SEMARAK ILMU PUBLISHING

Journal of Advanced Research in Fluid Mechanics and Thermal Sciences

Journal homepage:
https://semarakilmu.com.my/journals/index.php/fluid_mechanics_thermal_sciences/index
ISSN: 2289-7879

Thermal and Concentration Impacts on Tannin and Physical Properties Changes during Alkaline Soaking of Sorghum Grain

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ARTICLE INFO

Article history:
Received 21 April 2024
Received in revised form 28 July 2024
Accepted 11 August 2024
Available online 30 August 2024

Keywords:
Food processing; sorghum; soaking; thermal; tannin

ABSTRACT

Tannin is one of the phenolic components present in food ingredients, especially grains and nuts. The tannin content in sorghum grain up to 4.8% depending on the variety. In low concentrations, tannins have benefits if consumed by humans. Meanwhile, at high amounts, tannin acts as an anti-nutritional substance that can inhibit the absorption of protein in digestion. In this study, the process of degrading tannin levels in this study used the soaking method using potassium hydroxide and calcium hydroxide solutions with concentrations (0.05-0.15%) with soaking temperatures of 30, 40, and 50^{o}C. Soaking was carried out for 8 hours with sampling every 1 hour. The use of Calcium hydroxide alkaline solution of 0.15% obtained in the highest dissolved tannin solution in sorghum grain soaking process. The results of soaking process also obtained data on the Water Adsorption Index, Water Solubility Index, and Swelling Power. From this research it is expected to obtain low concentrations tannin of sorghum grain as a quality food ingredient.

1. Introduction

Sorghum *(Sorghum bicolor (L.) Moench)* is a grain plant which is an important source of food and feed in various tropical and subtropical regions [1]. Sorghum plant has the distinctive features of hollow stems, serrated leaves, and long flower clusters. On the other hand, sorghum has diverse roles being used as food, animal feed, and even for biofuel production [2-4]. Sorghum plants are more resistant to drought conditions and extreme heat than other food crops, thus providing solutions to environmental challenges [5]. The concentration of nutrients in sorghum is quite high depending on the variety, growing conditions and processing method. In general, sorghum grains contain up to 64.30-73.80% carbohydrates, 8.19-14.02% protein, 8.00% fiber value, and 9.00% bioactive

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https://doi.org/10.37934/arfmts.120.2.115124

compounds [6]. Therefore, sorghum grains have the potential to be used as a substitute for staple foods such as rice, corn, cassava, wheat, and others [1]. On the other hand, sorghum is one of the grains that has high phenolic compounds such as tannins whose concentration reaches up to 4.8% [7].

Tannins are secondary metabolites widely distributed in plants which are polyphenolic compounds that have complex molecular sizes and structures and are generally easily soluble in water [8]. Tannins function to protect these plants from pests and other plant diseases [9]. At low concentrations, tannins are compounds that have beneficial to human health due to they can function as antioxidants, anti-cancer, anti-inflammatory, immunomodulatory, and antithrombotic [10-14]. Whereas at high concentrations, tannins are anti-nutritional which is detrimental due to they can bind to proteins so that the proteins cannot be absorbed optimally in the human body [15]. Moreover, high tannin concentration can cause to have a slightly bitter and chelating taste and make them becomes less desirable to consumers [16]. The maximum concentration of tannin in food that can be tolerated by humans is less than 1%. Therefore, sorghum grains need to be further processed to reduce their concentration.

In general, food processing can degrade its nutritional quality, thus requiring appropriate treatment to address tannins. In general, food processing can degrade its nutritional quality, thus requiring appropriate treatment to address tannins, biological, and chemical treatments [17-19]. Even for the chosen methods, the processing conditions play a crucial role in determining the overall nutritional losses. In terms of physical treatment, dehulling has been reported to reduce the tannin content in sorghum grains due to the outer husk of the sorghum grain also contains relatively high amounts of tannin [17]. In biological treatment, the fermentation process plays a role in reducing tannin concentration up to 30% [20]. Meanwhile in the chemical process, the method employed were soaking in alkaline, acidic, or saline solutions to reduce tannin concentration.

Research on soaking sorghum grains using alkaline solutions has not been found much. This research was focused on studying the effect of the type of alkali, the concentration of the alkali, and the soaking temperature on the concentration of tannins and their effect on the physical properties of sorghum grains. This research is expected to obtain new food materials as food substitution ingredients with good nutritional value and low tannin concentrations.

