SOME CHANGES ON ENZYMIC ACTIVITIES, COMPOSITIONAL AND ANTINUTRITIONAL FACTORS OF AN INDONESIAN COWPEA (*VIGNA UNGUICULATA* (L) WALP) CULTIVAR UNDERGOES GERMINATION AND A MILD HEATING PROCESS

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ABSTRACT

Changes during 48 hours germination at room temperature followed by a mild heating process on the seeds of an Indonesian cowpea cultivar were evaluated. The results indicated that the activity of amylases and proteases increases until 36 hours of germination and then decreases. The levels antinutritional factors, namely trypsin inhibitor, phytic acid, haemagglutinins were reduced somewhat, while the protein content and the digestibility value increase. Germination for 36 hours followed by mild heating produces germinated seeds (10% moisture) with 26.27% protein, 9.63% reducing sugars, and relative enzymic activity levels of 37.63 (unit/mL) for amylase, and 78.81 (μmole tyrosine/minute/mg) for proteases. The levels of phytic acid, haemagglutinins and trypsin inhibitors were reduced to about 50%, 40 % and 70% of their original levels respectively. The in vitro protein digestibility value is 74.79%.

Key words : cowpea, germination, changes, antinutritional factors

Introduction

Legume seeds have played in a signicant role as protein source in the human diet of the peoples in Africa, Asia and Latin America, and currently being a focus of research in many parts of the world. In the Orient, legume seeds may be consumed in the forms of fermented products as well as unfermented ones. Intensive studies have been carried out on soybean, the most popular legume, however an increase attention has emerged to lesser legume seeds such as cowpea (*Vigna unguiculata* (L) Walp).

It is known that cowpea is easily adapted to various soil regimes and climatic conditions (Karsono, 1998). It may be cultivated in less fertile types of soils, and is also tolerant to drought (Adisarwanto, Riwanodja and Suhartina, 1998). Cowpea is mainly composed of carbohydrates, protein and at smaller of fat. Maia *et al.* (2000) recently reported that cowpea contains 67.8-76.1 % db (dry basis) carbohydrates, 19.5-26.1 % db protein and only about 1.2-3.6 % db fat. Like other legume seeds, cowpea also contains antinutritional factors such as trypsin inhibitors (TI), haemagglutinin, phytic acid (Oluwatosin, 1999; Maia *et al.*, 2000). The report of Oluwatosin (1999) indicated that the levels of antinutritional factors in cowpea depends on genotypes and environmental conditions. TI content is mostly affected by genotypes, while tannin, phytic acid and haemagglutinin levels are majorly governed by environment.

Research on the changes of antinutritional factors of legume seeds during germination has been carried out by many investigators, but mostly conducted on soybean seeds (Vasconcelos *et al*., 1997; Bau *et al*., 1995) and an excellent review on this matter was prepared by Bau, Villaume and Mejean (2000), also a textbook presented by Liu (1997). Such changes were also observed in the germination of pigeon pea (Godbole, Krishna and Bhatia, 1994; Ambekar *et al*., 1996). However, very limited published data is found related to the status of antinutrional factors during germination of cowpea (*Vigna unguiculata* (L) Walp).

The current study is aimed at evaluating the changes of some components and antinutrional factors of an Indonesian cowpea, a cultivar KT-6 small bean undergoes 48 hr germination process an a mild heat treatment.

Materials and Methods

The seed-grade of cowpea KT-6 small bean cultivar, obtained from BALITKABI (Research Institute on Legumes and Tubers), Malang, harvested in 2001 were used in the experiment. The chemicals used are analytical grades unless stated otherwise. The seeds were cleaned, sorted, washed in running water and then soaked for 12 hr prior to germination.

The soaked seeds were then germinated on an aluminimum tray layered with three-pieces wetted tissue paper for 48 hr at room temperature. Periodically, the tissue paper was wetted with water to keep the paper moist. The lot of samples of the germinated seeds were taken at 12 hour intervals. The germinated seeds were then dried in vacuum oven at a temperature of about 40 ^oC for 12 hour and then ground into flour to pass a 100 mesh-sieve. The biochemical and compositional changes of the seeds during germination at 12 hr intervals followed by a mild heating treatment were evaluated. The parameters were the levels of protein (AOAC, 1990), trypsin inhibitor (Ambekar *et al*., 1990), phytic acid (Makower dan Wheeler dan Ferrel as described by Sudarmadji, Haryono and Suhardi, 1997), reducing sugars using specrophotometric method of Nelson-Somogyi (Sudarmadji, *et al*., 1997), in vitro disgestibility value (AOAC, 1990), proteases, amylases and lipase activities, and hemaglutinin (Muhtadi, 1989; Noor, 1992).

