EFFECT OF DIFFERENT COOKING PROCEDURES ON IN VITRO AMYLOLYTIC ACTIVITY, INHERENTLY PRESENT AMYLASE AND AMYLASE INHIBITORS IN BLACK GRAM

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Amylolytic activity, inherently present amylase activity and levels of amylase inhibitors, as affected by common processing and cooking methods, were studied in the grain legume *Vigna mungo* L. (black gram). Soaking reduced the inhibitor activity while amylolytic activity and inherent amylase activity were improved. Cooking increased the amylolytic activity, while the inherent amylase activity and the inhibitor activity were almost completely depressed on cooking. Germination increased the amylolytic activity appreciably. Inherent amylase activity increased significantly in 24-hour sprouted seeds, but decreased later. Inhibitor activity increased with increase in germination period. Negative correlation was found between amylase inhibitor activity and amylolytic activity.
Key words: black gram, cooking procedures, inherent amylase, in vitro amylolytic activity

INTRODUCTION

Grain legumes constitute an important part of human diet in many parts of the world. Black gram *(Vigna mungo* L.) is one of the most commonly consumed food legumes in Pakistan. Carbohydrate composition of the legume grain varies from 50-75 %, starch being the major component (Bourne, 1989). Availability of energy from the dietary legumes is, therefore, dependent to a significant extent on the digestibility of carbohydrates in these foods. During seed germination increase in amylolytic activity in endosperm is mainly contributed by the secretion of alpha-amylases. Such secretion of à-amylases mobilizes ttarch (Subbarao et al., 1998). Owing to several reasons including chain length and amount of amylose, and presence of antinutrients like amylase inhibitors, phytic acid and phenols, starch in legumes possesses low digestibility (Singh et al., 1982; Thompson and Yoon, 1984; Feng et al., 1996). Levels of different available carbohydrates and antinutrients responsible for lowering the digestibility of carbohydrates, have been reported to be affected by various processing and cooking methods (Kataria and Chauhan, 1988; Gahlawat and Sehgal, 1994).

A large variation in the inhibitor activity of pancreatic amylase has been reported by Jaffe et al. (1973). The growth inhibiting properties of raw beans may be due to the presence of heat labile factors which inhibit the in vitro activity of pancreatic amylase (Mulimani and Supriya, 1993a). Various biochemical, nutritional and toxicological aspects of alpha-amylase inhibitors from different plant foods have been reviewed by Buonocore and Silano (1986). Amylase inhibitor activity, both in raw and processed black gram seeds was studied. Inherently present amylase activity and effect of processing on amylolytic activity in the black gram seed was also studied and reported in the present paper.

MATERIALS AND METHODS

The seeds of black gram (Vigna mungo L.) were obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. Processing of seeds included the following methods :

1. Soaking: Seeds were freed from dust and other foreign material and then soaked in water for 12 and 18 hours at room temperature. A seed to water ratio of 1:5 (w/v) was used. The seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in an oven at 70°C for 36 hours.

2. Ordinary Cooking : The seeds after soaking for 12 hours, were rinsed in distilled water and put in round-mouthed tall beakers fitted with condensers. Having added water (three times the weight of dry seeds), the soaked seeds were cooked until sufficiently soft as felt between fingers. After mashing, the cooked seeds along with cooking water were dried at 70°C for 36 hours to a constant weight. The unsoaked seeds were rapidly rinsed with water and then cooked, using seed to water ratio of 1:7 (w/v) and dried in the same manner as mentioned above for soaked seeds.

