

**EFFECT OF COMBINED GERMINATION, DEHULLING AND BOILING
ON MINERAL, SUCROSE, STACHYOSE, FIBRULOSE, AND PHYTIC ACID
CONTENT OF DIFFERENT CHICKPEA CULTIVARS**

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ABSTRACT

Chickpea is a good source of high quality protein, carbohydrates, vitamins (thiamine and niacin), and minerals. However, its use in industry has been limited by variation in composition with cultivar and also the presence of oligosaccharides, trypsin inhibitors, phytic acids, tannin, and haemagglutinin. Different technologies have been studied to eliminate or minimise the undesirable factors in chickpeas. None of the studied traditional technologies has been found to effectively eliminate or minimise all the undesirable factors in chickpeas. It is not clear whether a combination of these traditional technologies, more especially cooking of germinated and dehulled chickpeas, will significantly reduce all the antinutritional factors. The physical characteristics, stachyose, sucrose, phytic acid, fibrulose, and mineral content of different chickpeas cultivar were determined and compared with reference to infant and child nutrition. The selected cultivars were (1) dehulled and boiled before drying; (2) dehulled followed by soaking and boiling before drying; (3) boiled without dehulling before drying; and germinated, boiled followed by drying and dehulling . The effects of the processing on mineral, sugar, dietary fibre content were evaluated. *Desiwere* found to have lower seed weight, hydration capacity and swelling capacity compared to *kabuli*. Seed density, hydration index and swelling index did not vary with cultivar. The mineral density, stachyose, fibrulose, and hull content increased significantly ($p < 0.05$) with the decrease of seed weight whereas phytic acid content did not vary. All processes resulted in an increase in calcium, phosphorous, zinc, and phytic acid and a decrease in potassium, iron, magnesium, sucrose, stachyose and fibrulose content regardless of cultivar type. Germination for 72 hrs followed by boiling, drying and dehulling resulted in highest reduction in antinutritional factors with minimal nutrient loss. It is feasible to use chickpeas as an excellent source of infant follow-on formula/weaning food with minimal mineral fortification and use of low phytic acid cultivars.

Key words: Chickpea, antinutritional factors, germination, dehulling

INTRODUCTION

Chickpea is one of the most important legumes in the Mediterranean and south Asia diet. Chickpea is a good source of high quality protein, carbohydrates, vitamins (thiamine and niacin), and minerals such as calcium, phosphorous, iron, magnesium, and potassium [1]. Consumption of 100 g of chickpea each day can meet nutritional requirements for most nutrients for children below 3 years of age. However, its use in industry has been limited by the presence of oligosaccharides, trypsin inhibitors, phytic acid, tannin, and haemagglutinin [2].

Various traditional technologies have been reported to eliminate or minimise the undesirable factors in chickpeas. For example, germination for 72 hours eliminates oligosaccharides and results in 56.1% reduction of phytate [2, 3]. Cooking was found to eliminate saponin and haemagglutinin whilst reducing trypsin inhibitors by 80%, tannin by 77% and phytate by 30% [2, 4, 5]. Additionally, germination or cooking also improves protein and starch digestibility and mineral bioavailability which is a direct result of a decrease in antinutritional factors [2]. The aforementioned results suggest that combination of germination and cooking might effectively eliminate or reduce the antinutritional factors. It is not clear whether cooking of germinated and dehulled chickpeas will significantly reduce all the antinutritional factors as literature is scarce.

In an effort to use chickpea as a food product for children with lower levels of antinutritional factors, we investigated the combined effect of different technologies on antinutritional factors, mineral and sucrose content of chickpea. The following combinations were studied (1) cooking of germinated and dehulled chickpea; and (2) cooking of dehulled and soaked chickpeas.

MATERIALS AND METHODS

Materials

Five different *kabuli* cultivars of chickpea (*Cicer arietinum* L), namely *raz*, *zehavit*, *bar*, *HA500* and *yarden*, were obtained from Volcani Center, Israel Agricultural Research Institute and one *desi* cultivar of chickpea was obtained from the Institute of Plant Sciences at The Hebrew University of Jerusalem. All reagents were bought from Sigma-Aldrich Israel.