Results and Discussion

Yields, Amylases Activity and Reducing Sugars

It is obvious that the yield, the amount of flour obtained after the process, continually decreases along with germination (Table 1). It indicates that some drastical metabolism-induced changes do occur during germination of the cowpea seeds. Liu (1997) stated that germination involves vigorous metabolisms, driven by mobilization of the food materials stored in the seeds.

Table 1. The yields, relative amylase activity and the level of reducing sugars of the germinated and mild heated cowpea seeds

Germination time (hour)	Yields (g/50 g)*	Relative Amylase Activity (unit/mL)	Reducing Sugars (%)*
0	40.95 e	23.09 a	2.52 a
12	40.37 d	27.62 b	2.70 a
24	38.20 c	30.04 d	5.59 b
36	36.95 b	37.63 e	7.13 b
48	34.91 a	29.51 c	5.69 b
$LSD_{0.05}$	0.30	0.46	1.63
Ungerminated seeds	-	15.87	1.80

*) 10% moisture

The reduction in yields is concomitant with the increase of the activity of seed amylases as well as the reducing sugar content. The long chain carbohydrates seems to be the major source of energy for germination process of cowpea seeds. The conversion of such carbohydrates, mostly associated with the increase in the activity of amylases, produces free sugars. In turn, the sugars are further converted into energy and simpler compounds for germination. A similar result was reported by Kikunaga *et al.* (1991) on the germination of rice.

Soaking for 12 hr results in the swelling of the bean due to an abundant water absorption and as a consequence the amylases begins to active hydrolizing the starch, as indicated by the increase of reducing sugars. As the germination proceeds to 48 hr, however, the amylase activities are slightly reduced and that probably the main cause of the reduction of the reducing sugar level. The reduction in the activity of amylases may possibly be related to the presence of amylases inhibitors in the cowpea seed, as found in the germinated pigeon pea (Ambekar *et al.*, 1996). They observed that a proteinaceous inhibitor of amylases in pigeon pea was still present after 10 days of germination.

A continual formation of reducing sugars and smaller molecular weight compounds in the germination eventually brought about to the reduction of the total dry matter of the seeds. Therefore, the yield also continually decreases with germination. Zhuang and Xu (1989) in Liu (1997) also reported that the dry matter loss occur during germination of soybean seeds.

Protease and trypsin inhibitor activities, protein content and digestibility

Similar to that of amylases, the activity of proteases in the first 36 hr of cowpea seed germination process increases, and then decreases somewhat after 48 hr of germination. It is also applied to protein content. However, as the germination onsets the TI content was consistently reduced, thus allowing enzymic hydrolysis of protein to its composing amino acids. As a result, the digestibility of the protein is simultaneously improved (Table 2).

Reports by Godbole *et al.* (1994) and Ambekar *et al.* (1996) on the germination of pigeon pea also noted similar results. The activity of TI of the pigeon pea were reduced to about 50% of its initial activity after 5 days of germination (Ambekar *et al.*, 1996) and they predicted that a new TI may be synthesized during germination. The current study shows that despite the TI activity was reduced, the activity of proteases also decreased after 48 hr of germination.

Compared with the ungerminated cowpea seeds, the germinated ones contains higher level of protein. The results resemble the one that was reported by Zhuang and Xu (1989) in Liu (1997) on soybean seed germination. The increase in the relative protein content is most probably due to a concomitant decrease of the dry matter, which is mainly composed of carbohydrates. The protein was possibly reduced. However, its reduction is much less than that of the dry matter. Other possibilities are the amount of absolute protein does not significantly changes, and the breakdown products of protein is N-containing compounds, including amino acids.

