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The effect of Sprouting on the *in vitro* Digestibility of Maize and Cowpea

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Abstract: Despite the high protein content of cowpeas, their maximum contribution to nutrition has not been fully exploited in many parts of the world because of the following problems: the presence of anti-nutritional factors, such as trypsin inhibitor, which are common with legumes; flatulence factors; low level of sulphur amino acids, particularly methionine; and, in many instances, the inconvenience involved in their long preparation into local dishes. Moreover, there is the problem of the beany off-flavour. Grinding treatments that break most of the cells and release the cell contents of raw legumes prevent the subsequent development of the characteristic beany flavor on cooking. An off-flavour develops when ground raw legumes are suspended in water probably because of mixing of the cell contents enzyme lipoxygenase and could be controlled by adjusting the pH of the slurry towards the acid side.

Germination is widely claimed as a means of correcting nutrient deficiencies of particular seeds, especially through alterations in the amino acid balance of the proteins and enhancement of the content of vitamins. This wide belief is emphasized and investigated in this research. In maize, however, the various food enzymes exited during germination had already played vital roles in breaking down the higher molecular components to simple molecules especially protein, which eases the digestibility as depicted in this investigation.

Keywords: *In vitro* Digestibility, Sprouting, Germination, Protein.

1. Introduction

The practice of sprouting legumes like mung beans and soybean has long been known. It has recently been advocated as a means of improving the nutritional worth of many other seeds (Elwood, 1971; Whyte, 1973; Blanchard, 1975; Fordham *et al*., 1975; Muimui 2010; Wang *et al*., 2003). Legumes have been part of the human diet since the early ages of agriculture. Many legume species are still an irreplaceable source of dietary proteins for humans, especially in the mainly vegetarian diets of developing countries (Wang *et al*., 2003). However, researches have been mainly devoted to the dry seeds. Legume seeds provide an exceptionally varied nutrient profile, including proteins, fibres, vitamins and minerals (Mitchell *et al.,* 2009). Yasmin *et al.,* (2008) found a 48-hour germination period to be the optimum for soybeans if the grains are to be consumed after sprouting. On the other hand, Fordham (1975) found that 4-5 days sprouting period produced organoleptically acceptable peas and beans. However, 3 days were considered appropriate for lentils, mung beans and soybean by Kylen and McCready (1975). Germination is a natural process occurred during the growth period of seeds in which they meet the minimum condition for growth and development (Sangronis *et al*., 2006). Vandersoep (1981), comparing the proximate analysis of peas, pinto beans, navy beans, lentils, mung beans, soybeans and alfalfa seeds, before and after germination, found a marked increase in moisture content during germination. Protein, fat, fibre and surprisingly ash contents (on dry matter basis) decreased with increasing germination period. Several studies on the effect of

