionally, both primary bile salts disrupt the formed gluten. So, It is concluded that gluten interacts with the bile salt in its formation and on formed gluten.

Keywords: Bile salt; Gluten formation; Sodium glycocholate hydrate; Sodium taurocholate hydrate; Wheat flour; Formed Gluten; Gastrointestinal molecules; Immunological disorders.

1. Introduction:

Wheat-based products, a staple in many diets worldwide, derive their unique properties from viscoelastic gluten, the primary protein in wheat grain. However, gluten is also associated with a range of health concerns, including suspected links to immunogenic and non-immunogenic disorders and malnourishment(1-3).

Gluten is a protein composed of gliadin and glutenin [4,5]. These constituents interact via various intra- and inter-non-covalent and covalent bonds to form gluten[6]. As the gluten constituents can interact with themselves, they can possibly interact with other digestive and gastrointestinal molecules.

Previous study has been carried out to understand the interaction of negatively charged gluten peptides with positively charged HLA molecules, leading to the various immunogenic reactions in Celiac disease and leaky gut[5]. Another study demonstrated about fifty different types of epitopes on gliadin residues of

gluten that can have either immunomodulatory, cytotoxic or change in gut permeability-related interactions[2]. However, no broad study has been done on how gluten could interact with various other biomolecules, leading to malabsorption and intestinal immunological interaction.

Bile salts are one of the important digestive biomolecules; they are derivatives of cholesterols, synthesized in the liver, stored in the gallbladder and secreted into the small intestine [7]. Primary bile salts chenodeoxycholic acid and cholic acid are conjugated with the taurine and glycine, forming sodium glycocholate and sodium taurocholate. They play a crucial role in absorption of lipid and lipid soluble vitamins. They are also important signalling molecule and modulated the cell proliferation, gene expression and glucose and lipid metabolism[7,8].

The interaction of bile salt with protein molecules can disrupt bile salt functions, potentially leading to the malabsorption of bile salt and, consequently, lipid and lipid soluble vitamins. Notably, no study to date has explored the interaction of gluten constituents with primary bile salts such as sodium taurocholate hydrate and sodium glycocholate hydrate, highlighting the potential health implications of this unexplored area.

So, in this paper, we reported the invitro effect of primary bile salts on gluten formation and on formed gluten. The paper also discussed the health consequences of these reactions.

2.Materials and methods:

Soft red wheat grains (Triticum aestivum) were purchased from the local shop in Pondicherry, India. After removing all other impurities from the grains, they were dried in sunlight for 48 hours to remove moisture. Then, around ten thousand wheat kernels were taken from that and milled.

Chemicals such as, sodium taurocholate hydrate, sodium glycocholate hydrate, phosphate buffer saline of himedia were used. All other chemicals used for the experiment were of analytical grades.

Removal of fat and soluble proteins such as albumin and globulin from the wheat flour:

To remove the fat and fat-soluble molecules present in wheat four, the wheat flour was washed with Chloroform at room temperature, and the process was repeated three times [9].

Albumin and globulin protein were removed 0.04 M NaCl containing NaH2Po4 buffer (200ml) were mixed with the defatted wheat flour and the suspension was shaken for 10 min and centrifuged at (15,000g) to separate the supernatant, which contained the albumin and globulin protein, procedure was repeated twice [10].

Extraction of wheat gluten:

Gluten was extracted by a slightly modifiedmethod. 500mg of wheat flour was mixed with the 350 µl of phosphate buffer (pH 7.0) and kneaded for 30 minutes to form proper dough. The kneaded dough was immersed in the phosphate buffer for 60 minutes, which is shown in (figure no.1). After soaking in the buffer, the dough was washed under a stream of tap water over the laboratory sieve of 32 µm size. Moreover, it was washed until all the soluble starch was removed. The remaining viscoelastic proteins were considered wet gluten. Wet gluten yield was calculated by the formula given below [11].

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

Figure 1:Soaking dough in the buffer for 60 minutes

Iodine test:

The starch content was measured by iodine test. An iodine solution was prepared by mixing 2 gm of iodine and 4 gm of potassium iodide in 200 ml of distill water, and the solution was used to check the presence of starch [12].

Dry gluten yield and Moisture content measurement:

Dry gluten was measured by drying the wet gluten at 120 C in a hot air oven for 24 hours. Dry gluten yield was measured by the formula below [11].

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

Measurement of Moisture content: Moisture content was measured by the formula given below [11].

