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The Effect of Germination on the Physico-Chemical Properties of Black Gram (*Vigna mungo* L.)

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ABSTRACT

Rehydration of dry black gram seeds activated metabolic processes that caused changes in the chemical composition and allowed growth of the seeds. Further changes were recorded when germination occurred and the components that were found to be affected were the protein, fat, vitamin C, total carotenoid (provitamin A) and simple sugar (glucose, fructose and sucrose) contents. The protein and fat contents decreased following germination. Vitamin C content, on the other hand, increased significantly when seeds were soaked and allowed to germinate. Although higher in value than the dry seeds, the vitamin C content decreased after germination for 1 day. The total carotenoid content of dry seeds was higher than either the soaked seeds or the sprouts. Soaking caused a substantial loss in total carotenoid but the content slowly increased upon germination. Sucrose was the major simple sugar of dry seeds. Upon imbibition of water, glucose and fructose were completely lost. Germination led to an increase in the contents of all three sugars.

INTRODUCTION

Germination is defined as a process in which the hypocotyl is encouraged to develop and grow in length. During germination, seeds undergo pronounced metabolic changes whereby storage nutrient reserves in the cotyledons are broken down into more usable forms and are translocated to the growing shoots and roots for utilisation.

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Several sprouted grains are eaten as vegetables and are popular eastern dishes. Sprouts of mung bean and soybean are easily available in the Malaysian market. Many bean sprout producers in the country still employ the traditional sprouting procedure for producing sprouts. Beans that have been washed are placed in banana leaves in lined wooden boxes and washed at 4 hourly intervals for 6 days using well water. A recent innovation in the sprout industry is the automatic bean sprout growing equipment which is capable of producing bean sprouts in a shorter time.

Vigna mungo L. (formerly *Phaseolus mungo* L.) or black gram, as it is commonly known, is sometimes used in place of mung bean (*V. radiata*/*P. aureus* Roxb.) to produce bean sprouts. Although many reports have been published on the effects of germination on green gram (e.g. Kylen & McCready, 1975, Mohd. Ismail Noor *et al.*, 1980), very little work has been done on black gram. However, some work has been carried out on the dry, raw gram itself (e.g. Watson, 1977; Reddy *et al.*, 1982). In this paper, we report on the physico-chemical changes that took place when black gram was soaked and then germinated for a period of 3 days.

MATERIALS AND METHODS

Materials

Raw, dry black gram (*Vigna mungo* L.) seeds were obtained from a sprout cultivator in Penang and the seeds were kept at room temperature during the course of the study.

Germination procedure

Germination (sprouting) was carried out batch-wise over a period of 3 days in the dark at room temperature. One hundred and fifty grams of seeds were soaked in approximately ten times their volume of deionised water for 1 h, after which seeds that floated were discarded and the remaining seeds washed several times. The seeds were soaked for a further 10 h (hereafter referred to as soaked seeds) in ten times their volume of deionised water, following which the water was drained and the seeds placed in the sprouting compartment of a home-type automatic seed sprouter that has the capacity to produce a maximum of 900 g of sprouts. The seeds were sprayed with deionised water every 4 h and this function was performed automatically by the sprouter. Perforation at the bottom of the sprouting compartment ensured that the water was not retained in the compartment between waterings and the water was drained out via an outlet pipe situated at the bottom of the sprouter.

Sampling

Samples of dry, raw seeds and soaked seeds were obtained for physico-chemical analyses through random sampling. Sampling of germinating seeds was carried out by obtaining at least two lots of samples each from the top and bottom of the sprouting compartment. Results presented are the means of at least four determinations.

Analyses

Dry, raw seeds, soaked seeds and germinating seeds (24 h, 48 h and 72 h) were analysed for pH, total titratable acidity and crude protein, crude fat, vitamin C, total carotenoids, fructose, glucose and sucrose contents. The average hypocotyl lengths of 1st, 2nd and 3rd day sprouts were also measured. Except for the determinations of crude fat and crude protein which were carried out on freeze-dried samples (dry raw seeds were not freeze-dried), all other analyses were carried out on fresh samples. Freeze-dried samples were ground using a pestle and mortar and were kept in an airtight container at 4°C. Moisture contents of dry seeds, soaked seeds, germinated seeds and freeze-dried samples were determined by drying ground samples in an oven at 105°C to a constant weight.

Titratable acidity was determined by blending 10 g of sample with 60 ml of distilled water and titrating to pH 8·3 with 0·1N NaOH solution. Protein was estimated by the Micro Kjeldahl procedure (Pearson, 1976) and a nitrogen conversion factor of 6·25. Crude fat was determined using the Soxhlet extraction method. Vitamin C was determined titrimetrically according to AOAC (1970) where 0·5% oxalic acid solution was used as the extractant. The method of Hsieh & Karel (1983), but with slight modifications, was used to determine the total carotenoid contents of the samples. An acetone–hexane mix (50:50) was used as the extraction solvent instead of an acetone–light petroleum (50:50) mix. Extraction of carotenoids was carried out several times on each sample until the extractant was colourless. Pooled extracts of each sample were evaporated to dryness *in vacuo* at 30°C and the residue was redissolved in 100% hexane to 10 ml. The absorbance was read immediately at 436 nm for an index of total carotenoids. A calibration curve was obtained using *trans* β-carotene (0–7·0 μg/ml) as the standard carotenoid.

