Study on Red and White Sorghum (*Sorghum bicolor*) Germination Flour: Physicochemical Properties and It's Correlation

D Kristanti[1] [1], *W* Setiaboma[1], *A C* Iwansyah[1], *A* Pangestu[1], *S N* Permadi[1], *A* Ningrum[2], *and E L* Puspitaningtyas[3]

[1]Research Center for Food Technology and Processing, National Research and Innovation Agency, Yogyakarta, Indonesia, 55861
[2] Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia, 55281
[3] Department of Food Science and Technology, Faculty of Agricultural, Universitas Jenderal Soedirman, Central Java, Indonesia, 53123

Abstract. Sorghum is a local commodity that has the potential as an alternative ingredient to replace the use of wheat flour in food products. Food security can be accomplished by replacing wheat with sorghum, which is supported by sustainable agriculture. Germination is one of the economical and environmentally friendly modification techniques, it affects the physicochemical properties of seed flour as well as increase the lightness, water and oil holding capacity (WHC/OHC), swelling, and solubility. The goal of the study is to ascertain how germination affects the physicochemical characteristics of sorghum flour and to use principal component analysis (PCA) and hierarchical clustering analysis (HCA) to assess the link between each parameter and its cluster. The germination had an impact on the moisture content, ash, redness (a*), yellowness (b*), viscosity, final viscosity, holding strength, breakdown, and setback of germinated sorghum, but it also increased lightness (L*) and solubility. In white sorghum germination, WHC, OHC, and swelling capacity were increased; but these did not significantly different in red sorghum germination. The protein, fat, breakdown, redness value (a*), peak viscosity, pasting temperature, and peak time were all positively connected, according to PCA, while OHC and lightness (L*) were negatively correlated. Keywords : Sorghum flour, Germination, Physicochemical properties, Principal component analysis (PCA), Food security

1 Introduction

Food security is carried out to realize food supply, ensure good health and nutrition for the community, as well as to encourage healthy economic growth, so this is an important issue in Indonesia. According to data from the official statistics agency [1], Indonesia imported

[1] Corresponding author: dita.kristanti@gmail.com

approximately 11 million tons of wheat in 2021. Replacing wheat with sorghum can accomplish the food security, which is supported by sustainable agriculture. The President of Indonesia encourages sorghum production through the sorghum roadmap (2022-2024) in order to reduce wheat imports [2]. To boost sorghum consumption, efforts must be made to diversify food products in addition to expanding production.

Sorghum (Sorghum bicolor L.) is popular scalpels besides wheat, maize, or rice in Asian and Africa that is mostly growth in semiarid (tropical and subtropical) region because of its tolerance less water [3]. Types of sorghum that are widely cultivated in Indonesia are white and red sorghum. White sorghum contains 11.20-12.50% moisture, 6.14-11.84% protein, 2.23-3.15% fat, 0.76-1.61% ash, 65.56-73.17% carbohydrate, and 5.37-8.35% total dietary fiber, while red sorghum contains 11.08-11.92% moisture, 6.85-11.94% protein, 2.00-3.10% fat, 0.88-1.83% ash, 63.32-71.32% carbohydrate, and 6.46-9.94% total dietary fiber [4]–[6]. This cultivar be highly consumed due to it nutrients. Sorghum is an Indonesian commodity that has the potential as an alternative ingredient to replace the use of wheat flour in food products. Sorghum have been applied as alternative food for wheat allergen, is popular in product non-gluten free. Applied of sorghum in food product is easily process as a flour that it should be done modified or unmodified.

Modifications through both physical and chemical treatments can be made to improve the nutrition and physical capabilities of a flour. Germination is one of the chemical modifications by utilizing natural enzymes contained in a seed, which is natural and environmentally friendly. Germination does not use harmful chemicals and does not cause residues that can damage the environment. Several studies related to germination in cereals have been conducted on rice [7], brown rice [8], [9], oats [9], millet [10], buckwheat [10], [11], and sorghum [12]–[16].