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germination on legumes also found that germination can increase protein content and dietary fibre; reduce tannin and phytic acid content and increase mineral bioavailability (Rao and Prabhavathi, 1982; Ghanem and Hussein, 1999; Ghavidel and Prakash, 2007). Germination also was reported to be associated with increase of vitamin concentrations and bioavailability of trace elements and minerals (El-Adawy *et al*., 2004). Kaushik *et al*., (2010) found that germination improves calcium, copper, manganese, zinc, riboflavin, niacin and ascorbic acid content. In cereal grains, germination increase oligosaccharides and amino acids concentration as observed in barley (Rimsten *et al*., 2003), wheat (Yang *et al*., 2001), oat (Mikola *et al*., 2001) and rice (Manna *et al*., 1995). Chen *et al*., (1977) discovered that the water content of 8 varieties of legumes studied increased rapidly up to the first day of germination and slowly afterward. Tabekhia and Mohammed (1971) also noticed a similar trend of water absorption. Hsu *et al*., (1980) found no significant change in the protein content of dry peas, lentils and faba beans after germination. Cunningham *et al*., (1978) observed a slight increase in both protein and oil content of the germinated cottonseed. This increase, they attributed to large dry weight loss observed during germination. This large loss in weight had been earlier observed by White (1958) who noticed a loss of over 12 percent after 6 days of germination. Fat content was decreased in all germinated samples with significant decrease found in germinated soybean, peanut, white, black, red and brown rice ($p < 0.05$). Megat *et al*., (2011), Similar results occurred in a study by Dhaliwal and Aggarwal (1999), El-Adawy *et al*., (2004), Ghavidel and Prakash (2007) and Hahm *et al.*, (2008) where the fat content decrease with an increase in the time of germination. This is because fat was used as the major source of carbon for seed growth (Bau *et al*., 1997). Hahm *et al.*, (2008) also suggested that fatty acids are oxidized to carbon dioxide and water to generate energy for germination. This was also confirmed by Megat *et al*., 2011, Subbulakshmi *et al*., (1976) observed a progressive increase in non-protein nitrogen with a corresponding decrease in the protein nitrogen in cowpea, horse bean and moth bean during germination. Kumar and Venkataraman (1975) also reported a progressive reduction in protein nitrogen of cowpea during germination. Protein content was significantly decreased in germinated legume and rice varieties ($p < 0.05$) Megat *et al*., (2011). Vellupillai *et al*., (2009), observed that the decreased in total protein content is simultaneous with increased in amino acid content caused by increased levels of protease activity. They found that the protein nitrogen decreased from 3.2 percent in ungerminated seed, to 2.4 percent after 72 hours of germination. With chickpea and green gram, similar trend of reduction was noticed with increasing germination period. A decrease from 3.4 percent in ungerminated to 2.8 percent at 72 hour germination was recorded with chickpea seed protein nitrogen, while green gram showed a reduction in protein nitrogen from 4.1 percent in ungerminated to 3.3 percent in 72 hours germinated seed. There are wide applications of germinated food products besides as ingredient in normal food preparation. Some of the identified uses of germinated legumes and cereals include flour (Pomeranz *et al*., 1977; Giami, 2004; Charoenthaikij *et al*., 2010), beverage (Tonella and Berry, 1987) and weaning food (Marero *et al*., 1988).

2. Materials and Methods

The method used in this study was based on that of Saunders *et al*., (1973), details of which are described below.

One gram of defatted sample was weighed into a 50ml screw-capped polypropylene centrifuge tube (du Pont) and suspended in 20ml of 0.1M HCI and mixed with freshly prepared 50mg of pepsin in 1ml HCI. The mixture was gently shaken at 37^0C for 48 hours and then centrifuged for 5 min. at 200,000g.

After removal of the supernatant, the solids were resuspended in 20ml of 0.1M sodium phosphate buffer, pH 8.0 (84ml 0.2M $Na_2HP0_4.12H_20$ plus 16ml 0.2M NaH_2P0_4 made up to 200ml with distilled water), and treated with fresh 5mg of trypsin (Type II, Sigma Chemical Co.). The mixture was gently shaken at 37^0C for 26 hours. The solids were finally separated by centrifuging at 20,000 x 5 min. and washed once with 30ml distilled water. The solids were collected on a Whatman No. 541 filter paper and analysed for nitrogen.

Calculation: The result was calculated as follows:

$$\text{Protein digestibility (\%)} = \frac{\text{N in sample} - \text{N undigested residue}}{\text{Nitrogen in sample}} \text{x } 100$$

2.1 Determination of the Calorific Value of Test Samples

The calorific values of the test samples were determined using a Gallenkamp ballistic bomb calorimeter (model CBB-330-01). The operating procedure of the instrument manual was used a reported below:

2.1.1 Sample Preparation

About 3-2g of corn sample and 0.5g in the case of cowpea were weighed into a silica crucible. The weight of the crucible and contents was noted. The sample was pressed gently after weighing to form it into a smooth and compact material suitable for combustion. The sample was reweighed to obtain an accurate weight. The sample was then dried at 50^0C for 16 hours. It was cooled after drying in a desiccator and then weighed. The drying was to provide a completely dry sample.

2.2.2 Operational Procedure

The dried sample was carefully placed in the calorimeter metal crucible. This was placed on the pillar of the bomb, a standard length of 5cm of sewing cotton was fitted with the firing wire and its free end was dipped into the sample. The bomb body was then fitted, the thermocouple inserted, and the bomb charged to a pressure of 25 atmospheres. The galvanometer zero was adjusted and the firing button was pressed afterward. About 5 unit increases in the readings of the pressure gauge and the galvanometer showed that the firing had been successful, and after about a second the peak reading was attained. This peak reading was recorded as the galvanometer deflection. The gas pressure was then released and the bomb body was removed and cooled in water, in preparation for the next test.

