The Effect of Sodium Chloride with Varying Concentration on Characteristics and Rheology of Extracted Gluten from Wheat Flour

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ABSTRACT

Gluten is a vegetable protein with the potential to be developed into functional food because of its high protein content. Gluten is viscoelastic which gives chewiness characteristics. In Indonesia, gluten is still imported and limited to processing food or to substitute for animal protein. This study aims to determine the effect of NaCl addition on the characteristics and rheology of extracted gluten. Addition of NaCl to the dough, 1%, 2% and 3%, respectively. The results showed that the extracted gluten had a protein content of 83.23-86.93%, moisture content of 1.17-1.54%, ash content of 0.13-0.16%, and fat content of 0.28-0.89%. These results are in accordance with the Codex International Standard. This showed that the gluten contained in wheat flour is good as a commodity in the food industry. Increasing the NaCl amount would also increase the tensile strength and the % elongation of gluten produced in the range of 88.71-201.90 gf/mm^2 and 7.10-14.51%, respectively. It showed that this addition affects the empirical rheological quality of the dough, strengthening the gluten network so that the extracted gluten from wheat flour in addition to food fortification can also be used for non-food purposes. The FT-IR spectrum showed the presence of bands, including β-sheet (1612 and 1633 cm^{-1}) in the intermolecular and intramolecular structures of gluten, α-helix (1658-1650 cm^{-1}), and β-turn (1668 cm^{-1}) linked to the glutamine side chain. These contribute to the secondary structure of the gluten protein. The presence of β-sheet and β-turn in the FT-IR spectrum of gluten indicates that the gluten contained in wheat flour is more elastic and stable. The gluten in this study has functional groups in the same wavenumber range as the functional groups from the literature. The addition of NaCl to the dough did not have a significant effect on the absorption bands on the gluten spectra.

Keywords: *Wheat flour, Gluten, Sodium chloride, Characteristics, Rheology.*

1. INTRODUCTION

Wheat flour contains a complex and unique protein mixture because it can form a thick dough when mixed with water and gives it viscoelastic and cohesive [1]. Up to 85% of the protein contained in wheat flour contains gluten, the largest contributor to the elasticity and resilience of a dough [2]. Gluten is formed by its main protein fractions, glutenin, and gliadin when wheat flour is mixed with water. These two proteins play an important role in the formation of dough that is elastic, fluffy, and soft like a sponge [3]. These properties make gluten an important commodity in the food industries.

As a commodity, gluten must contain a minimum of 80% protein, water content <10%, fiber content <1.5%, ash content, and fat content <2.0% respectively as specified by the International Codex Standard [4]. As with other types of protein, the structure of gluten and its interactions are also influenced by the solvent environment including the presence of salt [5].

The addition of salt at low concentrations shields the charges of the gluten thereby reducing the electrostatic repulsion between the proteins and allowing the salt to associate. Whereas at higher concentrations, NaCl interacts more with the solvent molecules. In these circumstances, NaCl will affect the characteristics and rheology of gluten [5] [6].

Technologically, NaCl has an important function in the formation of dough and bread, which is a significant sensory contribution to processing in the bakery industry. NaCl affects the rheological behavior of the dough - if the amount of NaCl is reduced, a sticky dough phenomenon will appear. This will cause processing problems and the final product quality will be poor. Experiments to find a substitute for NaCl in the bread-making process to reduce sodium have been carried out, but they often have a negative effect on sensory properties [6].

This study aims to study the effect of variations in NaCl concentration on the characteristics and rheology of gluten extracted from wheat flour.

2. BACKGROUND

One of the alternatives to fulfill functional food is by consuming vegetable protein. One plant that has a high enough protein content is wheat (*Triticum aestivum*). Wheat seeds are composed of 60-80% carbohydrates, 10-20% protein, 2-2.5% fat, 4-4.5% minerals, and a number of other vitamins [7]. In Indonesia, the product of this wheat is wheat flour. The main proteins that makeup wheat flour are gliadin (± 20-25%) and glutenin (± 35-40%). When wheat flour is mixed with water, those two proteins will form gluten [7].