The protein digestibility of the 36 hr-germinated seeds increased almost two-folds of the ungerminated ones. This suggests that nutritional improvement of cowpea seeds may be carried out simply by germinating them, and that is ideal in the making of formulated foods for children, eldery persons, as well as for those recovering from illness. In the Orient, legume sprouts are very common consumed as vegetables (Liu, 1997), and therefore cowpea sprouts is also recommended for consumption as it is nutritious.

Table 2. The protease and trypsin inhibitor activities, protein content and protein digestibility of the germinated and mild heated cowpea seeds

Germination time (hour)	Protease activity (µmol tyrosine/ min/mg)	Trypsin Inhibitor (TIU/100g)	Protein content (%)*	Protein digestibility value (%)**
0	61.87 b	31.5 c	21.30 a	60.44 a
12	67.26 c	29.75 c	22.18 a	69.88 b
24	68.08 c	24.75 ab	23.47 ab	71.48 c
36	78.82 d	21.25 a	26.26 c	74.79 e
48	33.88 a	17.00 a	24.98 c	73.55d
LSD $_{0.05}$	2.131	4.476	0.212	9.018
Ungerminated seeds	49.50	31.75	20.26	44.06

*) 10% moisture **) in vitro

Haemmaglutinin and phytic acid

The haemmaglutinin and phytic acid contents of cowpea seeds during germination is presented in Table 3. The results indicate that soaking for 12 hr slightly reduced the phytic acid content, but the haemmaglutinin content was relatively stable. The following germination process results in the reduction of both haemmaglutinin and phytic acid contents. After 36 hr of germination, the reduction of the values are 60% for haemmaglutinin and 50% for phytic acid.

Table 3. The levels of haemmaglutinin and phytic acid of the germinated and mild heated cowpea seeds

Germination time (hour)	Haemmaglutinin level *	Phytic acid (mg/g)
0	100 b	332.08 e
12	80 b	303.78 d
24	60 ab	284.91 c
36	40 a	166.99 b
48	40 a	152.84 a
LSD 5%	25.56	30.41
Ungerminated seeds	100	391.04

*) compared to non germinated seeds

Maia *et al.* (2000) observed the presence of haemmaglutinins in Brazilian cowpeas, while Oluwatosin (1999) reported that the levels of haemmaglutinin and phytic acid are largely dependant on the environment where the plant is grown. Bau *et al.* (1997) stated that in the germination of soybean seed, the level of haemmaglutinin decreased to various degrees depending on the germination conditions. It was also noted in the germination of cowpea seeds, indicating that proteinaceous haemmaglutinins were broken down, possibly due to the increase in the activity of various proteases, during germination.

Phytate-phosphorus accounts for a large proportion of the total phosphorus existing in grains (Kikunaga *et al.*, 1991). During germination such a compound is hydrolyzed by phytase (Ayet *et al.*, 1997) to produce inorganic phosphorus needed in the metabolism, and is therefore, the phytic acid content of the cowpea seeds decreased with germination. The reduction of phytic acid will improve the availability of minerals in foods (Beal, Finney and Mehta, 1984; Hurrell *et al.*, 1992; Davidsson *et al.*, 1994).

Conclussion

Based on the overall evaluation, it may be concluded that soaking for 12 hr and followed by germination for 36 hr and subsequently dried at 40 ^{o}C for 12 hr for cowpea seeds is quite enough to improve the nutritional quality of the seeds. By such treatment, the yield of the process (flour), is about 73% and producing maximum reductions in antinutritional factors. The flour of the germinated seeds is probably suitable for the development of nutritious formulated foods for children, as well as for the eldery persons and those recovering from illness, as its protein digestibility value is relatively high.

References

Adisarwanto, T., Riwanodja dan Suhartina. 1998. Budidaya tanaman kacang tunggak in Kasno, A. dan Winarto, A. 1998. Kacang Tunggak. Monograf BALITKABI No 3-1998. Badan Penelitian dan Pengembangan Pertanian. PUSLITBANG Tanaman Pangan. Balai Penelitian Tanaman Kacang-kacangan dan Umbi-umbian. Malang. pp : 73-83.

Ambekar, S.S., S.C. Patil, A.P. Giri and M.S. Kachole. 1996. Proteinaceous inhibitors of trypsin and of amylases in developing and germinating seeds of pigeon pea (*Cajanus cajan* (L) Millsp). J. Sci. Food Agric. 72 : 57-62.