Germination causes physical and chemical changes in cereal flour. Germination increases protein content, antioxidant capacity (FRAP, DPPH, ORAC), WAC and OAC, foaming capacity, and foaming stability in rice flour [7], millet, and buckwheat [10]. However, germination causes a decrease in color profile (L, a, b), and profile gelatinization in rice [7], and brown rice [8]. In sorghum, germination increases WAC, OAC, swelling, and solubility [12], [14], [15] but reduce protein content, fat, ash, peak viscosity, trough, holding capacity, peak time, breakdown, setback, and pasting temperature [12], [13], [17].

There has been investigation on how germination affects the physical and chemical properties of sorghum flour, however there is limit information on the correlation of physicochemical parameters in germinated sorghum flour. Thus, the goal of this study is to ascertain the impact of germination on the physicochemical characteristics of sorghum flour, this study will also investigate the association between each parameter and its cluster using PCA and HCA. The physicochemical characteristics of sorghum flour analyzed include proximate content (moisture, ash, fat, protein, carbohydrate), color properties (L*, a*, b*), functional properties (WHC, OHC, swelling, solubility), and gelatinization properties.

2 Methods

2.1 Materials

A smallholding in Bantul, Yogyakarta, Indonesia (-7.810860° S, 110.356310° E) provided the sorghum (Sorghum bicolor). Samples used in this research were red and white sorghum seeds. Each sample soaked in water to obtain good seeds so that they could germinate. The floated seeds were discarded, while the submerged seeds were collected.

2.2 Sorghum germination process

Samples were put in a plastic container and submerged 1:2 (w/v) in distilled water for a full day. After soaked process, the sample while rinse in the running water for 1 minute. The germination took place for 2 days in the dark at room temperature. Samples (RS: red sorghum; WS: white sorghum; GRS: germinated red sorghum; GWS: germinated white sorghum) were dehydrated in a dehydrator at 50°C for 3 hours. Sorghum grain samples that had germinated and hadn't both crushed into powder using a blender (HR2116) and sorted on a 60 Mesh screen. Until they were used for analysis, the samples were kept at -18°C in a plastic bag.

2.3 Analysis of germinated sorghum flour

The proximate content examined in this study includes moisture, ash, fat, protein, and carbohydrate. The parameters of chemical qualities were determined using a proximate analysis, which included the gravimetric method [18] for analyzing the moisture and ash content, the KjelDigester K-446 (Buchi, Switzerland) and KjelMaster K-375 (Buchi, Switzerland) for determining the protein content, and the Soxhlet method using fat extractor E500 (Buchi, Switzerland) for analyzing the fat content.

The color properties including lightness (L*), redness (a*), and yellowness values were analyzed in this study. The Spectrophotometer CM-5 (Konica Minolta, Japan) was used to measure the color characteristics of the samples according to the CIE technique. Each time a sample was changed, the sample was placed on a cuvette and cleaned with a dry tissue. Each treatment replication underwent the duplicate analysis.

The WHC, OHC, swelling, and solubility of the sample were its functional characteristics. All of analysis refer to Elkhalifa et. al. [15] with some modification. For 30 minutes, samples were centrifuged at 3000 g. The WHC, OHC, swelling, and solubility were expressed in units of g/g.

Using the Modular Compack Rheometer MCR 302 (Anton Paar, German), the sample's gelatinization characteristics were evaluated. Sample (1.3 g, wet basis) was put in to Rheometer canister and 15 mL of distilled water. The following instrument settings were used to measure the gelatinization properties: 50 °C for 50 s, 95 °C for 450 s, 95 °C for 450 s, 50 °C for 450 s, and 50 °C for 120 s. There were measurements made for peak viscosity (cP), peak time (min), pasting temperature (°C), holding strength (cP), breakdown (cP), final viscosity (cP), setback from peak (cP), and setback from trough (cP).

The FTIR spectra of all samples were assessed by FTIR Spectrometer Vertex 80 (Bruker instrument, Billerica, MA-USA). The measurement was performed in region spectra from 400 to 4000 cm-1 using Zn-Se detector. A sample was measured out and clamped with a sharp tip onto a diamond crystal sampling plate. Every five samples, the background scan was obtained using an empty sample plate, and the reading was taken twice for each sample.