2. Methodology

2.1 Materials

The sorghum grains used in this study were white sorghum varieties obtained from farmers in the Gunung Kidul district, Yogyakarta, Indonesia which were harvested during June-July 2023. The raw material for sorghum obtained was sorghum grains which had been milled beforehand and had been sorted from dirt such as leaves, small stones, and other foreign materials. The selected sorghum grains are intact and unbroken grains.

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2.2 Soaking Process

The soaking trials were carried using randomly selected size of 100 sorghum grains. The sorghum grains were soaking in 200 ml of alkaline solutions of Potassium Hydroxide (Merck, Germany), Calcium hydroxide (Merck, Germany), and Natrium Hydroxide (Merck, Germany) at varying concentration (0.05–0.15%) and soaking temperatures (30, 40, and 50°C). The chosen soaking solution concentration was below the safe limit for chemical usage in food materials. While, the temperature chosen in the soaking process is the temperature below the gelatinization temperature. Soaking process were carried out for 8 hours with sampling every 1 hour. Distilled water is used as a comparison in the process of soaking sorghum grains. In addition, at every hour of sampling, an analysis of water uptake and tannin degradation is carried out. Furthermore, sorghum grains were washed with distilled water and dried by oven (merk, manufacture) at 55°C for 2 hours and milled for further analysis (nutritional, physical and morphological properties).

The water absorption capacity of sorghum grains was evaluated using the Eq. (1) [21].

$$W_a = \frac{W_f - W_i}{W_i} \times 100\ \% \tag{1}$$

where W_a represents the water absorption (on a dry basis, %), W_f is the weight of seeds after soaking (in grams), and W_i is the initial weight of seeds before soaking.

2.3 Tannin Analysis

The tannin analysis method on sorghum grains was conducted using the method described by Elgailani *et al.,* [22]. The Folin-Denis reagent was prepared by dissolving $Na_2WO_4{\cdot}2H_2O$ (20.66 g), dodeca-molybdo-phosphoric acid (4.13 g), and phosphoric acid (85%, 10 mL) in 150 mL distilled water with stirring for 1 hour. The Folin-Denis reagent is prepared by dissolving $Na_2WO_4{\cdot}2H_2O$ (20.66 g), dodeca-molybdo-phosphoric acid (4.13 g), and phosphoric acid (85%, 10 mL) in 150 mL of distilled water with mixing at 100 rpm for 2 hours. The final solution is then diluted with distilled water to a total volume of 500 mL in a volumetric flask. A solution of sodium carbonate was created by dissolving 106 g of Na_2CO_3 in 1000 mL of water. Subsequently, a tannic acid solution with a water content of 6% was prepared by dissolving 250 mg of tannin in 500 mL of double-distilled water. To prevent fungal and bacterial contamination, a small quantity (2-3 drops) of a 0.1% sodium azide solution was introduced. Before application, a portion of this solution was diluted to 1:100 with double-distilled water. All analytical solutions were kept at 4°C for further analysis.

The extracted solution from the soaking process was transferred using a pipette to sterile tubes. Then, 7.9 mL of destilled water and 0.5 mL of Folin-Denis reagent were put and the mix for a duration of 3 minutes. Subsequently, 1.5 mL of a 20% sodium carbonate solution was incorporated to the solution. The mixture solution was vortexed and allowed to rest for 2 hours within a dark cabinet at room temperature. Arsobancy was measured using UV-VIS spectrophotometry (GENESYS™ 20 Thermo Fisher Scientific, Karlsruhe, Germany) at a maximum wavelength of 725 nm then the concentration was obtained from the standard curve callibration of tannic acid. A calibration curve was created by diluting tannic acid at a concentration (0-140 ppm) then read for its absorbance and the absorbances value was used as a standard curve and regression equation. All experiments and absorbance readings were performed in duplicate.