Physical characteristics determination

The physical characteristics of chickpea were determined by selecting randomly 3 samples of 100 seeds from each cultivar. Seed size was determined by weighing the 100 seeds and converting it to grams per seed. Seed volume was determined by transferring the 100 seeds into 100 ml measuring cylinder followed by addition of 50 ml water. The seed volume (ml/seed) was the quotient of the gain in volume by 100 seeds. The ratio between seed weight and seed volume was the seed density. Hydration capacity (g/seed) was calculated as gain in weight after soaking 100 seeds for 24 hours whereas swelling capacity (ml/seed) was the gain in volume after 24 hours soaking. Hydration index was determined by dividing hydration capacity by

seed size and swelling index was calculated as the ratio between swelling capacity and seed volume [6].

Chickpea flour Processing

The chickpea grains were processed in four different ways as shown in Figure 1 and followed by drying and grinding to flour using a coffee grinder (Model RM100, Duisburg, Germany) as follows.

Germination: Alcohol (70% ethanol) disinfected chickpea samples were soaked in water in the ratio 1:10 (w/v) for 12 hrs and rinsed in water. The seeds were later placed in tray lined with absorbent paper and was later allowed to germinate at room temperature under dark environment. The seeds were rinsed with water at 12 hours interval and germination was terminated after 72 hrs by freezing at -20 °C for 12 hrs.

Boiling: Chickpea grains were cooked in boiling water (95-97 °C) until soft (ready to eat). Germinated chickpea seeds were placed in boiling water for 3 minutes and rinsed in hot water in order get rid of the foam before boiling them. The cooked seeds were immediately rinsed with hot water.