2.4 Statistical analysis

Using IBM Statistic 20 for statistical ANOVA with Duncan's means test at a significant difference of 95%, data for all determinations were evaluated. The information was provided as mean and standard deviation. Using R-statistical software (an open-source platform), dendrogram heatmaps were used to illustrate the interrelationship patterns between parameters and treatment clusters, and PCA and HCA were used to assess the connection between parameters measured from the treatments. Prior to analysis, the data were standardized in the 0–1 range.

3 Result and discussion

3.1 Proximate content of germinated sorghum flour

The proximate content of germinated sorghum flour is displayed in Table 1. While white sorghum's protein and fat concentrations were lower than those of red sorghum, it had higher moisture and ash values. According to this finding, which is consistent with earlier studies, red sorghum has a higher protein content than white sorghum. This result in line with the previously studies, red sorghum had a higher protein content than white sorghum [4]–[6]. The germination affected the decrease of moisture, ash, and fat (but not significantly different). The protein of both red and white sorghum increased by germination process even though not significantly different. According to Phattanakulkaewmorie et. al. [12] sorghum germination increased moisture and ash levels while without significantly changing the amount of protein. The same trends also occur in the germinated rice flour [7] and buckwheat [11], the germination process causes the increase of protein and decrease of fat. The ash, crude fat, crude protein, and crude fiber of sorghum were found to reduce lead by germination [13].

Table 1. Proximate content of germinated sorghum flour

Parameter	Sample			
	RS	WS	GRS	GWS
Moisture (%)	19.08±1.30^{b}	22.74±0.82^{a}	14.72±0.11^{c}	18.37±0.66^{b}
Ash (%db)	1.22±0.02^{b}	1.28±0.02^{a}	1.15±0.01^{c}	1.21±0.01^{b}
Fat (%db)	3.08±0.02^{a}	1.86±0.04^{b}	2.94±0.24^{a}	1.78±0.05^{b}
Protein (%db)	8.26±0.08^{a}	5.51±0.11^{b}	8.15±0.26^{a}	5.58±0.13^{b}
Carbohydrate (%db)	68.08±1.52^{b}	68.6±20.8^{b}	72.78±0.40^{a}	72.83±0.57^{a}

The formula for values is mean standard deviation. Means with distinct letters differed significantly from one another at $p < 0.05$. RS = red sorghum; WS= white sorghum; GRS = germinated red sorghum, GWS = germinated white sorghum.

3.2 The color properties of germinated sorghum flour

Table 2 shows the germinated sorghum flour's color characteristics. Sorghum flour became whiter as a result of germination, which also decreased the redness (a*) and yellowness (b*) of the grain. It has been noted that germination increases the pea's lightness (L*), decreases the common bucket and pea's redness (a*), and also reduces the millet, common bucket, and millet's yellowness [8]. Germination decreased the redness (a*) and yellowness (b*) of rice flour, according to Enyinnaya et. al. [7]. The metabolic changes that occur during germination are what cause the reduction in redness and yellowness values [7]. Color changes in germinated sorghum flour may be caused by an increase in the activity of oxidative enzymes such as polyphenol oxidase and peroxidase, which are triggered during germination [19].

Table 2. Characteristics of the sorghum flour's color

Parameter	Sample			
	RS	**WS**	**GRS**	**GWS**
Lightness (L*)	68.67±0.35[d]	74.90±0.18[b]	72.01±1.03[c]	76.69±0.49[a]
Redness (a*)	7.17±0.56[a]	4.13±0.10[c]	5.42±0.27[b]	2.18±0.15[d]
Yellowness (b*)	12.68±0.46[a]	13.23±0.18[a]	9.00±0.22[b]	9.50±0.25[b]
The formula for values is mean standard deviation. Means with distinct letters differed significantly from one another at $p < 0.05$. RS = red sorghum; WS= white sorghum; GRS = germination red sorghum, GWS = germination white sorghum.				

