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Antinutrients in amphidiploids (black gram × Mung bean): varietal differences and effect of domestic processing and cooking

A. KATARIA, B.M. CHAUHAN* & D. PUNIA
*Department of Foods and Nutrition, Haryana Agricultural University, Hisar 125 004 India (*author for correspondence)*

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Abstract. Phytic acid, saponin and polyphenol contents in grains of various varieties of black gram (*Vigna mungo*) Mung bean (*Vigna radiata* L.) amphidiploids ranged from 697 to 750, 2746 to 2972 and 702 to 783 mg/100 g, respectively. Domestic processing and cooking methods including soaking, ordinary and pressure cooking of soaked and unsoaked seeds, and sprouting significantly lowered phytic acid, saponin and polyphenol contents of the amphidiploid seeds. Soaking for 18 h removed 31 to 37% of the phytic acid; the extent of removal was higher with long periods of soaking. Saponins and polyphenols were relatively less affected. Loss of the antinutrients was greater when soaked instead of unsoaked seeds were cooked. Pressure cooking had a greater effect than ordinary cooking. Antinutrient concentrations declined following sprouting; the longer the period of germination the greater was the reduction.

Introduction

Food legumes are rich and less expensive sources of proteins in human diet in several developing countries. Biological utilisation of pulses is limited due to deficient sulphur containing amino acids [1] and the presence of antinutrients including phytic acid, saponins, polyphenols, enzyme inhibitors, lectins etc. [2].

Hybridization has been known to be the most potent tool for increasing genotype variability among food crops through new recombinations. When the variability within species is exhausted or some characters sought are not present, interspecific hybrids are attempted with the view of producing new species through amphidiploidy. The amphidiploids, in addition to showing various characters of economic importance, also exhibit a wide range of variability and desirable genotypes could be selected by using suitable breeding techniques [3].

Some promising strains of amphidiploids (black gram × Mung bean) have been developed by Haryana Agricultural University, Hisar (India).

The present investigation was undertaken to determine the level of phytic acid, saponins and polyphenols of these new legume grains and also to know the extent to which the antinutritional factors survive the domestic processing and cooking treatments and finally remain in the food.

Materials and methods

Materials

Seed samples of four varieties of amphidiploids (T_1-12, T_1-19, T_2-10 and T_2-26) were obtained from the Department of Plant Breeding, Haryana Agricultural University Hisar (India).

Processing and cooking methods

Methods of processing and cooking included soaking in water for different intervals of time, ordinary and pressure cooking of soaked as well as unsoaked seeds and sprouting of the seeds.

Soaking

Seeds freed from broken seeds, dust and other foreign materials were soaked in water for 6, 12 and 18 h at 37 °C. A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were washed twice with ordinary water followed by rinsing with distilled water and then dried in hot air oven at 70 °C to a constant weight.

Cooking

Seeds after soaking for 12 h were rinsed in distilled water and put in round-mouthed tall beakers fitted with condensors. Having added distilled water (three times the weight of dry seeds) the samples were boiled until soft as felt between fingers. Cooked seeds alongwith cooking water were dried to a constant weight at 70 °C for 36 h. Unsoaked seeds were also cooked in the same manner, using seed to water ratio of 1 : 7 (w/v). For pressure cooking, the seeds were autoclaved at 1.05 kg/cm^2 pressure for 5, 10 and 15 minutes. For this, dry seeds to water ratio 1:2 (w/v) was used. The cooked samples were mashed and then dried at 70 °C.

Germination

The seeds soaked for 12 h were germinated in sterile petri dishes lined with wet filter paper for 24, 36, 48 and 60 h at 25 °C, with frequent watering. The sprouts were then dried at 70 °C to a constant weight.

The oven-dried unprocessed as well as processed samples were milled in a cyclone mill (Cyclotec, M/s Tecator, Sweden) to pass through a 0.5 mm sieve and stored in plastic containers until required for further analysis.

Chemical analysis

Phytic acid was extracted in 0.5 M nitric acid and determined colorimetrically [4]. Saponins were also determined colorimetrically [5]. Total polyphenols were extracted [6] and estimated as tannic acid equivalent according to the Folin-Denis procedure [7].

Stastistical analysis

The data were processed for analysis of variance to find the significant differences among various varieties and treatments [8].

Results and discussion

Antinutrients in parents and the amphidiploids

A perusal of the data in Table 1 and Table 2 indicated that the phytic acid content of all the amphidiploid varieties seemed to be higher than that of black gram but close to the values found in mung bean parent. Saponin content of the amphidiploids was less than that in black gram but close to the saponin content of mung bean parent. As regards polyphenol content, the grains of amphidiploid had relatively lower amount of this antinutrient than both the parents.