Table 1. Amylolytic activity and amylase inhibitor actiyity in *Vigna mungo*

	Amlolytic activity (mg maltose released/g sample)	Percent increase (due to processing)	Inhibitor activity (units inhibited/g sample)
Raw seeds	73.88j±3.4	--	3.70a±0.10
Ordinary cooking (unsoaked seeds)	148.68f±9.8	101.24	0.04h±0.00
Ordinary cooking (soaked seeds)	245.32b±14.2	232.05	0.03h±0.01
Pressure cooking (unsoaked seeds)	236.82c±14.3	220.55	0.06h±0.01
Pressure cooking (soaked seeds)	262.30a±15.6	255.04	0.04h±0.02
12h soaking	80.99i±2.3	9.62	2.47d±0.10
18h soaking	96.10h±1.49	30.07	2.90b±0.13
24 h sprouting	148.04f±4.3	100.37	1.72f±0.13
48h sprouting	173.34d±4.1	134.62	2.40e±0.12
60h sprouting	163.7ge±3.8	121.69	2.62c±0.11
Seed coat alone	135.55g±3.4	--	1.24g±0.09

Values are means ±t SD of three independent determinations. Means carrying the same alphabets are not significantly (P>0.05) different.

3. Pressure Cooking : The seeds soaked for 12 hours were autoclaved for 15 minutes. The amount of cooking water was twice the weight of dry seeds. Unsoaked seeds were also autoclaved for 15 minutes using seed to water ratio of 1:5 (w/v). The pressure cooked seeds were then mashed and dried at 70°C for 36 hours.

4. Sprouting: The seeds soaked for 12 hours were germinated in sterile petri dishes lined with wet filter papers for 24, 48 and 60 hours at 25°C, with frequent watering. The sprouts were then dried at 70°C. The oven dried processed as well as unprocessed samples were milled to pass through a 0.5 mm sieve and stored in plastic containers until further analysis.

Chemical Analysis : Amylolytic activity was assessed as in vitro starch digestibility by employing the method of Singh et al, (1982) by using pancreatic amylase, and incubating at 37°C for 'two hours. Liberated maltose was measured calorimetrically by using dinitrosalycylic acid (DNS) reagent. Inherently present amylase activity was determined by using soluble starch as substrate, and liberated maltose was measured calorimetric ally (Singh et aI., 1982). Amylase inhibitor was extracted by using phosphate buffer and determined on spectrophotometer by using DNS reagent (Mulimani and Supriya, 1993b).

RESULTS AND DISCUSSION

1. Amylolytic Activity : The amylolytic activity was assessed on the basis of mg maltose released /g sample. It was found to be 73.88 and 132.55 for unprocessed whole seed and seed coat respectively (Table 1). Singh et al. (1982) reported that chickpea had amylolytic activity in the range of 45.2 to 47.1. Processing significantly (P<O.Ol) affected the amylolytic activity of *Vigna mungo*. Soaking of seeds for 12 hours improved the activity up to 9.62 %, while 18 hours soaking increased it up to 30.07 % (Table 1). Factors like amylase inhibitors and tannins have been reported to inhibit alpha amylase. Level of these antinutrients in the legume seeds decreases during soaking (Rao and Deosthale, 1982; Mulimani and Supriya, 1993a) which may account for increased amylolytic activity i.e. increased starch digestibility of the soaked seeds.

Amylolytic activity increased more than three fold as a result of ordinary cooking of soaked and unsoaked seeds. The activity was increased

significantly when unsoaked seeds were autoclaved and was much higher (255.04%) when the soaked seeds were autoclaved (Table 1). Enhancement of amylolytic activity in cooked legumes may be attributed to swelling and rupturing of starch granules which facilitate more randomized configuration for alpha-amylase to affect hydrolysis. Similar results were also shown by Kataria and Chauhan (1988) and Kataria et al. (1992) who found 35 to 48% increase in amylolytic activity in amphidiploids (black gram x mungbean) after cooking. Increase in amylolytic activity is related to the reduction in antinutrients due to cooking (Sharma and Khetarpaul, 1995).

Germination of seeds increased the amylolytic activity by 100.37% after 24 hours germination. The activity increased even more after 48 hours germination and then decreased to some extent after 60 hours period (Table 1). The increased activity after germination may be due to decrease in complex polysaccharides as they are consumed during germination. Increased amylolytic activity after cooking and germination in chickpea and black gram seeds was also reported by Jood et al. (1988) and Garcia (1989). Marero et al. (1990) reported reduction in amylolytic activity produced during germination in flour (rice, corn, mungbean and cowpea) despite drying and roasting processes.