3.3 The functional properties of germinated sorghum flour

Germination affected the increase of WHC, OHC, swelling, and solubility of white sorghum (Table 3). An increase of WHC, OHC, swelling, and solubility has been reported in sorghum flour [12], [14], [15]. An incease of WAC and OAC that affected by germination could be associated to change in quality of protein, increase in the availability of amino acids, and break down in polysaccharide molecules [15]. This leads to increase of the interaction side with water or oil so the WHC and OHC also increase. Higher levels of protein, fat, and lipids, as well as a significant amount of amylose-lipid complex, can prevent the swelling of starch granules [12] which have an effect on solubility. The sorghum flours had a relative higher protein, and fat than the germinated sorghum flour (Table 1), so the swelling and solubility of germinated sorghum flour higher than non-germinated.

3.4 The gelatinization properties of germinated sorghum flour

The characteristics of germinated sorghum flour's gelatinization are shown in Figure 1. The germination process decreased the peak viscosity, holding strength, breakdown, final viscosity, and setback. These findings are consistent with research by Phattankulkaewmorie et. al. [12] and Singh et. al. [16], which found that germinated sorghum flour had lower peak viscosities, holding strengths, breakdown, final viscosities, and setbacks than native sorghum flour. As a result of the activation of starch hydrolyzing enzymes during germination, which causes starch breakdown, the reduction of peak viscosity, holding strength, breakdown, final viscosity, and setback occurs. When making frozen food products, flour with a low setback value is helpful [16].

Table 3. Functional properties of germinated sorghum flour

Parameter	Sample			
	RS	**WS**	**GRS**	**GWS**
WHC (g/g)	2.08±0.04[b]	1.80±0.08[c]	2.17±0.01[b]	2.39±0.06[a]
OHC (g/g)	1.91±0.05[bc]	1.93±0.08[b]	1.82±0.06[c]	2.16±0.01[a]
Swelling (g/g)	8.66±0.33[a]	7.46±0.25[b]	7.63±0.08[b]	8.77±0.16[a]
Solubility (g/g)	6.34±0.62[b]	3.79±0.13[c]	16.32±1.64[a]	15.91±0.52[a]

The formula for values is mean standard deviation. Means with distinct letters differed significantly from one another at $p < 0.05$. RS = red sorghum; WS= white sorghum; GRS = germinated red sorghum, GWS = germinated white sorghum.