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

Effect of STH on gluten formation:

In order to examine the effect of STH on gluten formation, different concentration of STH like a)100mg b)200mg c)300mg d) 400mg e)500mg, were separately mixed with the 500mg of wheat flour and 350µl of distil water and kneed thoroughly for 10 min to make the gluten to form. Doughs were immersed into the phosphate buffer pH (7.0). After immersing the dough into buffer gluten was extracted as described above.

Effect of SGH on gluten formation:

In order to investigate the effect of SGH on gluten formation, varying concentrations of SGH like a)100mg b)200mg c)300mg d) 400mg e)500mg were mixed with the 500mg of wheat flour and gluten was extracted by the AACC method (2005) described above.

Effect of STH and SGH on formed Gluten:

To investigate whether STH and SGH interact and disrupt the formed Gluten, the varying concentrations of STH and SGH like T-1)100mg T-2)200mg T-3)300mg T-4) 400mg T-5) 500mg were separately, mechanically mixed with the fixed amount of 230mg of extracted Gluten. After mixing with the spatula, 5ml of di-hydrogen phosphate buffer(pH-7) was added and allowed for 10 min to interact; after that stability, gluten integrity was observed visually and photographed and compared with the control.

Statistical analysis:

Each experiment was repeated three times to ensure the reproducibility of values. The mean values and standard deviation of each experiment were measured, and the p-value was calculated at the significant difference of (<0.05) using a one-way ANOVA test by Graph pad Prism software.

Result and Discussion:

Effect of sodium taurocholate hydrate on gluten formation:

After removing the starch and unaggregated gluten constituents, the remaining extracted formed gluten is shown in **Table no.1,** indicating that the augmentation of concentration of STH reduced the formation up to no gluten formation in (Concentration-5) from (44.94%) in control. Dry gluten shows the dry matter of gluten present in flour[13]. Dry gluten was also uniformly reduced as the wet gluten was reduced, showing that the remaining gluten constituents that do not interact with bile salt remains intact and form gluten. The constituents that interacted with the bile salt were washed away without interaction. No significant difference was observed in moisture content in the remaining gluten. No significant difference was observed in the moisture content of extracted gluten.

Concentration of STH	Weight of dough	Extracted Wet gluten(%)	Dry gluten(%)	Moisture content (%)
Control	640.0 ± 4.9	44.94 ± 1.19	14.71± 0.77	67.26 ±0.93
Concentration 1 (100mg)	707.3 ±11.19^{a}	34.67 ± 0.35^{a}	11.40 ± 0.12^{a,b}	67.11 ± 0.04^{a}
Concentration 2 (200mg)	715.4 ± 6.88^{a,b}	14.31 ± 0.43^{a,b}	4.73 ± 0.10^{a,b}	66.90 ± 0.33^{a}
Concentration 3 (300mg)	823.5 ± 25.7^{a,b}	10.39 ± 0.57^{a,b}	3.32 ± 0.15^{a,b}	67.94 ± 0.20^{a}
Concentration 4 (400mg)	844.3 ± 29.0^{a,b}	3.95 ± 0.29^{a,b}	1.22 ± 0.06^{a,b}	68.90 ± 0.72^{a}
Concentration 5 (500mg)	941.0 ± 33.8^{a}	-	-	-

Table 1: Effects of sodium taurocholate on gluten 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 sodium glycocholates hydrate on gluten formation:

Wet gluten was extracted using the hand wash method. The result in (**Table No. 2**.) is shown: As more SGH was mixed with the wheat flour, the gluten extraction was reduced. Wet gluten was extracted from (45.88%) in control to (4.23%) in (concentration-4) and not formed in (concentration-5). Dry gluten was significantly reduced (13.03%) to (1.23%). No significant difference was observed in moisture content in the remaining gluten.

Table 2: Effects of sodium glycocholate hydrate on gluten formation

Concentration of SGH	Weight of dough	Wet gluten (%)	Dry gluten (%)	Moisture content (%)
Control	643.6 ± 4.22	45.88 ± 0.23	13.03 ± 0.39[a]	70.93 ± 1.00
Concentration 1 (100mg)	716.2 ± 5.52[a]	35.45 ± 0.28[a]	10.30 ± 0.08 [b]	70.94 ± 0.25[a]
Concentration 2 (200mg)	764.4 ± 5.66[a,b]	15.26 ±0.14[a,b]	4.62 ± 0.22[a,b]	70.71 ±0.09[a]
Concentration 3 (300mg)	872.9 ± 3.19[a,b]	11.97 ± 0.21[a,b]	3.51 ± 0.06[a,b]	70.66 ±0.03[a]
Concentration 4 (400mg)	923.8 ± 5.52[a,b]	4.23 ± 0.24[a,b]	1.23 ± 0.07[a,b]	70.70 ±0.015[a]
Concentration 5 (500mg)	980.8 ±0.953[a,b]	-	-	

