

Germination Effect on Functional Properties and Antitrypsin Activities of Pigeon Pea (Cajanus cajan (L.) Millsp.) Sprout Flour

Ni Wayan Wisaniyasa[1] I Ketut Suter[1] Yustinus Marsono[2] IN. Kencana Putra[1]
1. Department of Food Science and Technology, Udayana University, Bukit Jimbaran, Badung, Bali, Indonesia
2. Department of Food and Agricultural Product Technology, Gadjah Mada University, Yogyakarta, Indonesia

Abstract
Germination is a series of changes in morphology, physiology and biochemistry. These changes are likely to affect the nutritional content, anti-nutritional and functional properties of sprout flour produced. The purpose of this study was to determine the effect the length of germination on the functional properties of the antitrypsin activity of pigeon pea sprout flour. After being soaked at 50 °C during the first 6 hours, then at room temperature in the next 30 hours, pigeon pea seeds were germinated at room temperature with germination time of 24; 36, 48; 60 and 72 hours respectively. The resulting sprouts were dried at 50 °C, then powdered. The results showed that the germination process increased water absorption capacity and the oil absorption 15.73% and 14.80% respectively. The longer the time of germination, the higher the water absorption capacity and the oil absorption were. The process of germination decreased pigeon pea flour swelling volume from 5.3% to 4.2% in pigeon pea sprouts flour, but increased the solubility of 13.3% (pigeon pea flour) to 19.0% (pigeon pea sprouts flour) and speeding wettability from 377 seconds (pigeon pea flour) to 188 seconds (pigeon pea sprouts flour). The longer the time of germination, the activity of trypsin inhibitor decreases. At the 72-hour germination, anti-trypsin activity decline reached 46%, from 573.090 UTI / g sample becoming 308.827 UTI /g sample.
Keywords: germination, functional properties, antitrypsin, pigeon pea

1. Introduction

Pigeon pea beans contain complete nutrients that are not inferior to soy. The contents of nutrients of pigeon pea in 100 g is energy 336 cal., protein 20.7 g, fat 1.4 g, carbohydrates 62.0 g, 125 mg calcium, phosphorus 275 mg, 4.0 mg iron, vitamin A 19 RE and vitamin B1 0.48 mg (Odeny, 2007). Until now, most of the pigeon pea has been used as a vegetable. Like other legumes, pigeon pea can also be processed into tempeh, concentrate, isolate protein and pigeon pea flour (Odeny, 2007). Among the processed products, pigeon pea flour has relatively good prospects.

One of the obstacles of the use of bean flour is the presence of anti-nutritional substances such as anti-trypsin which may decrease the bioavailability of nutrients in it. One of the efforts to overcome this is to give pretreatment before flouring to eliminate anti-nutritional substances, namely by means of germination (Sharma and Shegal, 1992; Savelkoul *et al*., 1994; Adawy *et al*., 2003; Ramakrishna *et al*., 2006; Valdez-Anguino, 2015)

Germination has been known as a process that is not expensive and is technologically-effective in improving the nutritional quality of beans (Steve, 2012 and Valdez-Anguino, 2015). Duenas *et al*. (2009) proved that germination is able to increase the nutrient content, digestibility and availability of free amino acids, dietary fiber and bioactive components of lupine seeds (*Lupinus angustifolius* L.). Adawy *et al*., (2003) found that the germination green beans, peas and lentils proven to increase mineral content of N, K, Ca, P, Mg, Fe and Mn ore. Germination can cause changes in the functional properties (Nowzu *et al*., 2010), chemical (El-Moneim *et al*., 2012) as well as the content of anti-nutritional substances such as anti-trypsin (Savelkoul *et al.,* 1994). No research has been done on the long period effects of germination on the functional nature and content of the flour antitrypsin of the pigeon pea sprouts produced. Therefore, this research is very necessary. The purpose of this study was to determine the effect of long germination time on the functional properties (water absorption capacity, oil absorption capacity, swelling volume, solubility and wettability) and anti-trypsin activity of the pigeon pea sprout flour produced.