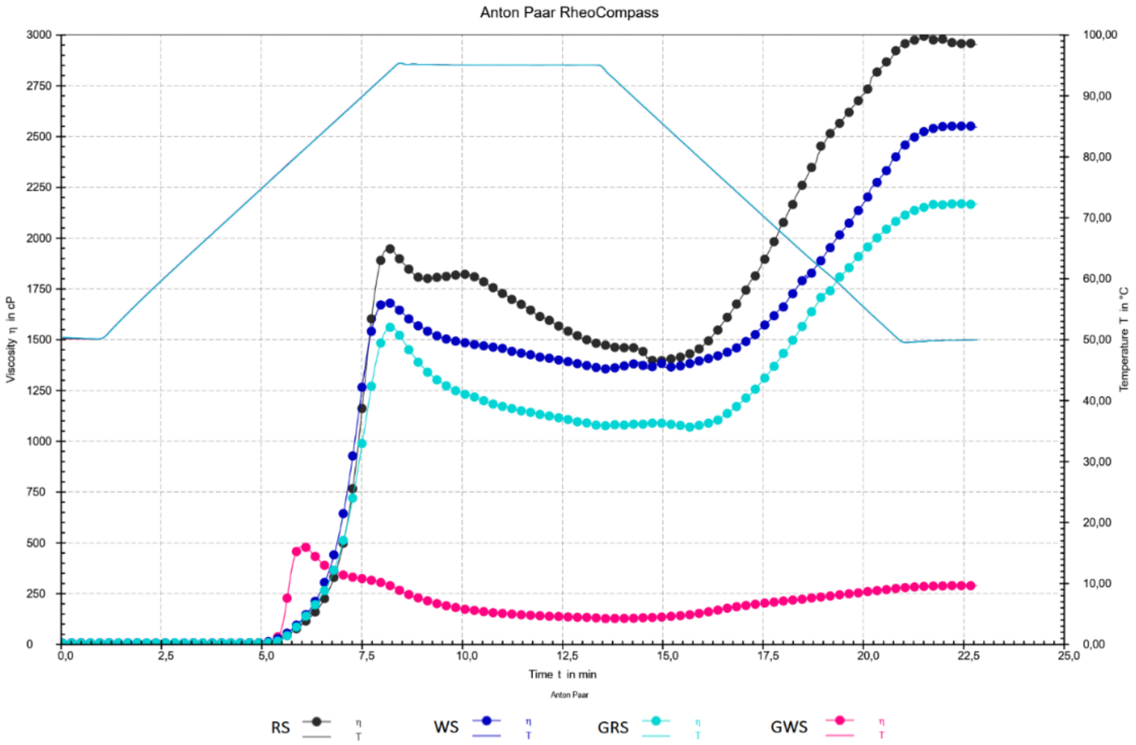

Fig. 1. Gelatinization properties of germinated sorghum flour.

3.5 The FTIR spectra of sorghum germination flour

The FTIR spectra of sorghum germination flour shown in Fig. 1. The strong absorbance of sorghum was at 3257, 2916, 2319, and 1700-600 cm^{-1}[20]. Specific spectra of peak at wave numbers of 3500-3100 cm^{-1} represented of presence hydrogen bond (OH) [21]. The intensity of its peaks at 3500-3300 cm^{-1} was decreased in GRS and GWS than RS and WS so that it may show decreased the hydrogen bond during germination. Presence of wave numbers of 3200-3000 was N-H amide, and it was in line with previous [20], however this wave numbers was not change. On the hand, the peaks numbers 2900-2700 cm^{-1} indicated of asymmetric C-H stretching. Region 1700-1680 cm^{-1} indicated C=O stretching, and the RS and WS was higher intensity of its peak compared to GRS and GWS. Lin et. al.[22] and Wahyono et. al. [21] stated that range 1745-1652 cm^{-1} was C=O stretching referenced to amide I band of peptide group of protein. The germination reduced the C=O stretching band and indicated that the aromatic bond like tannin was loss. This result was in line with Kaur and Lin et. al. [23][24], during the germination β-helix and α-helix turned position due to reduce the C=O stretching band.