The observed deflection was corrected for the heat generated by the firing current and the combustion of the cotton thread. The correction, which was found to be constant from test to test, was determined by the above procedure, but without any sample in the crucible.

Calculation: The calculation of the calorific value of the test samples was carried out as follows:

a) Determination of Calibration Constant
Weight of benzoic acid = 0.456g,
Heat released from benzoic acid = 6.32 kcal/g,

Heat released from 0.456g benzoic acid
= 6.32 x 0.4456 kcal = 2.86 kcal,

Galvanometer deflection of thread without sample = 0.3,

Galvanometer deflection of thread + benzoic acid = 6.55,

$$\text{Calibration constant} = \frac{2.86}{(6.55 - 0.3)}$$

$$= 0.4608 \text{ Kcal / Deflection}$$

b) Heat released from Individual samples

$$\text{Heat released from sample} = \frac{a \times 0.4608}{b} = \text{Gross energy}$$

Where a = Deflection from galvanometer when the sample was bombed;
b = Weight of sample g;

3 Results

Changes in the *in vitro* protein digestibility of maize and cowpea during sprouting are shown in Tables 1 and 2, respectively.

Both raw and sprouted maize grains show high digestibility, but digestibility increase with the length of the sprouting period (correlation, r = + 0.95).

Digestibility of raw cowpea protein is low (65%), but was rapidly increased by sprouting (90% after 2 days of sprouting). Protein digestibility of cowpea was also found to be highly correlated with sprouting (r = + 0.877). However, losses in total dry matter of the sprouted grains were not considered in the digestibility calculation; when considered it alters the image of the digestibility especially for sprouted maize.

4. Discussion

The *in vitro* digestibility of maize, although high (84.5%), was further increased by germination in proportion to the length of the germination period (Table 1). The length of sprouting period and the proportional increase indigestibility was also highly correlated with the percentage availability of total lysine of sprouted maize (r = + 0.97).

As in maize, there was an increase in the *in vitro* digestibility of cowpea during germination (Table 2). The increase in the *in vitro* digestibility was found to be highly correlated with the germination period (r = + 0.88), and well correlated with the availability FDNB lysine (r = + 0.95).

The observed increase in the *in-vitro* digestibility of both maize and cowpea during germination and the high correlation between the *in-vitro* digestibility of both maize and cowpea and their available (FDNB) lysine is presumably a reflection of the fact that during germination of seeds, stored materials are converted into forms more readily usable by the growing plants (Whyte, 1973; Chen *et al*., 1975). A similar progressive increase in *in-vitro* digestibility was reported by Subbulakshmi *et al*., (1976) for sprouted horse gram and moth bean.

Table 1. Changes in *In vitro* Digestibility of Maize during Sprouting.

	GERMINATION PERIOD (Days)				STANDARD ERROR OF DIFFERENCE (SED)	VARIATION RATIO (F)
	0	2	3	5		
% Digestibility	84.445	89.873	92.203	93.413	0.025	6303.54

Table 2. Changes in *In vitro* Digestibility of Cowpea during Sprouting.

	GERMINATION PERIOD (Days)				STANDARD ERROR OF DIFFERENCE (SED)	VARIATION RATIO (F)
	0	2	3	5		
% Digestibility	64.480	90.007	91.647	94.569	0.0813	6842.48

However, it should be noted that the total losses in dry matter were not taken into account in the calculation of the protein digestibility studies. Considering this, one might say that the increase in the *in vitro* protein digestibility had been caused by a reduction in the total amount of protein nitrogen present. As a result of the high losses in total dry matter. These are reflections of the losses to the total protein content as well. In other words, the percentage *in vitro* protein digestibility had been increased because of the enzyme attacking fewer quantities of proteins in the samples considered.

In maize, therefore, the percentage increase in the *in vitro* protein digestibility of the sprouted grains was insufficient to balance the losses in total dry matter. While on the other hand, with cowpea, the increase in the *in vitro* protein digestibility tends to outweigh the total dry matter losses. Previous workers did not consider these losses in the digestibility.

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