2. Materials and Methods

Materials used in this study were harvest dried pigeon pea in Kubu Village, Kubu District, Karangasem Regency, Bali. Materials for chemical analysis used were N-α-benzoyl-L-arginine-4 nitroanilide hydrochloride (BAPNA), NaOH, a solution of acetic acid, trypsin solution (Tripzin-1x-S from porcine pancreas, Sigma-Aldrich), BAPNA solution. The tools used included UV / Vis spectrophotometer to analyze the levels of anti-trypsin.

This research used completely randomized design, with a long treatment time of germination, namely 24, 36, 48, 60 and 72 hours with three replications. The research was preceded by the making of pigeon pea sprouts. The procedure of making pigeon pea sprouts was done in the following manner: pigeon pea was sorted and then soaked at 50 °C in the first 6 hours, then at room temperature for the next 30 hours. Replacement of water was done every 6 ours, then drained, and germinated in a leaf-base plastic basket container. The length of germination time was done according to treatment. Pigeon pea amount in each treatment was 150 g with a thickness of 1 cm at room temperature. Each treatment was evenly sprinkled with water of 10 ml every 12 hours. Having obtained the pigeon pea sprouts, then dried in an oven at 50 °C for 8 hours, then ground and sifted using a sieve of 60 meshes, in order to obtain flour pigeon pea sprouts. Three repetitions of the experiment were done. The observed parameters were the functional properties and anti-trypsin activity of pigeon pea flour sprouts. Analysis of functional properties include water absorption capacity by gravimetric method (Fernandez-Lopez *et al*., 2009), oil absorption capacity by gravimetric method (Fernandez-Lopez *et al*., 2009), swelling volume with volumetric method (Collado & Corke, 1999), solubility with gravimetric method (Collado & Corke, 1999), and wettability with hydration methods (Bhandari, 2000). Analysis of antitrypsin activity was made by means of enzymatic methods (Kakade *et al*., 1974 within Pazlopez, 2012). Data were analyzed using SPSS, when there was an effect of treatment; the analysis was continued with Duncant test (Steel and Torrie, 1995).

3. Results and Discussion

The influence of the length of time of germination on water absorption capacity, oil absorption capacity, swelling volume, solubility and wettability of pigeon pea flour sprouts can be seen in Table 1.

Table 1. The water absorption capacity, oil absorption capacity, swelling volume, solubility and wettability of pigeon pea sprouts flour at various lengths of time of germination

Length of germination (hours)	Water absorption capacity (% db)	Oil absorption capacity (% db)	*Swelling volume (% db)*	Solubility (% db)	*Wettability* (second*)*
0	216.65 ± 1.64 d	173.96 ± 1.64 d	5.3 ± 0.10 a	13.32 ± 0.91 b	377 ± 13 a
24	224.32 ± 2.73c	181.27 ± 2.87c	5.10 ± 0.10a	13.73 ±1.22 b	326 ±8b
36	237.71 ± 1.09b	182.71 ±2.26 c	4.77 ± 0.06 b	14.34 ± 1.98 b	275 ± 10c
48	248.56 ± 1.09 a	184.16 ± 2.73c	4.73 ± 0.12 b	16.95 ± 1.62ba	214 ±2b
60	250.01 ±1.66 a	192.48 ± 2.73b	4.57 ± 0.06 b	18.08 ± 1.39 a	204 ± 4b
72	250.73 ± 1.09a	199.72 ± 3.91a	4.23 ± 0.21 c	19.05 ± 1.04a	188 ± 2a

Note: the same letters behind the average value in the same column indicate no significant difference ($P < 0.01$)