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 Sodium Taurocholate hydrate and sodium glycocholate hydrate on formed gluten: As shown in (figure 1&2), Formed gluten (in control C2) remains intact, and no mechanical starring could disturb it. At the same time, T-1 to T-5 disrupted the formed gluten. T-5 showed the most disrupted, fragmented gluten.

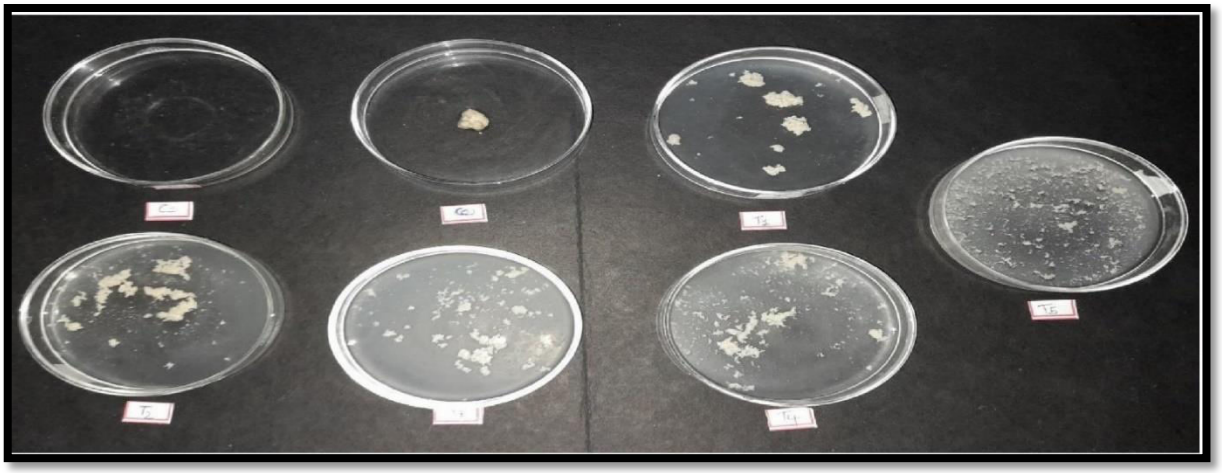

Figure 2: Effect of sodium taurocholate hydrate on formed gluten

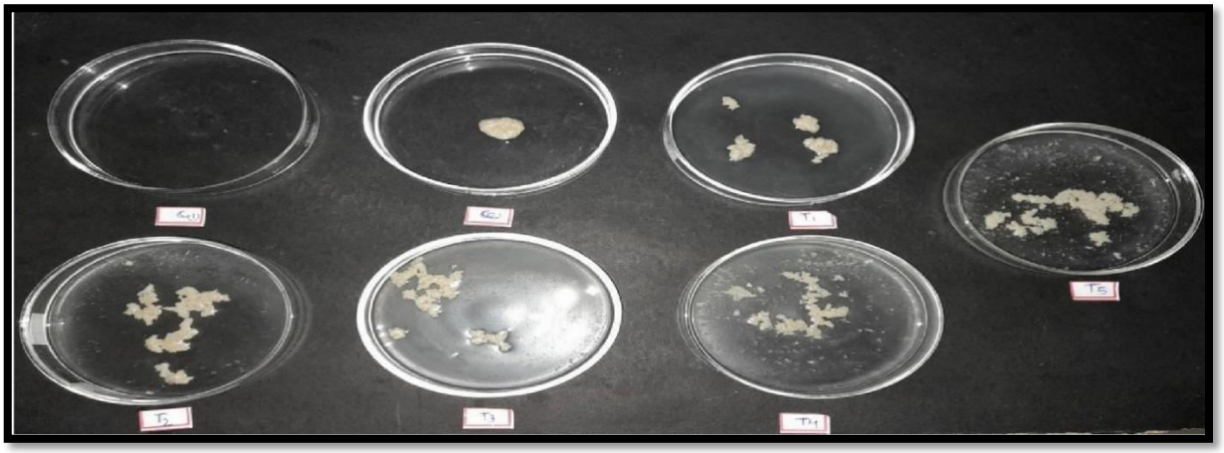

Figure 3: Effect of sodium glycocholate hydrate on formed gluten

Discussion:

Wheat products, a staple food worldwide, often contain gluten, which affects a significant portion of the population. The interaction of gluten with other biomolecules can profoundly alter their functions. A recent study has shown that gluten's interaction with amylase inhibits the function of this crucial

biomolecule, underscoring the importance of understanding gluten's effects [14].