Gluten is insoluble in water and is viscoelastic which gives food products the chewiness characteristic. In Indonesia, gluten is still imported, so its use is limited to a mixture of processed food or as a substitute for animal protein [8]. The commercial manufacture of gluten still uses variations from the Batter process and Martin process [7] where the protein content is 70-75% and fat 5-8% [9]. According to the Codex International Standard, however, as a commodity, gluten must contain a minimum of 80% protein and <2% fat.

In the production of gluten, its interactions are influenced by its solvent environment and the presence of salt. During the manufacture of food products NaCl acts as a co-solute and affects the hydration of flour dough under certain conditions [5], [6].

Most of the studies were focused on the effect of salt on the rheological properties of wheat flour dough. However, the effects of salt on the gluten protein network and the resulting rheological properties have not been adequately investigated [5]. To study the rheological properties and characteristics of gluten in relation of NaCl addition, quantitative testing based on international standards, tensile strength, % elongation and FT-IR can be carried out.

3. METHODOLOGY

3.1. Material and Chemicals

The high-protein wheat flour branded "Cakra Kembar" was purchased at a supermarket. The solvents used were distilled water and sodium chloride which were obtained from the laboratory of the Chemical Engineering Department, Politeknik Negeri Bandung.

3.2. Extraction of Gluten

Gluten extraction was carried out by weighing 100 grams of wheat flour and adding NaCl with varying concentrations of 0%, 1%, 2%, and 3% (w / v). The formed dough was kept for 40 minutes. The dough was washed under a stream of running water until most of the starch was washed out and the wash water was clear. The viscoelastic mass obtained was wet gluten.

3.3. Drying and Reducing the Size of Gluten

The extracted gluten was dried in a vacuum oven at 60 ° C for 8 hours. Dry gluten was powdered using miller up to 100 mesh in size and packed in polyethylene bags and stored in an airtight container at 4 to 7 ° C until further analysis.

3.4. Characterization of Extracted Gluten

To determine the quality of gluten, the characterization of gluten can be carried out in accordance with the provisions of the Codex International Standard. This characterization consisted of determining the protein content using the Kjeldahl method, the moisture content using the gravimetric method, the ash content using the dry ashing method, and the fat content using the Soxhlet method.

3.5. Determination of Tensile Strength and % Elongation

Determination of tensile strength and% elongation using the Tarno Crocki, Wilhelm Herm Holm, German apparatus, operated according to the ASTM Standard Method. Four specimens (100 mm long, 20 mm wide) from each sample were measured and cut using a cutter. The tensile strength (gf/mm^2) and % elongation are calculated according to the ASTM method.

3.6. Identification of Functional Group Using FT-IR

This stage aims to identify the presence of a functional group in the extracted gluten based on its wavenumber (cm $^{-1}$), then compare it with the functional groups in some literature.

4. RESULTS AND DISCUSSIONS

The extracted gluten powder is determined for its protein content, moisture content, ash content, and fat content. The test results can be seen in the following table.

Table 1. The Characterization Result of the Extracted Gluten

No	Specification	Extracted Gluten (%)	Codex International Standard (%)
1	Protein	83.23-86.93	> 80.0
2	Moisture	1.17-1.54	< 10.0
3	Ash	0.13-0.16	< 2.0
4	Fat	0.28- 0.89	< 2.0

Based on Table 1 gluten extracted from wheat flour has a specification that is in accordance with the Codex International Standard. This showed that the gluten contained in wheat flour has good quality as a commodity in the food industry.

Most of the fat content in flour is associated with gluten protein during the washing process. The hydrophobic part of the gluten protein will bind very strongly to fat. The presence of this fat can reduce the quality of gluten because it affects functional properties and taste. After all, fat is easily oxidized [7].