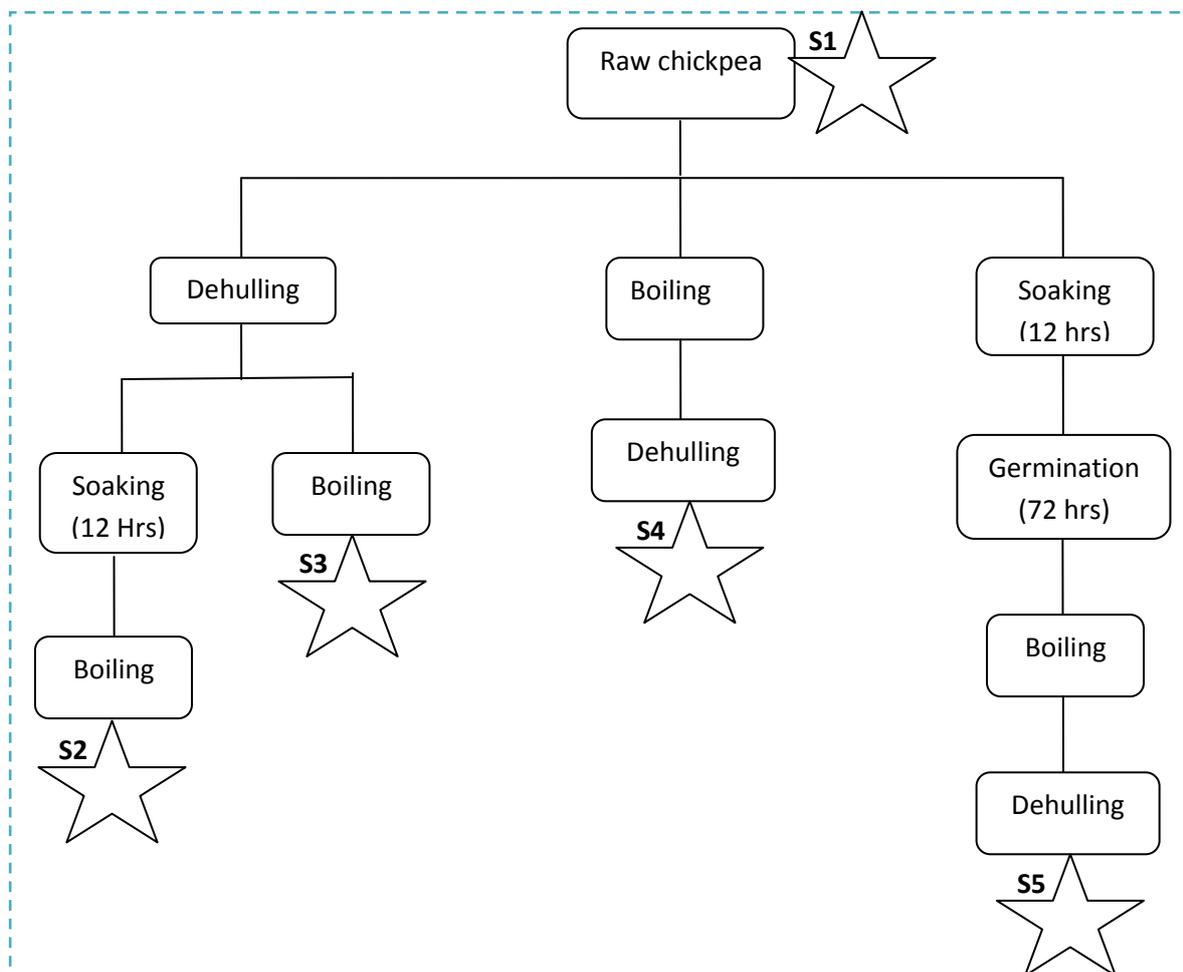


Figure 1: Experimental design for chickpea flour processing

Dehulling: All seeds were manually (using hands) dehulled at different points as shown in Figure 1. Raw chickpea grains were dehulled for S2 and S3 before soaking or boiling, respectively, whereas cooked chickpeas were dehulled for S4. Similarly, boiled germinated (S5) were dehulled after drying.

Drying and milling: All grains were dried at 50 °C for 20 hrs in an oven. Milling was done using a heavy duty blender (model RM100, German) to pass through a 200 micrometer sieve.

Mineral determination

Mineral composition was determined using inductively coupled plasma mass emission spectrometry (ICP-MES) (ICP-AES model "Arcos", SOP, manufacturer - "Spectro Limited", Kleve City, Germany). The measurements were taken after microwave acid digestion of the sample as per EPA method 3052 (EPA, 1996).

Oligosaccharides determination

Disaccharides (sucrose, stachyose, and fibrulose) and oligosaccharides were determined by HPLC. Chickpea flour (3 g) was mixed with 20 ml of water (85 °C), 0.5 ml carrez reagent 1 and 0.5 ml carrez reagent 2. The mixture was heated at 85 °C for 20 minutes in a water bath after which it was allowed to cool down. Water was later added to make it to 25 ml and centrifuged at 6000 rpm for 7 minutes. The supernatants were filtered through a 0.45 µm membrane paper and the filtrate to HPLC. HPLC column (6.5 X 300mm, HPX-87P BIO-RAD) was used at 80 °C, using HPLC grade water as a mobile phase at the flow rate of 0.5 ml/minute and the injection volume of 40 µl. The running time was 18 minutes and detection temperature of 50 °C. Chickpeas sugar compounds were identified by comparing the retention times of respective standards.

Phytic acid determination

Phytic acid was determined using AOAC method after extraction with 3% trichloroacetic acid (TCA) [7, 8].

Statistical analysis

Data obtained was analysed using one-way analysis of variance and difference was considered significant at $p < 0.05$. JMP07 program was used for statistical calculations.

RESULTS

Physical Characteristics

Seed weight, volume, hydration capacity, and swelling capacity varied significantly ($p < 0.05$) across the cultivars where as seed density, swelling index and hydration index did not vary. The results also indicate that cultivars with higher seed weight and volume are likely to have higher hydration capacity and swelling capacity (Table 2).

Nutrient composition

Chickpeas have been shown to vary greatly in their nutrient distribution composition depending on the cultivar, geographical area and planting time or season [6, 9-12]. We compared the mineral, sugars (sucrose and stachyose), phytic acid and dietary fibre (fibrulose between the *desi* and *kabuli* cultivars. Raw chickpea's sucrose, stachyose and fibrulose content varied with cultivar as shown in Table 1. *Desi* had less sucrose content but more stachyose and fibrulose compared to *kabuli* (*bar*). On the other hand, phytic acid content did not vary significantly with cultivars such that both contained about 77 mg/100 g (Table 1). The mineral composition content in chickpea varied greatly with cultivar (Table 3). It was observed that *desi* (which has low seed weight) had higher mineral density compared to *kabuli* cultivars. Furthermore, within the *kabuli* cultivar, a similar trend was observed such that *yarden* and *raz* with the least seed weight had on average highest mineral content compared to *bar* and *zehavit*.