3.1. Water Absorption Capacity

Table 1 shows that the germination process can increase water absorption capacity of pigeon pea sprouts flour. The smallest water absorption capacity was 224.32% (db) obtained from the pigeon pea sprouts flour with germination time of 24 hours long, while the largest water absorption capacity was 250.73% (db) obtained from pigeon pea sprouts flour with long germination period of 72 hours but not significantly different from pigeon pea sprouts flour with germination time of 48 hours and 60 hours. Increased water absorption capacity was probably due to the fact that the process of germination can increase levels of dietary fiber from wheat flour germinated. This is supported also by the results of research on the chemical composition of this research, that is, the levels of dietary fiber flour pigeon pea sprouts germinated for 48 hours was 18.46% (db), while those not germinated 16.85% (db). One property of dietary fiber is the ability to bind water (Marsono, 1995). Increased water absorption capacity may also be due to increased levels of protein and quality of protein so that absorption capabilities and increased interaction with water (Chauhan and Sing, 2013). This study also found that pigeon pea flour protein content was 23.12%, while the protein content flour pigeon pea sprouts with germination time of 48 hours was 25.32%. Previous researcher (Adedeji *et al*., 2014) reported that germination increases water absorption capacity of cornstarch. The same was found in sprouts amaranth flour (Chauhan and Sing, 2013) and tigernut sprouts flour (Chinma *et al*., 2009).

3.2. Oil Absorption Capacity

Germination was also able to increase the oil absorption capacity of pigeon pea flour bean sprouts. The smallest oil absorption capacity was 181.27% (db) obtained from the flour pigeon pea sprouts with germination time of 24 hours, but it was not statistically significantly different from germination time of 36 and 48 hours. Largest oil absorption capacity was 199.72% (db) obtained from pigeon pea sprouts flour with the germination period of 72 hours. Increased oil absorption capacity of pigeon pea sprouts flour can be caused by a decrease in fat content. This study found that the germination for 48 hours was able to lower the fat content of 4.9%. Likely due to decreased levels of fat because fat is used as an energy source for the growth of sprouts. So the longer the germination, the more oil can be tied so that the oil absorption capacity is increasing. Similar results were reported by Adedeji *et al*. (2014) who found that the germination increase oil absorption capacity of cornstarch. A similar case was reported by Chinma *et al* (2009) who proves that the germination can increase the absorption capacity of the tigernut flour oil.

3.3. Swelling volume

Germination reduces the ability swelling volume of pigeon pea sprouts flour. The smallest swelling volume 4.23% (db) was obtained from pigeon pea sprouts flour with germination period of 72 hours; while the largest swelling volume was 5.10 (db) obtained from the pigeon pea sprouts flour with germination time of 24 hours. The decreased capability of flour swelling volume was possibly due to the fact that, during germination, starch hydrolysis became simpler compounds. The bond of α 1-4 in a starch was damaged by amylase and subsequently by α-glycosidase. This reaction caused the starch to change into simpler sugar (Egwim and Ademonom, 2009). The decreased level of starch possibly caused swelling volume to decrease. Adedeji *et al*. (2014) also found the same thing, that is, germination decreased the ability of cornstarch swelling power. In the non-germinated corn flour, swelling power capability was 19.81 mg/g, while in the flour of the corn germinated for 24, 48 and 72 hours, the swelling power became 19.1, 18.8, and 15.0 mg / g respectively. Elhkalifa and Bernhard (2013) also reported that germination of sorghum flour caused swelling power to decrease. Swelling power of sorghum which was not germinated was 6.3 g / g, while sorghum which was germinated for 24, 48 and 72 hours was 5.5, 5.2 and 4.9 g/g respectively.

3.4. Solubility

Germination increases the solubility of pigeon pea sprout flour. The smallest solubility of 13.73 % (db) was obtained from pigeon pea sprout flour with germination of 24 hours but not significantly different from the germination of 36 hours, while the largest solubility of 19.05% (db) was obtained from bean sprouts pigeon pea flour with germination period of 72 hours, but not significantly different from germination of 48 and 60 hours. Increased solubility of starch as a result of germination is likely due to the fact that during the germination process there was hydrolysis from complex compounds into simpler compounds that are more water soluble and increased the workability of several enzymes such as amylase and protease enzymes. Enzymes involved in the hydrolysis of food reserves were α-amylase, β-amylase and protease. According to Phattanakulkaewnorie *et al*. (2011), increased amylase enzyme activity was due to germination causing more and more complex molecules such as carbohydrates break down into sugar molecules that are simpler and thus easier to dissolve.