2.4 Water Solubility Index, and Swelling Power Analysis

To analyze the Water Solubility Index (WSI) of treated sorghum grains**,** 10 grams of the sample were added to 100 mL of distilled water in a test tube with a cap and for a 30 minute in water bath at 60 °C. The slurry then colled until room temperature then the tube was centrifuged for 30 minutes at 2000 x g (Thermo Fisher Scientific in Waltham, Massachusetts, USA). The sorghum sediment was then filtered using filter paper (Whatman, United Kingdom) and weighed. Furthermore, the sediment is dried using an oven at 50°C until the weight becomes constant. The supernatant was dried in an oven at 100°C until the weight remained constant. The weights of the dried paste and the sediment were recorded to analyze swelling and solubility.

$$\text{WSI (\%)}: \quad \frac{(\text{weight of dry solids in the supernatant})}{(\text{dry weight of extrudates})} \times 100 \tag{2}$$

$$\text{Swelling Power (g/g)}: \quad \frac{\text{weight of formed paste (g)}}{\text{dry weight of sample (g)}} \tag{3}$$

3. Result and Discussion

3.1 Effect of Alkaline on Tannin Degradation

The effect of the type and concentration of alkaline solutions on the concentration of dissolved tannins in soaking sorghum grains is presented in Figure 1. The results show that both types of potassium hydroxide and calcium hydroxide solvents are able to degrade tannins in sorghum grains properly. The effect of the concentration of the two solvents showed the same results. The higher the concentration of the solution, the more tannins can be degraded and the faster the dissolution process compared to solvents with low concentrations at 30°C. In the potassium hydroxide solutions with a low concentration (0.05%), tannins dissolved significantly after the soaking process for 5 hours. Meanwhile, at the same concentration in the alkaline solvent calcium hydroxide, the concentration of tannins was significantly degraded after 4 hours of soaking process. At the highest concentration (0.15%) tannin was significantly degraded after soaking for 1 hour in both types of solvents. The maximum average degraded tannins by alkaline solution were obtained in alkaline calcium hydroxide at the three temperature variations. The highest yield of tannins could be degraded up to 44,923 ± 0,577 ppm. This is due to calcium hydroxide has the ability to degrade tannins with strong ionic bonds. The group present in tannins is a phenolic hydroxyl group which is reported to be easily soluble in dilute alkaline solutions [23]. On the other hand, the use of calcium hydroxide is easier to remove by washing with distilled water.