Effect of combined processes

The aim of using combined process was to eliminate or reduce to acceptable level the antinutrition factors (stachyose and phytic acid) in chickpea while maintaining its nutritive value. The nutrition profile of chickpea was erratically affected depending on nutrient (or antinutrition factor) type, process type and cultivar (Tables 1 and 4). Stachyose content in chickpea was reduced by at least 90% following process S5 suggesting that boiling of germinated seed followed by dehulling is more effective in reducing stachyose compared to other processes. Process S4 and S3 were conducted in order to establish whether dehulling prior to cooking is better with reference to antinutrition factor reduction. Our results indicate that stachyose reduction was higher when cooking dehulled chickpeas (38 - 50%) compared to cooking un-dehulled chickpeas (2%). Our results on stachyose reduction during cooking of whole chickpeas are similar those reported in other studies [2, 13].

Sucrose content decreased by almost 50% in all process types studied but process S4 regardless of cultivar. Reduction in sucrose following boiling has been reported in previous studies [14]. However, our result indicate that boiling of dehulled seeds results in more sucrose loss than boiling of whole grains (Table 1). The phytic acid content increased in all process type but the degree of increment varied with cultivar. Phytic acid increased with 35% in all process type in *desi* but with 8- 20% in *kabuli* (Table 1). In contrast, fibrulose content decreased in all process types and *desi* resulted in more % reduction compared to *kabuli* cultivar (Table 1). The effect on mineral content was erratic as some mineral registered an increased while others decreased in all processes (Table 4). In particular calcium, copper, iron, potassium and magnesium decreased in all processes. Process S5 resulted in moderate loss or increase in mineral content compared to other processes.

DISCUSSION

Physical characteristics

Kabuli cultivars as expected had higher weight, volume, hydration capacity, and swelling capacity compared to *desi* cultivars. The lack of variation in seed density, hydration index and swelling index indicates that the flours obtained from chickpeas will also have same water absorption capacity regardless of the cultivar. Furthermore chickpea seems to have the same seed density (1.2 g/ml), swelling index (1.26) and hydration index (0.97) as our results were identical to previous reports [1, 6].

Comparison of nutrition composition between chickpea cultivars

The raffinose family oligosaccharides (RFOs), namely ciceritol, raffinose, stachyose, and verbascose are not desirable as usually result in bloating or flatus when consumed due to human inability to digest 1-6 galactosidic linkage [15]. The variation observed could be as a result of difference in thickness of the seed coat which can be supported by high percent stachyose reduction in *desi* in all processes under study. Our results on stachyose are consistent with those reported in literature [5, 11-14]. The amount of fibre tends to increase with seed coat thickness in most grains due to their physiological function. In our unreported results, we found that *desi* had mean seed coat percent of 13.5 compared to 6.7 in *kabuli* cultivars which correlates with fibrulose

being higher in *desi* cultivars. Other researchers have also reported that *desi* cultivars have more fibre compared to *kabuli* cultivars [11].

The observed variation in mineral content in different strains of chickpea in our results is also reported in literature [1, 5]. The results indicate that mineral density of chickpeas is inversely related to the seed weight. Similarly, calcium content has been reported to decrease with increase in seed weight [16]. We suspect that the ratio of seed coat to total seed weight plays an important role in mineral density of chickpeas. It is widely accepted in legumes and cereals that seed coat or bran has higher mineral content compared to the endosperm. Despite the mineral content variation, the reported values indicate that daily consumption of 100g chickpea by 6 – 36 months old children will meet their mineral needs as per FAO mineral requirements. Calcium is the only mineral that might need supplementation to improve Ca:P ratio to >1 for efficient mineral absorption.

Effect of combined processes on chickpea

Previous studies including our unpublished data have indicated that germination eliminates stachyose. Germination for 72 hours followed by cooking was expected to completely eliminate stachyose but our results have shown only 90 – 98 % reduction. We suggest that cooking might have released bound stachyose due to deformation of protein structure. Han and Baik also found that soaking chickpeas for 12 hours reduced stachyose content from 27 to 8.8 mg/g but stachyose content of the soaked chickpeas increased to 25.6 mg/g after cooking [12]. We therefore stipulate that cooking and germination ensures complete reduction of oligosaccharides in legumes other than germination alone. It was also pleasing to note that highest reduction (98%) occurred in *desi* which apparently had the highest stachyose content. Literature suggests that cooking of soaked legumes including chickpea has an added advantage in reduction of stachyose. For example, stachyose content of cooked un-soaked chickpeas was 62.9 mg/g whereas that of cooked soaked chickpeas was as low as 25 mg/g [12]. However our results suggest that soaking of dehulled chickpeas does not have significant advantage in terms of stachyose reduction to not soaking. We suggest that reduction of RFO during soaking of raw chickpea is partly enzymatic mediated process (part of germination) and these enzymes are contained in the seed coat.

