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The study of germination and soaking time to improve nutritional quality of sorghum seed

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Abstract

Sorghum is rarely used as a raw material in the food industry as it contained anti-nutritive compounds, which lead to some negative effects in the human digestive system. Germination is a common practice in sorghum production. The results obtained in this study shows that the time of soaking and germination improves the nutritional value of sorghum. Soaking for 24 and germination for 36 h produced sorghum with higher nutritional values having characteristics such as protein digestibility (85.18%), non-protein nitrogen (0.28%), protein content (8.03%), fat content (1.64%), fiber (1.45%) and ash (2.24%). The results of this study indicated that soaking time and germination improved the nutritional properties of sorghum and this may leads to designing new foods using germinated sorghum.

Introduction

Sorghum is a plant that is very economical to grow in dry areas with a high yield (Anglani, 1998). Sorghum contains adequate nutritional values with 83% carbohydrate, 10% protein and 3.5% fat, however, sorghum also contains antinutrition compounds such as tannin and phytic acid which affects the human digestive system (Suarni, 2004; Elefatio *et al*., 2005).

Soaking is one of the method used to improve the nutritional value of sorghum as raw material in the manufacturing of food products. During soaking the process of fermentation also occurs simultaneously on the starch-containing involving several species of bacteria, which includes *Lactobacillus plantarum*, *Candida crusei* and *Lactobacillus delbruecki* (Ohenhen and Ikenbomeh, 2007). Soaking also leads to the breakdown several components into simpler compounds which alter the texture, flavor, aroma and taste (Parveen, 2003). Another process that can improve the nutrition of sorghum seed is by germination as it can helps in reducing starch component, induces hydrolytic enzymes synthesis such as phytate reduction and some flavonoid components. Hence, germination of sorghum is very important in preparing for the development of food with low viscosity and high energy (Dicko *et al*., 2006). Sprouting seeds contains high protein, low unsaturated fatty acids, low carbohydrate and vitamin compared with the ungerminated seeds. Mineral content such as phospour, kalium, zinc and copper were higher in sprouts as the hydrolysis of phytic acid by the phytic enzyme activated during germination.

There is very little information on the study on soaking and germination time of sorghum to improve its nutritional values for application as raw materials in food manufacturing. The objective of this study was to determine the combination of soaking and germination time of sorghum seeds to improve its nutritional values.

Materials and Methods

Sample preparation

Matured grains (≥ 60 days after anthesis) of a local variety of sorghum (*S. bicolor* (L.) Moench) were harvested from Pasuruan, Jawa Timur. The grains were sorted by removing broken kernals and other unwanted materials and were immediately washed in water and sun-dried. Peeled fresh sorghum seeds were then soaked for 24, 48, and 72 hours and were allow to germinate for 12, 24, and 36 hours by spreading the seeds on moist burlap and was covered with another moist burlap. The sorghum sprouts obtained were then washed thoroughly to reduce the

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sour taste.

Determination of protein digestibility

Protein digestibility of the sorghum samples were determined by the AOAC (1984) method. Sample (20 mg) was dissolved in 10 mL 0.1 N Walpole buffer (pH 2.0) containing 2% pepsin enzyme, incubated for 5 h at 370C in a shaking waterbath and was centrifuged at 3000 rpm for 20 minutes. 5 ml of the supernatant were transferred to a new tube and were neutralized by adding 5 mL of 20% TCA and incubated at ambient room for 15 h. The mixture was filtered using Whatman paper and the protein in the filtrate were analyzed by Kjedhal method. Protein digestibility was calculated as followed:

$$\% \text{ protein digestibility: } \frac{\text{mg N X 6,25X100\%}}{\text{mg sample x \% protein}}$$

Determination of non-protein nitrogen

Determination of non-protein nitrogen in sorghum were carried out according to the methods of AOAC (1984). Sample (2 g) was weighed and transferred into the Kjedhal containing 50 mL of aquadest and boiling stone and boiled for 30 minutes. 2 mL of alumunium sulfate were added to the mixture and upon reaching boiling temperature, 50 mL of copper sulfate was added and the solution was allowed to cool down, filtered and the filtrate was subjected to the Kjedhal method for the determination of protein content.