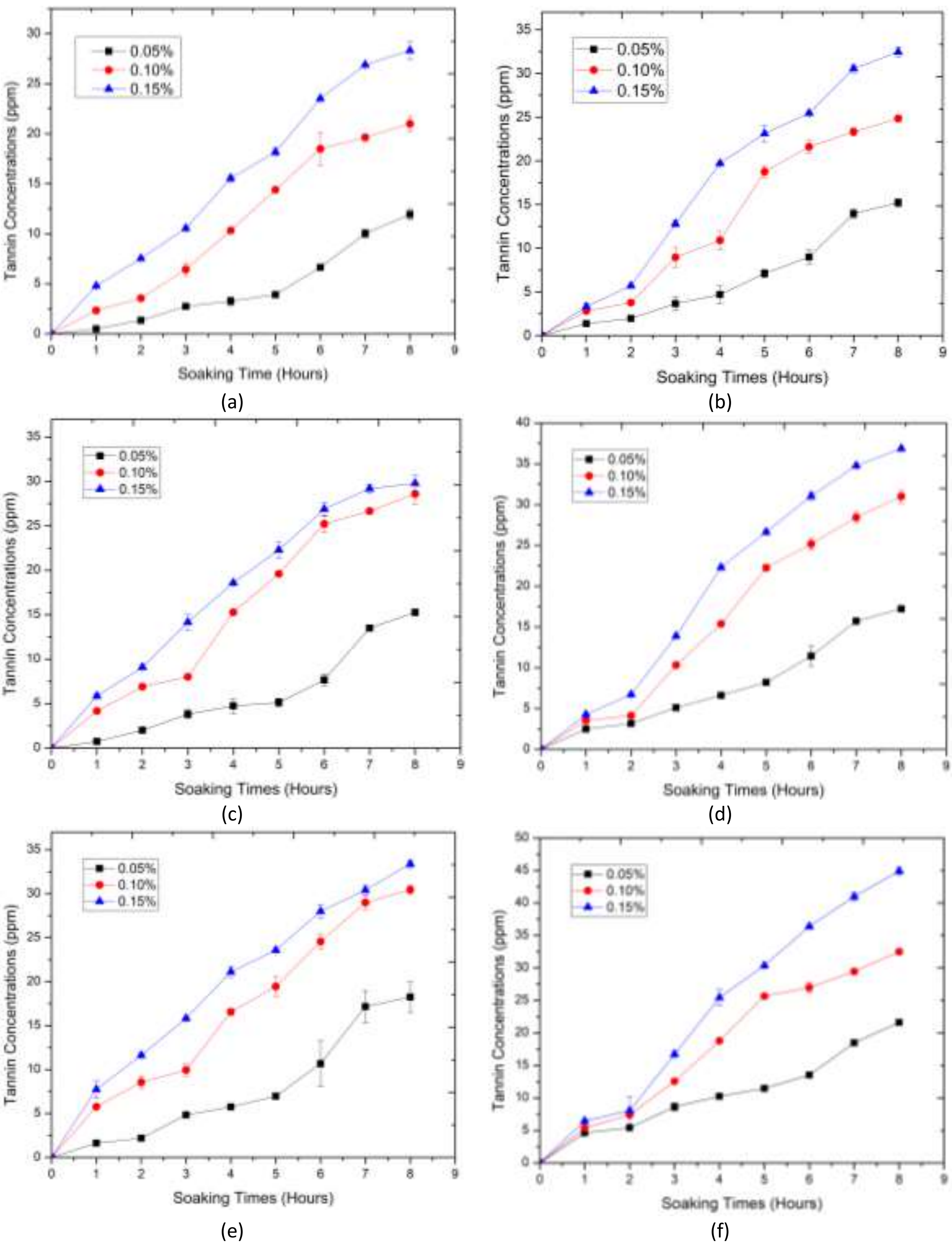

Fig. 1. Effect of the alkaline on the concentration of dissolved tannins at temperature of 30, 40, and 50°C. (a) Potassium Hydroxide at 30°C, (b) Calcium hydroxide at 30°C, (c) Potassium Hydroxide at 40°C, (d) Calcium hydroxide at 40°C, (e) Potassium Hydroxide at 50°C, (f) Calcium hydroxide at 50°C

3.2 Effect of Thermal on Tannin Degradation

The effect of soaking temperature on the concentration of dissolved tannins in sorghum grains is presented in Figure 1. The results show that the higher the temperature will affect the dissolving ability of the sorghum grains. The higher the temperature at the time of dissolution, the concentration of dissolved tannins will be more and more. At 50°C with calcium hydroxide solvent, the dissolved tannins reached 44.92 ppm ± 0.57. Whereas in potassium hydroxide solvent at the same temperature, the concentration of dissolved tannins reached 33.38 ppm ± 1.33. The tannins dissolved at 30 °C with calcium hydroxide and potassium hydroxide solvents resulted in lower tannin concentrations of 32.87 ppm ± 0.61 and 28.316 ppm ± 0.88 respectively. The heating process is carried out below the gelatinization temperature of 64–95°C [24]. The soaking process above the gelatinization temperature will affect the structure of the sorghum grains and their physical properties due to the absorption of much of solution [25]. The addition of thermal in the soaking process can speed up the soaking process. This is due to the higher the temperature will affect the rate of the diffusion process [26].

3.3 Effect of Alkaline Solutions and Thermal on Water Uptake

The effect of alkaline type and temperature in the soaking process on water uptake is presented in Table 1. The results obtained were that the difference in water uptake capacity of sorghum grains was significantly affected by the addition of thermal in the soaking process.