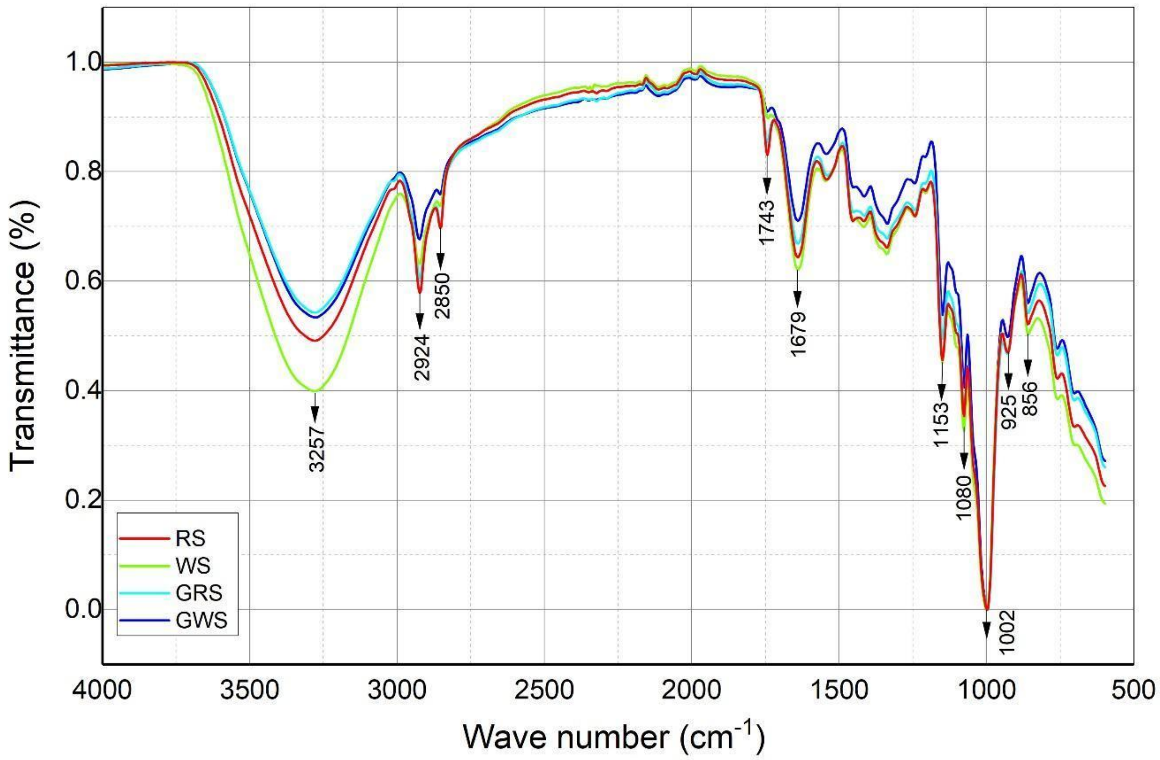

Fig. 2. FTIR of germinated sorghum flour.

3.6 The principal component analysis (PCA) and hierarchical clustering analysis (HCA)

Figure 3 shows the results of the main component analysis performed on the physicochemical properties of germinated sorghum flour. A loading plot of principal component 1 (PC1) and principal component 2 (PC2) can be used to describe the correlation between dependent parameters that have been observed. PC1 (58.86%) and PC2 (31.36%) together supplied 90.22 percent of the total PC variance. The same trend was shown by the protein, fat, breakdown, redness value (a*), pasting temperature, peak time, and peak viscosity. It suggests that there was a positive link between these characteristics. They had a bad correlation with OHC and L*, the lightness value. Peak time, peak viscosity, peak temperature, solubility, WHC, carbohydrate, swelling, and setback from peak all had only weak correlations or no correlation at all with the following parameters: protein, fat, breakdown, redness value (a*), pasting temperature, and peak time.

The interaction between the physical and chemical properties of the germinated sorghum flour is shown in Figure 4, along with variable clustering and treatment based on parameter similarity. Red sorghum (RS) and germinated red sorghum (GRS) flours were grouped together because they had the highest levels of protein, fat, redness value (a*), and gelatinization properties (pasting temperature, peak time, holding strength, breakdown, set back from trough, final viscosity, breakdown, peak temperature). White sorghum (WS) and germinated white sorghum (GWS) have a trend to be conversely correlation in some parameters. Germinated white sorghum having the highest of swelling, peak time, setback from peak, OHC, WHC, carbohydrate, and solubility, it was conversely with the white sorghum.