Results of this study were supported by the research of Hussein *et al*., 2013, which proves that the germination can improve the solubility of the starch of mung bean sprouts. Other studies have also proved that the solubility of the germinated sorghum flour increased from 14-50%, in line with the increase in temperature of 55-95 ^{0}C, whereas the solubility of non-germinated sorghum flour was lower that is, the increase of 10-24% was in line with increases in temperature 55-95 ^{0}C (Phattanakulkemorie *et al*., 2011). Research by Atify *et al*. (2012) also proved that the germination can increase the protein solubility of sorghum flour.

3.5. Wettability

Table 1 shows that the germination is capable of lowering wettability of pigeon pea flour sprouts. Wettability is the time required by the flour to absorb water or the time required by the flour to wet. The fastest wettability was obtained from flour 188.00 seconds pigeon pea sprouts with the germination period of 72 hours, while the longest was 326.00 seconds wettability obtained from flour pigeon pea sprouts with germination time of 24 hours. This is supported by research of Nwosu *et al.* (2010), which examines the effect of the germination on wettability of the asparagus seed flour. They found that the seeds of asparagus which have not been germinated had wettability of 189 minutes, while those germinated for 72 hours had the wettability of 79 minutes. The other research by Nwosu (2010), namely on seed germination of Oze also proved that the germination lowered wettability of Ozu flour. The study proves that the wettability of Ozu flour seed not germinated was 5 minutes, while Ozu seeds germinated for 24 hours were 4 minutes. The reduced wettability of flour was likely caused by germination which was able to increase hydrolysis of complex compounds into simpler compounds. The formation of compounds which were simpler due to germination can increase the number of compounds that are hydrophilic so that the time required to be wet was increasingly short.

3.6. Trypsin inhibitor activity

Effect of long germination time on the activity of trypsin inhibitor can be seen in Table 2. Pigeon pea beans that have not been germinated had trypsin inhibitor activity of 573.090 UTI / g sample. From Table 2 it appears that the longer the time of germination, the activity of trypsin inhibitor decreases.

Table 2. Activities of trypsin inhibitor of pigeon pea flour sprouts (UTI)

Length of germination (hour)	Activity of trypsin inhibitor (UTI)/g sample	Decrease of anti-trypsin activity after germination (%)
0	573.090 ± 37.903	0
24	406.923± 32.835a	29±3.08a
36	376.742± 13.400ab	34±2. 38 ab
48	354.042 ± 8.785b	38±4.88 b
60	348.754 ± 1.516b	39±4.14 b
72	308.827 ± 24.216c	46 ±2.27 c

Note: The same letters behind the average value in the same column indicate no significant difference (P <0.01)

Antitrypsin is a protein compound that acts as anti-nutrients, that is, it has the ability to inhibit the activity of trypsin enzymes in the digestive tract. The mechanism of inhibition of trypsin enzyme activity by antitrypsin occurs due to the formation of complex bonds between the two substances. In this study, a decrease in antitrypsin activity as a result of germination was possible because during germination process hydrolysis reaction took place. In the process, there was also a proteolysis by a more active endogenous protease enzyme. The presence of endogenous protease enzyme can hydrolyze antitrypsin during the development of sprouts to be used as a source of sulphur amino acids (Valdez-Anguino *et al*., 2015). This is in line with the research results of Shegal and Sharma (1992), which proved that *faba* seed germination for 2 days can lower the antitrypsin activity by 65%. Frias *et al*. (1995) proved that the lentil seed germination for 72 hours was able to lower antitrypsin activity by 10%. Seed germination of green beans, peas and lentils for 72 hours was able to significantly decrease the antitrypsin activity, but if germination extended to 120 hours, antitrypsin activity actually increased (Adawy *et al*., 2003). Other studies have reported that the germination of Indian bean (Delicos lablab) for 32 hours was able to lower antitrypsin activity by 17% (Ramakhrisna *et al*., 2006). Malomo *et al*., (2012) reported that soybean germination for 72 hours was able to lower flour antitrypsin activity of 29.82%.