Table 2. Amylase activity present inherently in *Vigna mungo* expressed as mg maltose re lease d/g sample/24 hours .

	Amylase activity
Raw seeds	47.79c ± 2.39
Seed coat	8.97f ± 1.08
12h soaking	52.47b ± 2.87
18h soaking	51.13b ± 2.88
24h sprouting	112.48a± 1.34
48h sprouting	34.20d ± 3.88
60h sprouting	19.7ge ± 1.73

Values are means ± SD of three independent determinations. Means carrying the same alphabets are not significantly (P>0.05) different.

2. Amylase Activity (inherently present) : Inherently present amylase activity was studied and expressed as mg maltose released *lg* sample/24 hours (Table 2). Higher activity was found in whole been seeds as compared to the seed coat alone. Marero et al. (1990) found that the highest concentration of amylase was present in rice followed by cowpea, corn and mungbean. No inherent amylase activity was present in cooked beans as alpha-amylases rapidly loose activity above 50°C (Khan, 1970). Soaking increased the activity from 47.79 to 51.13 and 52.47 for 18 and 12 hours soaking period respectively (Table 2). The increase was found to be significant with respect to the raw beans, but non-significant in relation to the soaking time.

Germination for 24 hours increased the amylase activity significantly. The activity was depressed with increase in the germination period. Increase in amylase activity on progressive germination in different legumes was found by Sumathi et al. (1995). They also showed that high amylase activity exhibited proportionately lower paste viscosity. The decrease and increase in inherent amylase activity during soaking and germination may be due to the concurrent decrease and increase in the antinutritional factors and enzyme inhibitors.

3. Amylase Inhibitors : The inhibitors of the pancreatic alpha-amylase, found in the legume seeds, are glycoproteins with molecular weights in the range of 45000-49000 and composed of subunits of three different types. Alpha-amylase inhibitor forms 1:1 complex with pancreatic amylase enzyme and binds at the site other than the active site of the enzyme thus inactivating the catalytic power of the enzyme through conformational change (Bourne, 1989). Amylase inhibitor activity was investigated in the raw and processed black gram seeds and expressed as units of the enzyme inhibited/g samples (Table 1). Very low inhibitor activity was found i.e. 3.7 D/g and 1.24 D/g for whole bean seeds and seed coat respectively. Savage and Deo (1989) reported that mungbeans contained 32 D/g inhibitor, while the inhibitor could not be detected in the Urd beans. Chickpea had pancreatic amylase inhibitor activity in the range of 7.4-9.0 D/g (Singh et al., 1982). Among inhibitory activity of alpha-amylase inhibitors against salivary amylase, maximum inhibitory activity of 124 units was found in sorghum, whereas rice had the lowest inhibitory activity (Mulimani and Supriya, 1993b).

By cooking almost all the inhibitor activity was lost (Table 1). It was also reported that the amylase inhibitors were inactivated at 100°C (Savage and Deo, 1989). Drastic reduction in alpha-amylase inhibitory activity on cooking raw and soaked seeds of sorghum was also reported by Mulimani and Supriya (1993a). However, application of dry heat to

sorghum seeds was not effective in inactivating the amylase inhibitory activity.

Soaking as well decreased the inhibitor activity, but to a lesser extent as compared to cooking. Highly significant (P<O.Ol) difference was found with respect to soaking period. Overnight soaking followed by heat treatment was found effective in destroying amylase inhibitory activity in sorghum (Mulimani and Supriya, 1993a). The inhibitor activity was depressed during .germination, but the activity increased significantly with increase in the germination period. The reduction in the levels of different antinutritional factors including a-amylase inhibitors after germination and fermentation was reported by Singh (1988).

The present results suggest that in case of unheated bean samples, some inhibition of starch digestion by amylase inhibitors may be expected. Highly significant and negative correlation (-0.796; P > 0.001) was found between amylase inhibitor activity and amylolytic activity.

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