In general, washed gluten usually still contains 75–85% protein, 5–10% lipids, and the rest is starch and non-starchy carbohydrates [10]. However, from Table 1, it can be seen that the extracted gluten contains quite low fat, namely 0.28-0.89%. The addition of a certain amount of NaCl during the mixing and washing processes of the dough will reduce the fat content in the dough.

Based on its rheology, gluten is a vegetable protein that is viscoelastic and can deform if forces are affecting it, one of these forces is tensile strength. Deformation can also cause gluten to stretch and change its length, which is known as % elongation.

The effect of adding various concentrations of NaCl on the rheology of gluten can be seen in the following Table 2.

Table 2. Tensile Strength dan % Elongation of Extracted Gluten

No	Extracted Gluten	Tensile Strength (gf/mm^2)	% Elongation
1	Without NaCl	88.71	7.10
2	1% NaCl	164.17	14.51
3	2% NaCl	126.44	6.32
4	3% NaCl	201.90	12.08
5	Gluten from whole wheat flour + 2% NaCl [1]	23.79-47,80	
6	Gluten from whole wheat flour without NaCl [11]	21.38-69.46	1.90-12.23

Tensile strength measurement aims to see the maximum pull that can be reached by gluten before breaking. This measurement depends on the maximum force and the cross-sectional area of the gluten. Percent elongation (ductility) is the ability of gluten to undergo deformation through elongation without breaking. At this stage, the cross-sectional area of the gluten used is not uniform because the gluten is very viscoelastic and cohesive. As a result, the tensile strength and % elongation obtained are inconsistent.

From Table 2, it is shown that the gluten extract from this study has higher tensile strength and % elongation compared to the literature [1], [11]. This could be due to the gluten from this study containing NaCl of varying concentrations and different sources of gluten. The gluten extract used in this study was from wheat flour, while the gluten in the literature came from whole wheat flour.

When salt is added to the mixture, Na^+ and Cl^- ions can interact with the polar parts of gluten. In this case, the ability of the protein to absorb water decreases, but the gluten network stabilizes, and the strength of the resistance to extensibility increases. In this case, the addition of NaCl will affect the empirical rheological quality of the dough, in particular strengthening the gluten network [6].

Based on the high tensile strength and % elongation and the viscoelastic of the extracted gluten from wheat flour, it can compete with other biopolymers for the fortification of food. It can also potentially wide use in non-food purposes such as films, plastics, and adhesives [7].

The FTIR spectra of gluten extracted from wheat flour added with various concentrations of NaCl can be seen in the following Figure 1

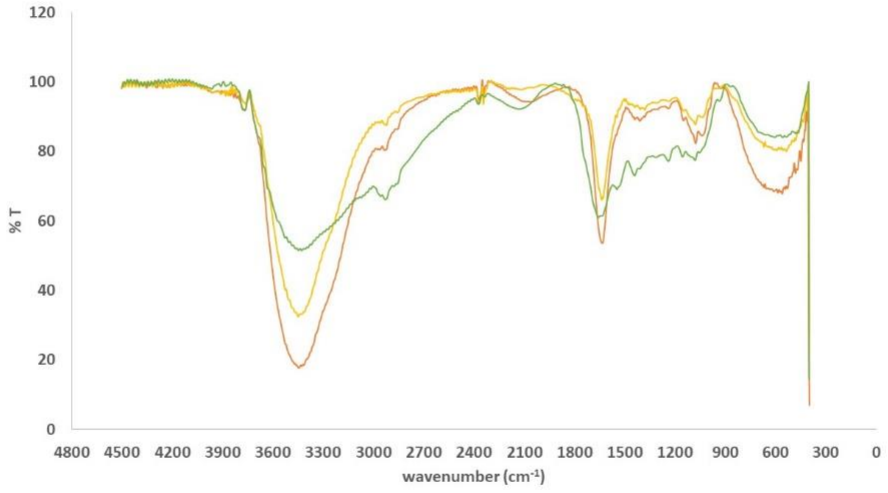

Figure 1 FTIR Spectra of Extracted Gluten Wheat Flour in Addition of Sodium Chloride with Varying Concentration (- 0% NaCl, - 2% NaCl, - 3% NaCl)

Figure 1 shows that there are absorption bands at a certain wavenumber (cm^{-1}). The spectra are then compared with some literature as shown in the following Table 3.