4. Conclusion

Germination process increased water absorption capacity, oil absorption capacity and solubility of flour pigeon pea sprouts. On the other hand, the process of germination decreased swelling volume, value wettability and anti-trypsin activity of pigeon pea flour sprouts. The percentage decrease in antitrypsin activity on the germination of 24-72 hours ranged from 29 to 46%.

Acknowledgments

The author would like to thank the Rector of Udayana University for financial support given to me during my studies at the Doctoral Study Program of Agricultural Sciences, Concentration of Agricultural Technoloy, Udayana University, Bali.

References

Adawy, TAE., Rahma E.H., Bedawey, A.A.E. & Beltagy, A.E.E. (2003), "Nutritional Potential and Functional Properties of Germinated Mung Bean, Pea and Lentil Seeds", *Journal Plant Foods for Human Nutrition*, 58 (3) :1-13.

Adedeji, OE., Oyinloye, O.D. & Ocheme, O.B. (2014), "Effects of Germination Time on The Functional Properties of Maize Flour and the Degree of Gelatinization of Its Cookies", *African Journal of Food Science*, 8(1): 42-47.

Afify, A.E.M., El-Beltagi H.S., El-Salam, S..MA. & Omran, A.A. (2012), "Solubility, Digestibility and Fractionation after Germination of Sorghum Varieties", Available:journals.plos.org/plosone/article?id=10.1371/journal.pone.0031154.

Bhandari, B. (2000), "Understanding Food: Principles and Preparation. Wadsworth Thomson Learning. USA", Available :www: Fst.edu.av/staff/bbhandari/teaching/brown.Amy.

Chauhan, A., & Singh, S. (2013), "Influence of Germination on Physico–chemical Properties of Amaranth (*Amaranthus Spp.*) Flour", International Journal of Agriculture and Food Science Technology, ISSN 2249-3050, 4 (3) : 215-220.

Chinma, C.E., Adewuyi, O. & Abu, J.O. (2009), "Effect of Germination on the Chemical, Functional and Pasting Properties of Flour from Brown and Yellow Varieties of Tigernut (*Cyperus esculentus*)", Food Research International, 42.p.1004–1009.

Collado, L.S., & Corke, H. (1999), "Heat Moisture Treatment Effect on Sweet Potato Starches Differing in Amylosa Content", *Journal of Food Chemistry,* 65: 339-346.

Dueñas, M., Hernández, T., Estrella, I., & Fernández, D. (2009), "Germination as a Process to Increase the Polyphenol Content and Antioxidant Activity of Lupin Seeds (Lupinus angustifolius L.)", *Journal of Food Chemistry*, 117(4) : 599-607.

Egwim E. & Ademonom. (2009), "Predicting α-amylase Yield and Malt Quality of Some Sprouting Cereals using 2nd Order Polynomial Model", African Journal Biochemistry, 3 (8): 288-292.

El-Moneim A., Afify, M.R. El-Beltagi, H.S., Samiha, M.A., El-Salam & Omran, A.A. (2012), "Protein Solubility, Digestibility and Fractionation after Germination of Sorghum Varieties", Research Article, Editor:Vladimir N. Uversky, University of South Florida College of Medicine, United States of America, PLoS ONE 7(2): e31154. doi:10.1371/journal.pone.0031154.

Fernandez-Lopez, Sendra-Natal J., Navarro, E., Sayas, C., Martos, E.V. & Perez-Alvarez. (2009), "Storage Stability of a High Dietary Fibre Powder From Orange by-Products", *International Journal Food Science and Technology*, 44:748-756.