Among the amphidiploids variety T_2-26 had significantly higher amount

Table 1. Phytic acid, saponin and polyphenol contents of black gram[a] and mung beans[b] (mg/100 g, on dry matter basis)

Antinutrient	Black gram	Mung bean
Phytic acid	645 ± 12	741 ± 4
Saponin	3335 ± 256	2848 ± 93
Polyphenol	866 ± 5	808 ± 4

[a]Taken from Kataria *et al.* [12].
[b]Taken from Kataria *et al.* [23].

Table 2. Effect of soaking on the antinutrients (mg/100 g) of black gram - Mung bean amphidiploids (on dry matter basis)[a]

Antinutrients	Soaking period (h)	Varieties			
		T_1-12	T_1-19	T_2-10	T_2-26
Phytic acid	0	706 ± 7	706 ± 5	697 ± 5	750 ± 8
	6	660 ± 10 (−6)	694 ± 20 (−2)	669 ± 4 (−4)	688 ± 9 (−8)
	12	624 ± 14 (−12)	623 ± 28 (−12)	621 ± 61 (−11)	646 ± 7 (−14)
	18	477 ± 6 (−32)	456 ± 11 (−35)	480 ± 22 (−31)	474 ± 20 (−37)
		CD ($P < 0.05$) = 15.6			
Saponin	0	2808 ± 20	2972 ± 18	2746 ± 33	2844 ± 38
	6	2734 ± 30 (−3)	2920 ± 39 (−2)	2622 ± 41 (−4)	2742 ± 66 (−3)
	12	2674 ± 34 (−5)	2834 ± 39 (−5)	2588 ± 24 (−6)	2554 ± 77 (−10)
	18	2602 ± 30 (−7)	2726 ± 30 (−8)	2484 ± 35 (−9)	2460 ± 40 (−13)
		CD ($P < 0.05$) = 76.2			
Polyphenols	0	728 ± 7	783 ± 4	702 ± 4	766 ± 5
	6	698 ± 8 (−4)	751 ± 7 (−4)	677 ± 4 (−3)	743 ± 0 (−3)
	12	669 ± 0 (−8)	733 ± 4 (−6)	651 ± 4 (−7)	727 ± 8 (−5)
	18	635 ± 5 (−13)	693 ± 5 (−11)	620 ± 0 (−12)	702 ± 4 (−8)
		CD ($P < 0.05$) = 10			

[a] Values are means ± SD of four replicates. Figures in parentheses indicate decrease (−) or increase (+) expressed as percentage of control values.

of phytic acid than the remaining three; the latter did not differ significantly among themselves. Highest amount of saponin as well as polyphenols was found in T_1-19 followed by T_2-26, T_1-12 and T_2-10 in descending order (Table 2). Significant variation in phytic acid, saponin and polyphenol content of varieties of various food legumes has been reported earlier [9, 10, 11].

Soaking

Phytic acid decreased by 2 to 8% when the seeds of amphidiploids were soaked in water for 6 h. The loss was higher when the period of soaking was raised to 12 and 18 h (Table 2). After 18 h soaking the seeds lost phytic acid ranging from 31 to 37%. The obvious decrease in phytate content of the

legume seeds during soaking can be attributed to leaching of phytate ions into water during soaking and rinsing under the influence of concentration gradient. The phytase inherent in the grains may also become active during soaking and may cause hydrolysis of phytic acid consequently leading to reduction in phytic acid content of the legume grains. Loss of phytic acid during soaking has been reported for black gram, chickpea, moth bean, cowpea and limabean seeds [9, 10, 12].

Twelve or eighteen hour soaking reduced saponin and polyphenols in the amphidiploids significantly ($P < 0.05$). Soaking for 6 h had a significant lowering effect on the saponin content only in T_2-10 whereas polyphenols were lowered significantly in all the varieties (Table 2). Soaking has been reported to lower the level of saponin and polyphenols in legume grains [10, 11, 12, 13].

Cooking

Cooking of soaked and unsoaked seeds of the amphidiploids decreased phytic acid by 15 to 22% and 3 to 15%, respectively (Table 3). There was reduction in phytic acid content by a margin of 14 to 21% when the soaked seeds were pressure cooked for 5 min at a pressure of 1.05 kg/cm^2: increase in period of pressure cooking did not seem to make large difference. Pressure cooking of unsoaked seeds also lowered the phytic acid content of the amphidiploid seeds; the loss was relatively higher when soaked seeds were pressure cooked. The decrease in phytic acid content during cooking can be attributed to the formation of insoluble complexes between phytate and other components [14]. A reduction in phytate content after cooking of dry beans [15], moth beans [10], horse gram [16] and black gram [12] has been reported earlier.