Dietary fibres like oligosaccharides might cause flatus when consumed in larger amounts and the problem can even be worse when taken by children. The trend in fibre reduction was almost similar in all process types which suggest that the reduction was due to dehulling which was common to all. The reduction was more pronounced in the *desi* than *kabuli* which can be explained by their difference in proportion of seed coat. Like fibre, phytic acid loss in our study might have resulted from dehulling and not germination nor cooking. Our results suggest that phytic acid content is mostly present in the cotyledon part of the seed and not in the hull. The bioavailability of iron and calcium is reported to vary with phytate content of chickpea such that cultivars with highest in vitro bioavailability of calcium and iron had low phytate content.

The in vitro bioavailability of calcium and iron was found to be 64 and 40%, respectively, when total phytic acid content of chickpea was 1070 mg/100 g and the in vitro bioavailability of that was 24 and 31%, respectively, when phytic acid content was 1341 mg/100 g [17]. Our results however indicate that the cultivar used had very low phytic acid content (100 mg/100 g) suggesting higher bioavailability of minerals. We have demonstrated as well that phytic acid cannot be eliminated by using the simple traditional technologies. Therefore, cultivars with low phytic acid content (like the ones we used) should be used for follow-on formula production to ensure high mineral bioavailability. It was important to note that the processed chickpea can still meet the mineral requirements of children despite reduction in some minerals. The decrease is the result of some minerals leaching into water during soaking or cooking and removal of the seed coat. Other studies before did report calcium, zinc and phosphorous increase following cooking and germination of legumes [18 - 20]. On the other hand, different studies have also reported decrease in calcium and zinc following cooking soaking or decortication. For example, potassium decreased by 10% following decortication and 50% after cooking whole chickpea grains [5]. Similarly, another study reported a decrease of 60% in potassium following boiling [13]. The content of all other minerals were reported to reduce following cooking or decortication by other researchers before [5, 13].

CONCLUSION

As shown in this study, germination for 72 hrs followed by boiling, drying and dehulling resulted in highest reduction in stachyose, fibres, sucrose and less negative effect on the mineral composition of chickpea compared to dehulling, soaking, and cooking; dehulling and cooking; and cooking of whole grain chickpeas. Phytic acid was not affected by any process combination which can be overcome by use low phytic acid cultivars. It is feasible to use chickpea as an excellent source of all minerals regardless of cultivar type.

Table 1: The effect of processing on sucrose, stachyose, fibrulose and phytic acid content

Process	Sucrose (%)	Stachyose (%)	Fibrulose (%)	Phytic acid (mg/100 g)
Raw <i>Desi</i> (Israel)	4.6	4.54	6.08	76.0
DS2	1.4	1.83	1.51	106.0
DS3	-	-	-	104.0
DS4	2.06	2.29	0.66	104.0
DS5	1.68	0.12	0.69	103.0
Raw <i>kabuli</i> (bar)	6.86	2.32	3.23	78.0
KS2	3.29	1.42	1.31	94.0
KS3	3.62	1.45	1.03	92.0
KS4	5.72	2.28	2.66	89.0
KS5	3.71	0.26	1.42	84.0

Note: K stands for kabuli cultivar and D for desi. S2 = dehulled, soaked and later boiled; S3= Dehulled and boiled; S4 = boiled and dehulled; S5 = soaked, germinated, boiled and dehulled