Table 3. FTIR Spectra Comparison Between Extracted Gluten and Gluten from Some Literature

References	Wavenumber (cm^{-1})				
	Amide III	Amide II	Amida I		
	NH bending and CN stretching	NH bending and CN stretching	β - sheet	α – helix (C=O stretching)	β -turn
[12]			1633	1650	1668
[13]		1541	1631	1651	1668
[14]		1520	1620-1625	1650	1660-1670
[15]			1633	1658-1650	1668
[16]	1229-1301				
Djenar. N.S et al, 2021	1238.30 ;1240.23	1500-1550	1639.49; 1633.71	1650 -1700	1650-1700

It can be seen in Table 3 that the FT-IR spectra of extracted gluten showed three characteristics absorption band groups, namely amide I (1650-1720 cm^{-1}), amide II (1480-1580 cm^{-1}), and amide III 1430-1480 cm^{-1} [12],[14], [15] and 1229-1301 cm^{-1} [16].

The most sensitive region of the gluten spectra is the amide I band which appears in the form of carbonyl stretching (C = O) of its peptide bonds. Amide I band is useful for the analysis of the secondary structural composition of gluten proteins and changes in their conformation. The components of the amide I bands are β-sheet (1612 and 1633 cm^{-1}) in the intermolecular and intramolecular structures of gluten, α-helix (1658-1650 cm^{-1}), and β-turn (1668 cm^{-1}) associated with side chains glutamine [12]. The sensitivity of amide II and amide III absorption bands is lower than that of amide I. These bands contain N-H bending and C-N stretching.

These three components contribute to the secondary structure of the gluten protein. The presence of β-sheet and β-turn in the FTIR spectra of gluten indicates that the gluten found in wheat flour is more elastic and stable. This is an important characteristic of gluten for quality bread [15].

In the FTIR spectra of gluten, there are several absorption bands of several other compounds that are still associated with gluten, as shown in the following Table 4.

Table 4. FTIR Spectra of Gluten

References	Wavenumber (cm^{-1})		
	C-O-C stretching and C-C stretching of starch	CH_3 and CH_2 stretching of fat	OH, deformation band of water.
[12]	1170-900		
[13]	1.020; 1.080 and 1.150	2958; 2928; 2854	3350; 1640; 2150
Djenar. N.S et al, 2021	1037.70; 1080.14; 1149.57	2922.16; 2933.73	3342.94; 3348.72; 2300-2200

Table 4 showed that the extracted gluten from this study still contains a small amount of starch and fat associated with gluten [12], [13]. Water has a characteristic in the infrared spectra where water is a very important solvent in the formation of dough [13].

Based on the results of the identification of functional groups by FT-IR, it showed that the gluten from this study has functional groups in the same wavenumber range as the gluten functional groups from some literature. The addition of NaCl to the dough did not have a significant effect on the absorption band on the gluten spectra.

5. CONCLUSION

Gluten extracted from wheat flour has specifications that comply with Codex International Standard so that it has good quality as a commodity in the food industry. The addition of a certain amount of NaCl during the mixing and washing process of the dough will reduce the fat content in gluten to the range of 0.28 to 0.89%.

The increase in tensile strength and % elongation of gluten indicated that the addition of NaCl affects the empirical rheological quality of the dough, in particular strengthening the gluten network, so that the extracted gluten from wheat flour in addition to food fortification can also be used more widely for non-food purposes.

Based on the results of the identification of functional groups by FT-IR, it showed that the gluten from this study has functional groups in the same wavenumber range as the gluten functional groups from some literature. The addition of NaCl to the dough did not have a significant effect on the absorption band on the gluten spectra.

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