Saponin reduction varied from 9 to 14% when the amphidiploid seeds were cooked after 12 h soaking and from 4 to 15% when unsoaked seeds were cooked. Pressure cooking of soaked seeds had a marked effect on lowering saponin content which increased following an increase in the period of pressure cooking. Pressure cooking of unsoaked seeds for 15 min lowered the saponin content considerably but it was less effective when compared with pressure cooking of soaked seeds for 15 min. Possible thermolabile nature of saponin and formation of poorly extractable complex [13] may account for the loss of saponin level during cooking. Reductions in saponin levels during cooking of moth bean [10], chickpea [13] and black gram [12] have been observed earlier.

Cooking of soaked as well as unsoaked seeds resulted in significant reduction of polyphenol contents of the amphidiploids; the loss was more

Table 3. Effect of cooking on the antinutrients (mg/100 g) of black gram – Mung bean amphidiploids (on dry matter basis)[a]

Antinutrients	Cooking method	Varieties			
		T_1–12	T_1–19	T_2–10	T_2–26
Phytic acid	Ordinary cooking of soaked seeds	602 ± 19 (−15)	550 ± 21 (−22)	546 ± 19 (−22)	586 ± 9 (−22)
	Ordinary cooking of unsoaked seeds	632 ± 19 (−3)	626 ± 15 (−11)	646 ± 20 (−7)	634 ± 21 (−15)
	Pressure cooking of soaked seeds				
	5 min	590 ± 19 (−16)	605 ± 11 (−14)	547 ± 10 (−21)	626 ± 5 (−16)
	10 min	588 ± 7 (−17)	580 ± 27 (−18)	539 ± 9 (−23)	565 ± 40 (−25)
	15 min	575 ± 3 (−18)	554 ± 6 (−21)	535 ± 9 (−23)	546 ± 17 (−27)
	Pressure cooking of unsoaked seeds				
	15 min	627 ± 13 (−11)	578 ± 7 (−18)	598 ± 25 (−14)	586 ± 22 (−22)
			CD ($P < 0.05$) = 15.6		
Saponin	Ordinary cooking of soaked seeds	2612 ± 86 (−9)	2602 ± 40 (−12)	2504 ± 30 (−9)	2590 ± 31 (−14)
	Ordinary cooking of unsoaked seeds	2494 ± 82 (−11)	2538 ± 65 (−15)	2512 ± 66 (−4)	2578 ± 21 (−9)
	Pressure cooking of soaked seeds				
	5 min	2380 ± 43 (−15)	2438 ± 39 (−18)	2250 ± 39 (−18)	2398 ± 34 (−16)
	10 min	2250 ± 48 (−20)	2364 ± 55 (−20)	2188 ± 33 (−20)	2238 ± 13 (−21)

	15 min	2162 ± 45 (−23)	2220 ± 46 (−25)	2122 ± 30 (−23)	2176 ± 38 (−23)
	Pressure cooking of unsoaked seeds				
	15 min	2290 ± 114 (−18)	2432 ± 54 (−18)	2406 ± 106 (−12)	2500 ± 60 (−12)
			CD ($P < 0.05$) = 76.2		
Polyphenols	Ordinary cooking of soaked seeds	608 ± 4 (−16)	684 ± 5 (−13)	595 ± 12 (−15)	685 ± 4 (−10)
	Ordinary cooking of unsoaked seeds	658 ± 9 (−10)	715 ± 11 (−9)	618 ± 12 (−12)	715 ± 8 (−7)
	Pressure cooking of soaked seeds				
	5 min	597 ± 10 (−18)	637 ± 8 (−19)	565 ± 8 (−19)	652 ± 4 (−15)
	10 min	592 ± 5 (−19)	616 ± 5 (−21)	542 ± 5 (−23)	626 ± 5 (−18)
	15 min	565 ± 11 (−22)	597 ± 5 (−24)	524 ± 7 (−25)	609 ± 4 (−20)
	Pressure cooking of unsoaked seeds				
	15 min	639 ± 11 (−12)	700 ± 7 (−11)	599 ± 11 (−15)	721 ± 12 (−6)
			CD ($P < 0.05$) = 10		

[a] Values are means ± SD of four replicates. Figures in parentheses indicate decrease (−) or increase (+) expressed as percentage of control values.