The present study indicated that gluten interacted with bile salt. Therefore, gluten formation was inhibited by both primary bile salt, which shows that it has a more binding affinity with bile salt than its constituents. In addition, they also interact and disrupted the formed gluten. These interactions might be due to the high binding ability of gluten peptides with hydrophobic molecules. Gluten contains 35%of hydrophobic amino acids. Some other studies have also demonstrated by showed that gluten has more hydrophobic affinity with arabinoxylans and other polysaccharides [15,17].

Comparatively, STH showed less percentage of wet gluten extraction and followed by dry gluten extraction than SGH. This might be due to the presence of more hydroxyl groups in SGH than in STH. Some research evidence, shows that the binding affinity of bile salt increases as the number of hydroxyl groups decreases [18].

The interaction of gluten with bile salt could potentially disrupt the function of bile salt, leading to the malabsorption of lipids and lipid-soluble vitamins. This, in turn, could result in malnourishment and other related health issues. The urgency of understanding the molecular mechanism and health consequences of these interactions in animal models is evident, emphasizing the need for further study.

Conclusion:

The present evaluation clearly indicated that both primary bile salts interact with gluten constituents and hinder gluten formation. They also interacted and disrupted the insoluble formed gluten.

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The authors declare that they have no competing of interest

Ethical approval:

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References:

1. Barak S, Mudgil D, Khatkar BS. (2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. Lwt.51(1):211–7
2. Cenni S, Sesenna V, Boiardi G, Casertano M, Russo G, Reginelli A, et al. (2023). The Role of Gluten in Gastrointestinal Disorders: A Review. Nutrients.15:1–14
3. Ciccocioppo R, Di Sabatino A, Corazza GR. The immune recognition of gluten in coeliac disease. Clin Exp Immunol. 2005;140(3):408–16
4. Sharma N, Bhatia S, Chunduri V, Kaur S, Sharma S, Kapoor P, et al. Pathogenesis of Celiac Disease and Other Gluten Related Disorders in Wheat and Strategies for Mitigating Them.Frontiers in Nutrition. 2020;7:1–26
5. Chiang JYL (2013). Bile acid metabolism and signaling. Comprehensive Physiology.3:1191–212.
6. Staels B, Fonseca VA. (2009). Bile acids and metabolic regulation: mechanisms and clinical responses to bile acid sequestration. Diabetes Care.329(2).
7. Barak S, Mudgil D, Khatkar BS.(2013). Relationship of gliadin and glutenin proteins with dough rheology, flour pasting and bread making performance of wheat varieties. Lwt.51:211–7
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). Approved methods of the AACC, 11th ed. Method 38-12A. St Paul, MN: American Association of Cereal Chemists.
10. Qualitative Analysis to College Students.World J. Chem. Educ. 6(2), 82–86.
11. Kaushik R, Kumar N. (2015). Isolation, characterization of wheat gluten and its regeneration properties.J Food Sci Technol.52:5930–7.
12. Xu H, Zhou J, Yu J, Wang S, Wang S. (2021). Mechanisms underlying the effect of gluten and its hydrolysates on in vitro enzymatic digestibility of wheat starch. Food Hydrocolloids.113:106507

13. Iwaki S, Hayakawa K, Fu B xiao (2021). Changes in Hydrophobic Interactions among Gluten Proteins.10–2.
14. Si X, Li T, Zhang Y, Zhang W, Qian H, Li Y, (2021). Interactions between gluten and water-unextractable arabinoxylan during the thermal treatment. Food Chem.345:128785
15. Huebner FR, Wall JS (1979). Polysaccharide Interactions with Wheat Proteins and Flour Doughs.Cereal Chem.Vol.56: 68–73
16. Saitoh T, Fakuda T, Tani H, Kamidate T, Watanabe H (1996). Equilibrium Study on Interactions between Micelles by Micellar Electrokinetic Proteins and Chromatography. Analytical sciences.12:569-573.