Proximate analysis of sorghum

Proximate analysis for ash, protein, crude fiber and fat were carried out by the methods of AOAC (2000).

Statistical analysis

All statistical analyses were carried out using Microsoft Excel 2003. Analysis of variance (ANOVA) followed by Duncan Multiple Range Test at a level of $P<0.01$ if there was significant differences between samples. The best treatment was determined by effectivity index method as described by Susrini (2005).

Results and Discussions

Protein digestibility

Table 1 shows the trend of the protein digestibility ranging between 84.50 to 88.77%, occurring within the soaking time between 24 to 72 h, whereas germination begins at 12 h to 36 h. The lowest digestibility of sorghum protein was 84.50% at 24 h of soaking time and 12 h of germination time, whilst the highest was 88.77% at 72 h of soaking time and 36 h of germination time. Antinutrient such as tannin can affect growth, digestibility and nutritional efficiency, in which sorghum tannins can reduce the digestibility and the efficiency of nutrient use by about 3-15%. Kulamarva (2005) suggested that the presence of tannins in seeds and other factors have a major impact on the digestibility and amino acid availability.

Table 1. The average of proximate analysis of soaking and germination processed of sorghum seeds

Time of soaking processing	Time of germination processing	Protein digestibility (%)	Non-protein nitrogen (%)	Crude protein content (%)	Crude fat content (%)	Crude fiber content (%)	Ash content (%)
24	12	84.50	0.27	5.36	2.32	1.68	1.95
	24	84.89	0.27	6.50	2.02	1.58	2.05
	36	85.18	0.28	8.03	1.64	1.45	2.24
48	12	85.37	0.24	4.45	1.51	1.37	1.41
	24	86.11	0.25	4.66	1.36	1.25	1.42
	36	87.00	0.26	5.19	1.23	1.16	1.57
72	12	87.67	0.22	2.70	1.11	1.03	1.18
	24	88.33	0.23	2.97	1.02	0.94	1.20
	36	88.77	0.24	3.73	0.94	0.87	1.32

Means ± standard deviation in the same row with dfferent accompanied letters are significantly different ($P \leq 0.01$)

Dewar (1998) noted that digestibility of the material can be improved by germination, due to the possibility of seed structure changes and protein hydrolysis enzyme becomes active. A similar research was reported by Idris *et al*. (2005), who suggested that the germination was more effective in reducing phytic acid than the reduction of tannins as phytic acid can be degraded by enzymes.

The combination of soaking and germination can increase the protein digestibility due to the presence of reserve protein catabolism in seed and a decrease in antinutrient compounds (Laetitia *et al*., 2005). Dicko *et al*. (2006) also noted that the improving of digestibility was the process which provides essential nutrients for growth through the hydrolysis reaction.

Non-protein nitrogen

An increase of non-protein nitrogen of sorghum occurs at soaking time of 24 h and germination begin at 12 to 36 h and this corresponded with the formation of nucleic acid during germination process.

Chen and Tracker (1978) noted that an increase in non-protein nitrogen during germination of bean seeds was followed by an increase in the number of amino acids and nucleic acids formation during proteolytic enzyme activity in germinating seeds as a result of increased protease enzymes activity or nucleic acids due to an increased of metabolic activity. Therefore, an increased in the protease enzyme activity in germinating seeds will increase the soluble compounds N through the hydrolysis of stored protein.

Protein content

The results obtained in this study showed that an increase of protein content of sorghum occurs at soaking time of 24 h and germination occurred at 12 to 36 h. Suhaidi (2003) suggested that soaking lead changes in the biology of the breakdown of the various components into simpler compounds. However, during germination, protease enzyme increases and is involved in the degradation of peptide component to amino acids and the amount of protein will increase. Inyang and Zakari (2008) also noted that germination may increase the protein content. In cereals and legumes, this increase is due to the presence of protein hydrolysis as well as the results of protease enzyme activity during germination the seeds.