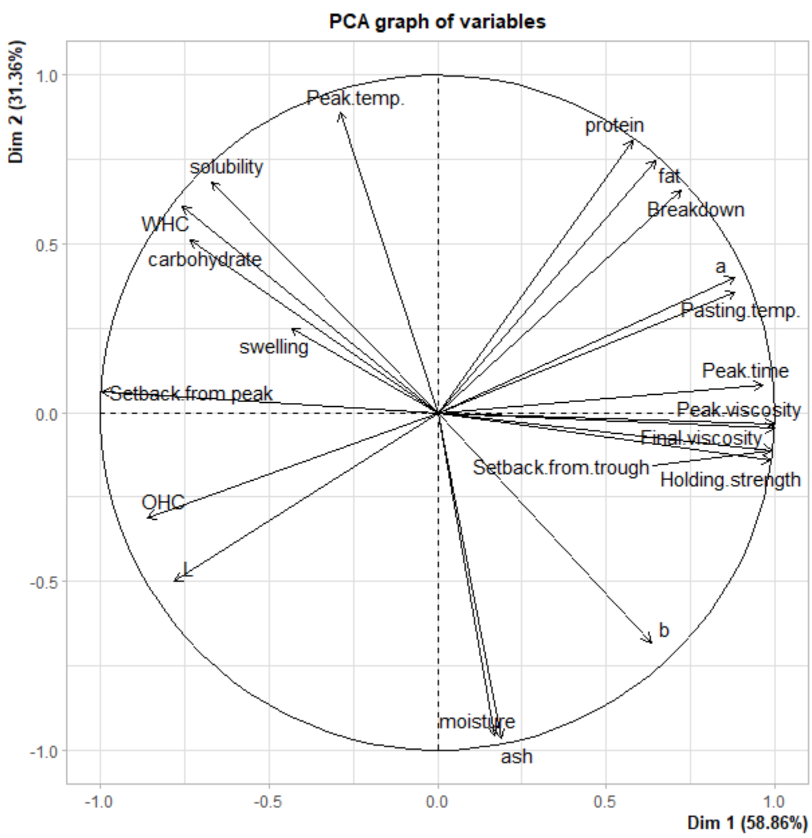

Fig. 3. Biplot derived from PCA of variables containing the physicochemical properties of sorghum flour that has been germinated

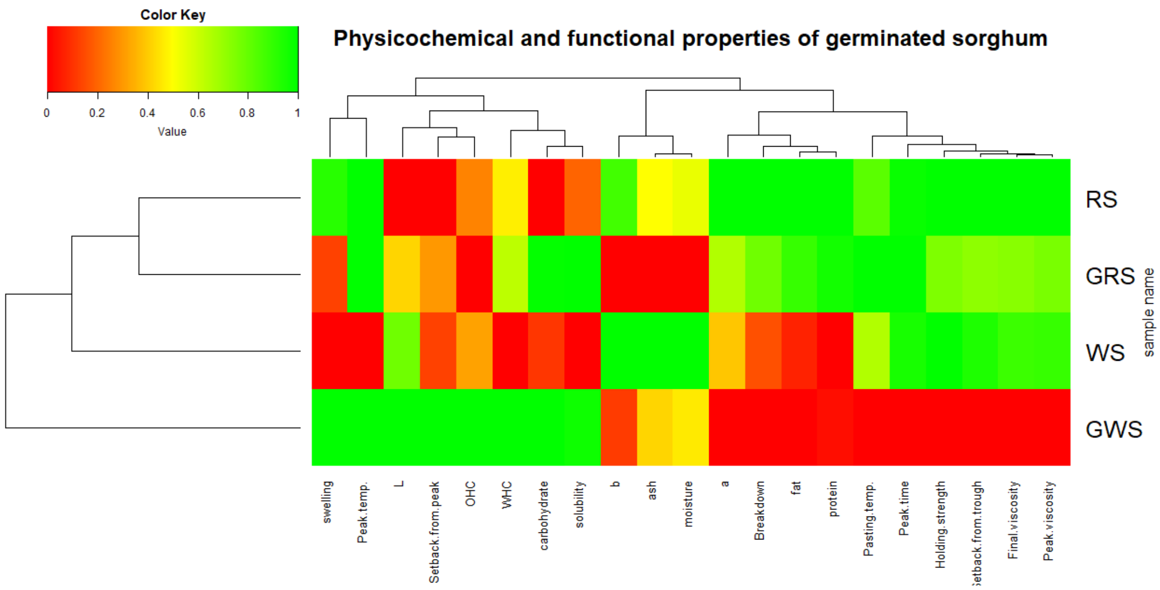

Fig. 4. Heat map of the relationships between treatments and response variables of germinated sorghum flour, as well as the clustering of these variables. The color represents the direction of the treatment impact, ranging from the lowest (red) to the highest (green).

4 Conclusions

Increased lightness (L*) and solubility of the germinated sorghum were results of the germination. Germination decreased the amount of moisture, ash, redness (a*), yellowness (b*), viscosity, holding strength, breakdown, final viscosity, and setback. WHC, OHC, and swelling capacity were all increased during the germination of white sorghum, whereas these factors were not substantially different during the germination of red sorghum. The protein, fat, breakdown, redness value (a*), pasting temperature, peak time, and peak viscosity were all positively connected, according to PCA, while OHC and lightness (L*) were negatively correlated. Germinated sorghum flour can be used in food products, it helps to reduce the consumption of wheat flour while also boosting food security.