Soluble sugars were extracted from 10 g of homogenised samples. Fifty millilitres of 85% ethanol was added to the sample and the mixture heated to boiling on a steam-bath. After 5 min, a further 25 ml of the extractant was added and allowed to boil. The process was repeated until a total of 100 ml of the extractant was added. The mixture was then filtered and the residue

washed several times with 85% ethanol. The filtrate was concentrated by evaporation *in vacuo* to a small volume, transferred quantitatively into a 10 ml volumetric flask and made up to volume with deionised water. The extract was clarified first through a C-18 Sep-pack cartridge (Waters Assoc.) and then through a 0·45 μ membrane filter. Sugars in the extract were identified and quantified by HPLC (Hewlett–Packard, Model 1084B) using an NH_2 (10 μm) column and acetonitrile–H_2 (80:20) as the mobile phase. The percentages of each sugar in the sample were calculated on the basis of peak area.

RESULTS AND DISCUSSION

Germination procedures that have been reported previously included the use of paper towels (Mohd. Ismail Noor *et al.*, 1980), cheese cloth (Labaneiah & Luh, 1981), cotton wool (Giri *et al.*, 1981) and cellulose sponge (Fordham *et al.*, 1975; Farhangi & Valadon, 1981) as the sprouting bed. Mould growth sometimes accompanied the growth of the young seedlings unless extra care was taken or the water used for the germination was pretreated with chemicals such as chlorinated lime (Fordham *et al.*, 1975) and sodium hypochlorite (Labaneiah & Luh, 1981). The sprouting apparatus used in the present study afforded the production of sprouts that were free from mould growth and rots without the need to use chemically-treated water. Regular watering of the germinating seeds and non-retention of water in the sprouting chamber of the apparatus are probable reasons for the success of the sprouting procedure. With the automatic sprouter it was possible to achieve a sprout yield of greater than 80%. Third day sprouts had hypocotyl lengths of about 7 cm (Table 1) and were much longer and thinner than commercial sprouts of the same age, probably due to the complete lack of any kind of nutrient in the deionised water. In general, sprout yield and germination rates of seeds are dependent on seed type and germination procedure (Fordham *et al.*, 1975; Farhangi & Valadon, 1981) and seed quality (Lovato, 1981).

The pH and titratable acidity of black gram was found to remain relatively unaffected by soaking and germination (Table 1).

Germination was observed to decrease the protein content of black gram when expressed in terms of fresh weight (Table 1). Such a decrease was also reported by Mohd. Ismail Noor *et al.* (1980) and Fordham *et al.*, (1975) for several types of legumes. The decrease was attributed to the large increase in the water content upon germination and the same reason seems to hold for black gram as well. The protein content on a dry weight basis, on the other

TABLE 1
Some Physico-chemical Properties of Black Gram during Germination

Sample	*Moisture (%)*	*pH*	*Titratable acidity (% citric acid)*	*Vitamin C (mg/100 g)*	*Total carotenoids (μg/100 g)*	*Crude fat (%)*	*Crude protein (%)*	*Hypocotyl length (cm)*
Dry seeds	17·9	6·41	0·61	trace	384 (467·4)	0·89 (0·92)	23·9 (29·2)	—
Soaked seeds	57·0	6·41	0·36	12·4 (28·9)	58·1 (135·0)	—	12·7 (29·5)	0·2
Germinated seeds								
Day 1	72·7	6·78	0·31	26·7 (97·8)	85·8 (314·0)	0·24 (0·87)	8·5 (31·0)	1·9
Day 2	81·5	6·53	0·32	22·9 (124)	111·5 (602)	0·14 (0·76)	5·9 (32·0)	3·9
Day 3	92·5	6·66	0·32	15·7 (209)	138·2 (1 843)	0·03 (0·36)	2·6 (34·0)	6·9

Values in parentheses are on dry weight basis. Otherwise, values are based on 100 g of edible portion (fresh weight).

hand, increased slightly during germination. Soaking appeared to have no effect. Similar results on protein content were also obtained by Kylen & McCready (1975) and Lorenz (1980) who studied the effect of germination on alfalfa, green gram, lentils and soybeans, and cereal grains, respectively. It has been suggested that the apparent increase in the protein content following germination could probably be due to loss of leachable sugars and seed coats (Kylen & McCready, 1975), protein synthesis (Kylen & McCready, 1975), losses of carbohydrates during respiration and/or an alteration of the nitrogenous substances rather than actual increase in protein (Lorenz, 1980). For the black gram, some or all of the above factors may have led to the increase in the protein content.