Table 4. Effect of germination on the antinutrients (mg/100 g) of black gram - Mung bean amphidiploids (on dry matter basis)[a].

Antinutrients	Germination period (h)	Varieties			
		T_1–12	T_1–19	T_2–10	T_2–26
Phytic acid	24	470 ± 3 (−33)	579 ± 12 (−18)	500 ± 9 (−28)	547 ± 39 (−28)
	36	468 ± 31 (−34)	563 ± 21 (−20)	488 ± 20 (−30)	504 ± 5 (−34)
	48	392 ± 5 (−44)	481 ± 6 (−32)	441 ± 8 (−37)	406 ± 41 (−47)
	60	369 ± 7 (−49)	430 ± 8 (−39)	419 ± 7 (−40)	394 ± 10 (−48)
			CD ($P < 0.05$) = 15.6		
Saponin	24	2624 ± 91 (−6)	2730 ± 33 (−8)	2598 ± 98 (−5)	2382 ± 127 (−16)
	36	2534 ± 66 (−10)	2642 ± 29 (−11)	2568 ± 50 (−6)	2664 ± 13 (−6)
	48	2414 ± 76 (−14)	2552 ± 96 (−14)	2516 ± 71 (−8)	2566 ± 106 (−10)
	60	2374 ± 106 (−18)	2310 ± 335 (−22)	2460 ± 93 (−10)	2520 ± 79 (−11)
			CD ($P < 0.05$) = 76.2		
Polyphenols	24	626 ± 5 (−14)	687 ± 48 (−13)	584 ± 8 (−17)	689 ± 10 (−11)
	36	585 ± 5 (−20)	677 ± 50 (−12)	543 ± 10 (−22)	658 ± 10 (−14)
	48	561 ± 8 (−23)	664 ± 10 (−15)	537 ± 8 (−23)	640 ± 10 (−16)
	60	554 ± 7 (−24)	644 ± 11 (−18)	513 ± 11 (−27)	626 ± 6 (−18)
			CD ($P < 0.05$) = 10		

[a] Values are means ± SD of four replicates. Figures in parentheses indicate decrease (−) or increase (+) expressed as percentage of control values.

when the soaked seeds were cooked. Pressure cooking of soaked seeds for 5 min decreased polyphenols to a larger extent as compared to the seeds which were ordinarily cooked after soaking. The effect of pressure cooking was greater when the period of pressure cooking was extended. A decreased amount of polyphenols recovered from cooked seeds could be on account of reduced extractability due to their changed chemical reactivity [17]. Autoclaving and ordinary cooking, involving moist heating, may destroy polyphenols. Cooking has been reported to decrease the polyphenol contents of Mung bean [18], pigeon pea and cowpea [19] and black gram [12].

Germination

Of all the processing methods studied, germination seemed to have the most pronounced effect on decreasing phytic acid content of the amphidiploid seeds (Table 4). A loss of 18 to 33% occurred during 24 h germination which increased further with an increase in period of germination. After 60 h germination the sprouts had 39 to 49% less phytic acid than that in unprocessed seeds. Loss of phytic acid during germination may be attributed to phytase activity in the germinating seeds as reported in faba bean [20] and Mung bean [21]. Decreases in phytic acid content of cowpea, soyabean and limabean [9], horse gram [16], moth bean [10] and black gram [12] during germination have been reported.

Germination of amphidiploids for 24 h lowered saponins by 5–16%. This lowering effect was more pronounced when the germination period was further prolonged to 36, 48 and 60 h. The saponin reduction after 60 h germination ranged from 10 to 22%. Enzymic degradation could be a possible explanation of the saponin loss during germination [13], but this is far from established. Loss of saponin from moth bean [10], chickpea [13] and black gram [12] during germination has been reported.

Germination for 24 h led to 11–17% reduction in the polyphenol contents of the amphidiploids. Increase in the period of germination also caused further reduction in polyphenols of the seeds; 18–27% reduction was noticed after 60 h germination. The presence of polyphenol oxidase may account for the loss of polyphenols during germination of food legume [11]. Germination has been shown to decrease the polyphenol contents of pigeon pea [11], chickpea and green gram [22] and black gram [12].

Conclusion

Phytic acid, saponin and polyphenols are present in significant amounts in amphidiploids as in other food legumes. They are significantly reduced during domestic processing and cooking. This can be expected to result in more effective utilisation of processed and cooked food legumes. Germination of amphidiploids seemed to be the most effective method of reducing the levels of these antinutrients.

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