Table 1
Effect of thermal and alkaline concentration on water uptake

Times (hours)	Water Uptake (%)					
	Calcium hydroxide 0.05 %	Calcium hydroxide 0.10 %	Calcium hydroxide 0.15 %	Potassium hydroxide 0.05 %	Potassium hydroxide 0.10 %	Potassium hydroxide 0.15 %
(a) Soaking temperature of 30°C						
1	4.54 ± 1.55	6.165 ± 0.44	8.12 ± 0.72	2.935 ± 0.68	3.66 ± 0.30	5.24 ± 0.52
2	11.56 ± 1.49	15.81 ± 1.30	17.44 ± 1.49	9.985 ± 0.78	11.21 ± 0.63	12.47 ± 1.51
3	21.37 ± 0.56	27.53 ± 1.50	29.57 ± 1.32	19.95 ± 0.71	21.17 ± 0.52	23.02 ± 2.15
4	37.99 ± 1.73	40.64 ± 1.47	44.12 ± 0.82	36.10 ± 1.00	37.39 ± 0.73	38.70 ± 1.19
5	50.56 ± 1.21	52.63 ± 1.22	55.16 ± 0.87	48.97 ± 0.65	50.36 ± 1.34	51.39 ± 1.86
6	53.52 ± 1.20	58.16 ± 1.91	59.31 ± 0.61	50.94 ± 0.11	54.38 ± 0.71	54.87 ± 0.76
7	58.15 ± 0.93	62.12 ± 0.91	65.20 ± 0.46	55.82 ± 0.56	57.91 ± 0.71	59.64 ± 0.33
8	59.16 ± 0.35	64.26 ± 0.78	67.02 ± 0.80	56.93 ± 0.71	58.72 ± 1.00	60.43 ± 0.02
(b) Soaking temperature of 40°C						
1	10.47 ± 0.22	13.68 ± 0.19	16.93 ± 0.14	4.42 ± 0.15	6.98 ± 0.77	9.89 ± 0.62
2	20.77 ± 0.27	23.21 ± 0.81	25.80 ± 1.03	12.54 ± 1.51	15.86 ± 1.49	18.01 ± 1.32
3	36.00 ± 0.82	37.16 ± 0.79	41.12 ± 0.83	22.61 ± 1.44	25.20 ± 0.81	25.20 ± 0.80
4	47.77 ± 1.08	50.91 ± 1.20	54.68 ± 0.18	38.15 ± 0.46	40.86 ± 0.26	46.57 ± 1.90
5	56.81 ± 1.04	57.77 ± 0.45	60.98 ± 0.60	49.77 ± 0.78	52.37 ± 0.05	56.15 ± 1.23
6	61.99 ± 0.63	62.97 ± 0.80	64.73 ± 0.91	54.11 ± 0.92	58.03 ± 0.82	59.67 ± 0.93
7	67.72 ± 0.40	68.80 ± 0.29	71.10 ± 0.53	57.74 ± 1.03	60.93 ± 0.59	64.19 ± 0.74
8	69.19 ± 0.92	69.94 ± 0.09	73.05 ± 0.85	60.14 ± 0.95	62.44 ± 0.01	68.39 ± 0.06
(c) Soaking temperature of 50°C						
1	14.78 ± 0.35	16.87 ± 0.94	20.78 ± 1.04	7.98 ± 0.75	17.24 ± 1.68	13.16 ± 0.63
2	25.96 ± 0.11	28.77 ± 1.03	31.13 ± 0.96	16.21 ± 0.71	22.00 ± 0.71	25.69 ± 0.11
3	37.96 ± 0.78	40.60 ± 1.11	44.08 ± 0.60	27.79 ± 1.72	35.67 ± 1.78	35.30 ± 0.97
4	51.75 ± 0.97	56.64 ± 1.24	59.23 ± 0.31	42.83 ± 2.51	48.63 ± 0.54	52.73 ± 0.45
5	62.46 ± 1.33	67.58 ± 1.09	69.93 ± 2.08	54.56 ± 3.01	57.02 ± 1.40	61.44 ± 0.14
6	68.21 ± 1.13	70.22 ± 0.26	76.05 ± 0.55	58.46 ± 0.82	62.47 ± 1.48	68.79 ± 0.18
7	72.21 ± 0.53	76.08 ± 0.50	78.11 ± 1.13	60.74 ± 1.01	68.87 ± 1.21	73.10 ± 0.98
8	75.93 ± 0.57	78.29 ± 0.89	82.98 ± 0.81	64.01 ± 0.80	70.82 ± 0.86	76.92 ± 0.17

The highest water uptake was obtained in an alkaline calcium hydroxide solution with a concentration of 0.15% at 50^{o}C reaching up to 82.98% ± 0.81. Increasing the temperature in the soaking process causes the pores of the sorghum grains to be more open. Thus, the alkaline solution will be easier to enter into the pores of the sorghum grains. In addition, at a higher soaking temperature process, it will affect the rate of change in volume caused by the coefficient which increases with increasing temperature [27]. However, the sorghum grain absorption solution has not been studied in thermodynamic equilibrium. In addition, the soaking process with or without thermal addition will cause damage to the outer layer of the grains thereby increasing the diffusion of water during soaking process [28].