The fat content of black gram was found to decrease as germination proceeded and third day sprouts were found to contain only 50% of the initial fat content, based on dry weight (Table 1). Other workers (Kylen & McCready, 1975; Mohd. Ismail Noor, *et al.*, 1980) have reported similar decreases in the fat content of green gram during germination. The overall decrease in the fat content of black gram during germination was probably the result of metabolism in order to meet the increased energy requirement of the developing plant tissues.

Vitamin C has been shown by several investigators to increase in content when seeds are germinated (Kylen & McCready, 1975; Fordham *et al.*, 1975).

In this study, the dry black gram itself was found to contain only trace amounts of the vitamin, although a value of 2·0 mg/100 g has been reported (Tee, 1985). Soaking of the gram led to a dramatic increase in the vitamin C content (Table 1) and may be due to *de novo* synthesis since many enzymes are generally activated during this period. Further increases in the vitamin C content were observed as germination proceeded (Table 1), when the values were based on dry weight. On a fresh weight basis, however, there appears to be an inverse correlation between the length of time of germination and vitamin C content since 100 g of fresh third day sprout has about 41% less vitamin C than 1 day old sprout.

It was observed that removing the seed coats from soaked beans revealed beans that were slightly yellow in colour. The tiny leaflets (shoots) that emerged from between the cotyledons of the beans were also observed to be yellow in colour. The amount of carotenoids were, therefore, determined. As shown in Table 1, the dry seed itself contained the pigment. Pigments in dry seeds of black gram are located both in the cotyledons and the outer seed coat. During soaking, soluble pigments were leached from the seed coats into the soaking medium and these were probably the anthocyanins. This left the seed coats yellowish green in colour. Loss of carotenoids during soaking can be partly associated with the removal of the seed coats. Upon germination, total carotenoids were found to increase in content and it is likely that the leaflets contributed significantly to the value. Carotenoids are

TABLE 2
The Simple Sugar Contents of Black Gram during Germination

Sample	*Total simple sugar* (%)	*Reducing sugar* (%)	*Fructose* (%)	*Glucose* (%)	*Sucrose* (%)
Dry seeds	0·76	0·07	0·01	0·06	0·69
	(0·93)	(0·08)	(0·01)	(0·07)	(0·84)
Soaked seeds	0·37	ND[a]	ND	ND	0·37
					(0·86)
Germinated seeds					
Day 1	1·13	0·44	0·27	0·17	0·69
	(4·14)	(1·61)	(0·99)	(0·62)	(2·53)
Day 2	1·82	1·03	0·50	0·53	0·79
	(9·84)	(5·57)	(2·70)	(2·86)	(4·27)
Day 3	2·34	1·53	0·73	0·80	0·81
	(31·2)	(20·4)	(9·73)	(10·67)	(10·80)

[a] ND—not detected.
Percents sugars are based on the fresh weight basis, while those in parentheses are on dry weight basis.

important to the diet as many members of the family are provitamin A, in that these can be converted to vitamin A by the human body.

The total simple sugar content (based on the sum of glucose, sucrose and fructose contents) of black gram seeds was 0·78% on a fresh weight basis or 0·93% on a dry weight basis. Reducing sugars constituted only about 10·8% of this and sucrose was found to be the major sugar in ungerminated seeds (Table 2). A decrease in the total simple sugar value was obtained upon imbibition of water during the soaking process and was due to complete losses of glucose and fructose but without any loss of sucrose. It cannot be ascertained as to whether the loss of the monosaccharides was due to leaching or to a dilution effect as a result of water absorption or utilisation. Germination of black gram led to a progressive increase in the total sugar content. Third day sprouts were found to contain 2·34 g per 100 g of fresh sprouts (31·4% on dry weight basis) of which 31·3% was fructose and 34·3% each was glucose and sucrose. Increases in the fructose and glucose contents of green grams (Silva & Luh, 1979; Jaya & Venkataraman, 1980) and the fructose contents of several other beans (Jaya & Venkataraman, 1980; Labaneiah & Luh, 1981) have been reported. Labaneiah & Luh (1981) observed that the sucrose contents increased during the germination of the seed under study. The increase in the contents of the different sugars during germination has been suggested to be the result of mobilisation of oligosaccharides and starch into simple sugars by enzymes (Matheson & Salini, 1977; Jaya & Venkataraman, 1980; Labaneiah & Luh, 1981). Starch constitutes 47·9% of black gram seed (Srinivasa, 1976).

CONCLUSION

The results obtained in this study showed that, as soon as black gram seeds became rehydrated, many physical and biochemical processes had begun to take place, allowing seeds that were initially dormant metabolically to become ones that were dynamically active metabolically and capable of growth. Some of the chemical changes that occurred, especially the vitamin C content, are of significant benefit to consumers since the values are higher in the germinated form of the seeds, whereas other changes, such as the protein content, can be regarded as losses to consumers.

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