Frias J., Diaz-Pollan, C., Hedley, C.L. & Vidal-Valvedley, C. (1995), "Evolution of Trypsin Inhibitor Activity During Germination of Lentils", *Journal Agricultural Food Chemistry*., 43(8): 2231–2234

Hussain, M.S., Anjum, Uddin, M.B., and Ali, S. (2013), "Optimization of Germination Effect on Functional Properties of Mungbean Flour By Response Surface Methodology", *Pakistan Journal of Science*, 65 (2) :214-218.

Malomo O., Ogunmoyela, O.A., Oluwajoba, B.S.O., & Kukoyi, I. (2012), "Effect of Germinated Soy Flour on The Sensory Acceptability of Soy-Wheat Composite Bread", *Asian Journal of Natural and Applied Sciences.*

Marsono, Y. (1995), "Fermentation of Dietary Fibre in the Human Large Intestine: A review", *Indonesian Food and Nutrition Progress,* 2: 48-53.

Nwosu, J.N. (2010), "The Effects of Processing on the Functional Properties of 'Oze' (*Bosqueia angolensis*) Seeds", *Pakistan Journal of Nutrition*, Asian Network for Scientific Information, ISSN 1680-5194 9 (8): 781-786, 2010

Nwosu, J.N., Ogueke, C.C., Owuamanam, C.I. & Iwouno, J.O. (2010), "Functional Properties and Proximate Composition of Asparagus Bean (Vigna Sesquipedalis) as Influenced by Malting", *Journal of American Science*, 6(9), p 376-82

Odeny, D.A. (2007), "The potential of Pigeonpea (*Cajanus cajan* (L.) Millsp.) in Africa", Natural Resources Forum. Blackwell Publishing Ltd 31. 297–305 .

Pazlopez, C.M. (2012), "Common Beans Cooked at High Altitudes Have Higher Trypsin Inhibitor Activity and Lower Protein Digestibility Than Beans Cookies at Sea Level", Presented to The College of Agriculture and Life Science, Physical Sciences, Cornel University, America.

Phattanakulkaewnorie, Paseephol, T., & Moongngarm, A. (2011), "Chemical Compositions and Physico-chemical Properties of Malted Sorghum Flour and Characteristics of Gluten Free Bread", Word Academy of Science, Engineering and Technology, Vol.5.

Ramakrishna, Rani, J.& Rao, P.R. (2006), "Anti-Nutritional Factors During Germination in Indian Bean (*Dolichos lablab L.*) Seeds", *World Journal of Dairy & Food Sciences*, © IDOSI Publications1 (1): 06-11.ISSN 1817-308X.

Savelkoul, F.H., Tamminga, S., Leenaars,P.P., Schering, J. & Maaat, D.W.T. (1994), "The Degradation of Lectins, Phaseolin and Trypsin Inhibitors During Germination of White Kidney Beans (*Phaseolus vulgaris* L)', *Plant Foods Hum Nutr.,* 45(3), p. 213-22.

Sharma & Shegal. (1992), "Effect of Domestic Processing, Cooking and Germination on the Trypsin Inhibitor Activity and Tannin Content of Faba bean (*Vicia faba*)", Plant Foods Human Nutrition, 42(2):127-33.

Steve, I.O. (2012), "Influence of Germination and Fermentation on Chemical Composition, Protein Quality and Physical Properties of Wheat Flour (*Triticum aestivum*)", *Journal of Cereals and Oil Seeds*, 3(3): 35-47

Valdez-Anguino, D.M., Herrera-Cabrera,E., Davila-Ortiz, G., OOMAH, B.D., Cardador-Martinez, A., & Jimenez-Martinez.C. (2015), "Content and distribution of Nutritional and Non-Nutritional Compounds During Germination of Three Mexican Faba Bean (*Vicia faba*)", *International Journal of Research In Agriculture and Food Sciences*,Vol. 2, No.9 ISSN 2311 -2476.