3.4 Effect of Alkaline Solutions and Thermal on Swelling Power and Water Solubility Index

Sorghum grain that has been treated by soaking process with various alkaline concentrations and thermal was processed into flour. From the treated sorghum flour, the physical properties of each treatment were analyzed in swelling power and water solubility index. From Table 2, it can be seen that the soaking process using alkali can change the physical properties of sorghum flour. When compared to Potassium hydroxide, soaking using calcium hydroxide (0.05, 0.10, and 0.15%) was able to reduce the swell power values up to 7.50%, 6.96%, and 4.73% at immersion temperatures of 30, 40 and 50^{o}C respectively. However, the results of the swelling power with two types of alkaline employed in the soaking process were not significant. The decrease in the value of swelling power occurred perhaps due to the soaking treatment using a greater alkaline concentration and a large temperature of soaking process would make the alkaline solution easier to diffuse into sorghum grain particle [29]. After being treated and converted into sorghum flour, the ability of sorghum flour to swell will be slightly reduced due to it having received quite a lot of solution in the previous process. Thus, the sorghum flour sample will produce a more hollow structure which results in a reduced degree of swelling [30]. In addition, the decrease in the value of the swelling power ratio due to immersion treatment with alkaline solution can also be related to the shrinkage of the micropores of sorghum and causes the collapse of the fiber capillaries [31].

Table 2
Effect of Alkaline Solutions and Thermal on physical properties

Times (hours)	Swelling power (%)			Water Solubility Index (%)		
	Soaking Temperature (^{o}C)					
	30	40	50	30	40	50
Calcium hydroxide						
0.05%	11.16	7.78	5.83	2.05	3.46	3.97
0.10%	8.71	7.58	5.30	2.00	2.89	3.40
0.15%	7.50	6.96	4.73	1.75	2.67	3.18
Potassium hydroxide						
0.05%	13.20	8.87	7.33	2.36	3.33	4.33
0.10%	9.83	7.55	5.57	1.79	2.94	3.32
0.15%	8.66	5.63	4.53	1.62	1.77	2.54
Not treated Sorgum flour	11.22			4.87		

The impact of the soaking process on the physical properties was also a change in the sorghum flour water solubility index. The greater the concentration of alkaline solutions in potassium hydroxide and calcium hydroxide, the solubility value were decreases. This phenomenon also occurs at all variations in soaking temperature. From Table 1, it can be seen that the greater concentration of alkaline solutions results in a decrease in the solubility with the lowest value obtained from soaking in 0.15% calcium hydroxide at a temperature of 30^{o}C, the WSI value reach 1.62%. The high WSI value of sorghum grains without the soaking process may be due to the presence of hydroxyl groups from polysaccharides that are accessible to water. In addition, the presence of hemicellulose content in untreated sorghum grains has high hydrophilic properties in sorghum grains so the tendency of their solubility in water causes the flour to swell when soaking in water [32]. However, hemicellulose lost due to the soaking process was not studied in this study. The hydrophilicity of sorghum grains is reduced through alkaline immersion which causes water to be difficult to enter into the sorghum particles after the soaking process [33]. From the effect of the soaking temperature, sorghum grains which are soaked at a higher temperature actually result in sorghum flour being more soluble in water. This may be because at high temperatures, the polysaccharides in sorghum grains begin to degrade. A low water solubility value has an advantage in food because not many particles are lost during the cooking process.

4. Conclusions

In this study, the degradation of tannins and the physical properties of soaking sorghum grains under the influence of type, alkaline concentration, and the effect of soaking temperature were evaluated. The addition of thermal in the soaking process is proven to accelerate the degradation of tannins in sorghum grains due to the pores of the sorghum grain layer will be more open so that the soaking solution will easily dissolve into the sorghum particles. The highest tannin degradation during the soaking process was obtained when soaking in 0.15% calcium hydroxide at a temperature of 50^{o}C reach 44.92 ppm ± 0.57 compared to potassium hydroxide solvent at the same concentration and soaking temperature which only reached 32.87 ppm ± 0.61. On the other hand, the addition of thermal in the soaking process to the sorghum grains also affects the water uptake of the sorghum grains because the higher the soaking process temperature will affect the rate of volume change caused by the coefficient which becomes greater as the temperature. The soaking process also affects the physical properties of sorghum grain. After the soaking process, the WSI value will decrease with the lowest value obtained of 1.62% while the swelling power value also decreases with the lowest value obtained of 4.53%. This study is expected to provide an overview of the processing of sorghum grains using the alkaline soaking method and its impact on the physical properties of sorghum grain so that it can provide insight into high quality modified food raw materials.

Acknowledgement
This research was funded by a grant from Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for the *Penelitian Disertasi Doktor* research grant, Faculty of Engineering Universitas Negeri Semarang with research grant No. Nomor: 119.22.3/UN37/PPK.05/2024, *Badan Riset dan Inovasi Nasional* (BRIN) Republic of Indonesia for the degree by research scholarship program, and Pusat Riset Teknologi dan Proses Pangan, BRIN.

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