AOAC. 1990. Official Methods of Analysis of The Association of Official Analytical Chemist. 25th Ed.. Publisher AOAC. Washington.

Ayet, G., Burbano, C. Cuadrado, C. Pedrosa, M.M. Robredo, L.M. Muzquiz, M. Cuadra, C.D. Castano, and A. Osagie. 1997. Effect of Germination, under Different Environmental Conditions, on Saponins, Phytic Acid and Tannins in Lentils (*Lens culinaris*). J. Sci. Food Agric. (74): 273-279.

Bau, H-M., Ch. Villaume and L. Mejean. 2000. Effects of soybean (*Glycine max*) germination on biologically active components, nutritional values of seeds, and biological characteristics in rats. A review. Nahrung/Food 44 (1) : 2-6.

Bau, H-M., Ch. Villaume, J-P. Nicolas and L. Mejean. 1995. Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soya bean (*Glycine max*) seeds. J. Sci. Fd. Agric. 73 (1) : 1-9.

Beal, L., P. Finney and T. Mehta. 1984. Effects of germination and dietary calcium on Zinc bioavailability from peas. J. Food Sci. 49 : 63-67.

Davidsson, L., P. Galan, P. Kastenmayer, F. Cherouvrier, M.A. Juillerat, S. Hercberg, R.F. Hurrell. 1994. Iron bioavailability studied in infants : the influence of phytic acid and ascorbic acid in infant formulas based on soy isolate. Pediatric Res. 36 (6) : 816-822.

Godbole, S.A., T.G. Krishna and C.R. Bhatia. 1994. Changes in protease inhibitory activity from pigeon pea (*Cajanus cajan* (L) Millsp) during seed development and germination. J. Sci. Food Agric. 66 : 497-501.

Hurrel, R.F., M.A. Juillerat, M.B. Reddy, S.R. Lynch, S.A. Dassenko and J.D. Cook, 1992. Soy protein, phytate, and iron absorption in humans. Amer. Journal Clinical Nutrition 56 (3) : 573-578.

Karsono, S. 1998. Ekologi dan daerah pengembangan kacang tunggak di Indonesia in Kasno, A. dan Winarto, A. 1998. Kacang Tunggak. Monograf BALITKABI No 3-1998. Badan Penelitian dan Pengembangan Pertanian. PUSLITBANG Tanaman Pangan. Balai Penelitian Tanaman Kacang-kacangan dan Umbi-umbian. Malang. pp : 59-72.

Kikunaga, S., Y. Katoh and M. Takahashi. 1991. Biochemical changes in phosphorus compounds and in the activity of phytase and α-amylase in the rice (*Oryza sativa*) grain during germination. J. Sci. Food. Agric. 56 (4) : 335-343.

Maia, F.M.M., J.T.A. Oliveira, M.R.T. Matos, R.A. Moreira and I.M. Vasconcelos. 2000. Proximate composition, amino acid content and haemagglutinating and trypsin-inhibiting activities of some Brazilian *Vigna unguiculata* (L) Walp cultivars. J. Sci. Food. Agric. 80 (4) : 453-458.

Muhtadi, D. 1989. Evaluasi nilai gizi pangan. IPB. Bogor.

Noor, Z. (1992) Senyawa Anti Gizi. Pusat Antar Universitas-Pangan Dan Gizi Universitas Gadjah Mada. Yogyakarta.

Oluwatosin, O.B. 1999. Genotype x environment influence on cowpea (*Vigna unguiculata* (L) Walp) antinutritional factors : 1-trypsin inhibitors, tannins, phytic acid and haemagglutinin. J. Sci. Fd. Agric. 79 (2) : 265-272.

Sudarmadji, S., B. Haryono, Suhardi. 1997. Prosedur analisa untuk bahan makanan dan pertanian. Liberty. Yogyakarta.

Vasconcelos, I.M., E.A. Siebra, A.A.B. Maia, R.A. Moreira, A.F. Neto, G.J.A. Campelo and J.T.A. Oliveira. 1997. Composition, toxic and antinutritional factors of newly developed cultivars of Brazilian soybean (*Glycine max*). J. Sci. Fd. Agric. 75 (4) : 419-426.