Laetitia *et al*. (2005) suggested that protease enzymes break down the peptide bonds into proteins and produces amino acids. An increasing number of proteins have occurred during germination as the presence of synthesis processes and reduced of dry matter in the process of soaking. Nzelibe and Nwasike (1995) also noted that protease activities during germination of "acha" will increase protein content, for example, the increase in the protein content during seed germination of corn will increase the mobilization of nitrogen fixing and thus improving the quality of proteins used for the development of young plants.

Fat content

The range of fat content in the sorghum seeds analyzed was 2.32-0.94% (Table 1). As the soaking and germination time increases, the fat content decreased. The lowest fat content of sorghum was 0.94% found at soaking time of 72 h and germination time of 36 h, and the highest fat content was 2.32% found at soaking time of 24 h and germination time of 12 h. The soaking process can decrease the fat content of sorghum grain due to absorption of water after the enzyme is activated and then into the endosperm and digest food reserve substance. Lipase enzymes break down fats into glycerin and fatty acids and since these compounds are water soluble, they can diffuse into the cells tissue. As noted by Inyang and Zakari (2008), during germination the seed, the decreasing in the amount of fat is due to the increased activity of lipolytic enzymes during germination, which hydrolyzed the fats into fatty acid and glycerol. Whilst Kiranawati (2002) noted that fatty acids is reduced as a result of reforms in the cell. Glycerol dissolved in water and transported by the krebs cycle to metabolism in cells whereas fatty acids also dissolved in water.

Lipase activity increased during germination and the proportion of lipid bodies during germination will decrease due to the synthesis of lipase. The lipase activity increased during germination possibly by the synthesis of the aleurone and scutellum (Uvere and Orji, 2002). Germination process enhance the hydrolysis of complex organic compounds which are insoluble in the seeds and form more simple organic compounds that are water soluble. In addition, fat will be degraded to produce energy and will be used for respiration (Sukamto, 1992).

Fiber content

With regards to the fiber content of the sorghum seeds, the lowest was 0.87% found at soaking time of 72 h and germination time of 36 h and the highest of fiber content in sorghum was 1.68% found at soaking time of 24 h and germination time of 12 h. Soaking process could decreased fiber content as sorghum contained soluble and insoluble fiber in water. Therefore, the longer soaking process may reduce water-soluble fiber content of sorghum seed namely β-glucan.

The increase in the activity of β-glucanase enzyme can reduced the fiber content of sorghum. Dicko *et al*. (2006) suggested that germination may increased activity of β-amylase enzyme which hydrolyze cell wall of carbohydrates during germination. While the study of Munck (1991) reported that during the first stage of barley seed germination process, β-glucanase enzymes degrades the endosperm cell walls and α amylase degrades starch.

Ash content

The lowest ash content of sorghum was 1.18% found at soaking time of 72 h and germination time of 12 h and the highest was 2.24% found at soaking time of 24 h and germination time of 36 h. This is in agreement to the findings by Lalude and Fashakin (2006) who reported that the mineral content of sorghum seed was about 2.39%. Hurell and Reddy (2003) noted that phytic acid found in cereals would lead to the low absorption of iron. Whilst Sukamto (2003) suggested that soaking will dissolve some nutrients such as vitamins and minerals, therefore the longer the soaking, the lower the mineral content of a material

Inyang and Zakari (2008) reported that germination and fermentation would increase the mineral content due to an increase in fitase enzyme activity during germination. The enzyme will hydrolyze the bond between the protein-enzyme minerals become free, therefore increasing the availability of minerals.

The best treatment according to proximate analysis using effectivity index method (Susrini, 2005) was soaking time of 2 h and germination time of 36 h. It had the following properties: protein digestibility 85.18%, non-protein nitrogen was 0.28%, protein content was 8.03%, fat content was 1.64%, fiber was 1.45% and ash content was 2.24%.

Conclusion

The soaking treatment can decrease several nutritional value of sorghum as most compounds were soluble in water, however by the germination treatment the nutritional values increases due to degradation process of carbohydrates, proteins and fats. Treatment with the combination of soaking for 24 h and germination for 36 h increases the nutritional value of sorghum.

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