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References

[1] B. P. Statistik RI, "Impor Biji Gandum dan Meslin Menurut Negara Asal Utama 2017-2020," [Online]. Available: https://www.bps.go.id/statictable/2019/02/14/2016/impor-biji-gandum-dan-meslin-menurut-negara-asal-utama-2017-2020.html. [Accessed: Jul. 13, 2023].
[2] E. Sutrisno, "Menjadikan Sorgum sebagai Alternatif Gandum?" [Online]. Available: https://indonesia.go.id/kategori/editorial/5603/menjadikan-sorgum-sebagai-alternatif-gandum?lang=1. [Accessed: Jul. 13, 2023].
[3] Y. Xiong, P. Zhang, R. D. Warner, and Z. Fang, "Comprehensive Review of Food Science and Food Safety," vol. 18, pp. 2025-2046, 2019.
[4] O. B. Eburuche, R. N. Attaugwu, H. E. Ufondu, and P. O. Uvere, "Journal of Food Science and Technology," vol. 56, pp. 3513-3523, 2019.
[5] A. Batariuc, M. Ungureanu-Iuga, and S. Mironeasa, "Applied Sciences," vol. 11, pp. 1-17, 2021.
[6] P. Pontieri et al., "Foods," vol. 11, pp. 1-11, 2022.
[7] C. E. Chinma, J. C. Anuonye, O. C. Simon, R. O. Ohiare, and N. Danbaba, "Food Chemistry," vol. 185, pp. 454-458, 2015.
[8] F. Wu, H. Chen, N. Yang, J. Wang, X. Duan, Z. Jin, and X. J. Xu, "Journal of Cereal Science," vol. 58, pp. 263-271, 2013.
[9] C. Li, S. G. Oh, D. H. Lee, H. W. Baik, and H. J. Chung, "International Journal of Biological Macromolecules," vol. 105, pp. 931-939, 2017.
[10] Q. Yang et al., "Innovative Food Science & Emerging Technologies," vol. 67, pp. 1-11, 2021.
[11] G. Zhang, Z. Xu, Y. Gao, X. Huang, Y. Zou, and T. Yang, "Journal of Food Science," vol. 80, pp. H1111-H1119, 2015.
[12] N. Phattanakulkaewmorie, T. Paseephol, and A. Moongngarm, "World Academy of Science, Engineering and Technology," vol. 81, pp. 454-460, 2011.
[13] A. Singh, S. Sharma, and B. Singh, "Journal of Cereal Science," vol. 76, pp. 131-139, 2017.
[14] O. B. Ocheme, O. E. Adedeji, G. Lawal, and U. M. Zakari, "Journal of Food Research," vol. 4, pp. 159-165, 2015.
[15] A. E. O. Elkhalifa and R. Bernhardt, "Food Chemistry," vol. 121, pp. 387-392, 2010.

[16] A. Singh, S. Sharma, B. Singh, and G. Kaur, "Journal of Food Science and Technology," vol. 56, pp. 3077-3089, 2019.
[17] R. Yang, P. Wang, M. F. Elbaloula, and Z. Gu, "Bioscience Journal," pp. 378-383, 2016.
[18] "Official Methods of Analysis of The Association of Official Analytical Chemist, 14th ed," AOAC Inc, Airilington, 1995.
[19] C. Li, D. Jeong, J. H. Lee, and H. J. Chung, "Food Science and Biotechnology," vol. 29, pp. 1223-1231, 2020.
[20] V. J. Sharanagat, R. Suhag, P. Anand, G. Deswal, R. Kumar, A. Chaudhary, L. Singh, O. S. Kushwah, S. Mani, Y. Kumar, and P. K. Nema, "Journal of Cereal Science," vol. 85, pp. 111-119, 2019.
[21] T. Wahyono et al., "IOP Conference Series: Materials Science and Engineering," vol. 546, pp. 1-7, 2019.
[22] S. Hainil, S. Suhaera, and L. Lirtri, "Borneo Journal of Pharmacy," vol. 2, pp. 82-86, 2019.
[23] H. Lin, S. R. Bean, M. Tilley, K. H. S. Peiris, and D. Brabec, "Food Analytical Methods," vol. 14, pp. 268-279, 2021.
[24] H. Kaur and B. S. Gill, "Journal of Food Science and Technology," vol. 58, pp. 2313-2324, 2021.