Table 2: Physical characteristics of studied chickpeas

	Seed weight (g)	Seed volume (ml)	Seed density (g/ml)	Seed weight after 24 hrs soaking (g)	Seed volume after 24 hrs soaking (ml)	Hydration capacity (g)	Hydration index	Swelling capacity (ml)	Swelling index
Bar	0.424 ^b	0.338 ^b	1.253 ^a	0.837 ^b	0.740 ^b	0.413 ^b	0.973 ^a	0.402 ^b	1.187 ^a
Zehavit	0.367 ^c	0.283 ^c	1.296 ^a	0.733 ^c	0.653 ^c	0.366 ^{cd}	0.998 ^a	0.370 ^{bc}	1.308 ^a
Raz	0.336 ^d	0.253 ^d	1.327 ^a	0.663 ^d	0.597 ^d	0.327 ^d	0.974 ^a	0.343 ^c	1.356 ^a
HA500	0.505 ^a	0.397 ^a	1.273 ^a	0.988 ^a	0.860 ^a	0.483 ^a	0.957 ^a	0.463 ^a	1.169 ^a
Yarden	0.336 ^d	0.310 ^c	1.333 ^a	0.813 ^b	0.710 ^b	0.400 ^{bc}	0.970 ^a	0.400 ^b	1.294 ^a
Israel desi	0.132 ^e	0.1 ^e	1.326 ^a	0.273 ^e	0.234 ^e	0.141 ^e	1.061 ^a	0.134 ^d	1.343 ^a

Note: Means on the same column with different superscripts are significantly different ($p < 0.05$).

Table 3: Comparison of mineral content in different chickpea cultivars

Sample name	Calcium (mg/100 g)	Iron (mg/100 g)	Potassium (mg/100 g)	Magnesium (mg/100 g)	Manganese (mg/100 g)	Phosphorous (mg/100 g)	Zinc (mg/100 g)
Desi Israel	142.18 ^a	2.76 ^f	1030.24 ^b	144.77 ^a	1.92 ^b	397.10 ^b	1.86 ^e
Bar	87.75 ^d	2.33 ^g	941.82 ^e	120.09 ^e	1.57 ^c	358.41 ^d	3.06 ^c
Malawi kabuli	92.86 ^c	6.95 ^a	1011.84 ^c	130.80 ^b	1.36 ^d	368.25 ^{cd}	3.73 ^a
Malawi desi	134.22 ^b	6.66 ^b	1016.12 ^c	131.43 ^b	2.44 ^a	374.36 ^c	3.63 ^b
Zehavit	68.22 ^f	4.63 ^d	1070.10 ^a	124.88 ^d	1.93 ^b	347.08 ^e	1.52 ^f
Raz	82.21 ^e	3.05 ^e	952.36 ^d	130.84 ^b	1.53 ^c	407.54 ^a	3.77 ^a
Yarden	92.99 ^c	5.1 ^c	1032.91 ^b	127.88 ^c	1.96 ^b	354.67 ^{de}	2.22 ^d
DRI (6 – 36 months children)	270 - 500	11 - 7	700 - 3000	75 - 80	0.6 – 1.9	275 - 460	3

Note: Means on the same column with different superscripts are significantly different ($p < 0.05$). DRI (Dietary Reference Intake) are based on United Nations Food and Agriculture Organization [21]

Table 4: Effect of different processes on mineral composition

Sample name	Calcium (mg/100 g)	Copper (mg/100 g)	Iron (mg/100 g)	Potassium (mg/100 g)	Magnesium (mg/100 g)	Manganese (mg/100 g)	Sodium (mg/100 g)	Phosphorous (mg/100 g)	Zinc (mg/100 g)
a) Desi Cultivar									
Raw desi	142.18 ^c	0.65 ^b	2.76 ^c	1030.24 ^a	144.77 ^{cd}	1.92 ^c	94.25 ^a	397.10 ^d	1.86 ^e
DS2	175.10 ^b	0.63 ^c	2.56 ^d	278.30 ^c	133.07 ^d	1.86 ^d	57.20 ^b	450.89 ^b	3.56 ^b
DS3	176.79 ^b	0.64 ^{bc}	2.59 ^d	247.83 ^d	146.94 ^c	1.80 ^e	58.46 ^b	447.44 ^{bc}	3.01 ^d
DS4	183.96 ^a	0.85 ^a	2.90 ^b	303.21 ^b	163.61 ^a	2.38 ^a	57.06 ^b	475.75 ^a	3.25 ^c
DS5	181.59 ^a	0.49 ^d	3.03 ^a	306.48 ^b	150.40 ^b	2.28 ^b	60.75 ^b	435.66 ^c	3.69 ^a
b) Kabuli Cultivar									
Raw Kabuli	87.75 ^d	0.60 ^b	2.33 ^a	941.82 ^a	120.09 ^a	1.57 ^c	13.99 ^d	358.41 ^c	3.06 ^d
KS2	163.11 ^a	0.60 ^b	2.23 ^{ab}	339.21 ^e	115.34 ^c	1.36 ^d	36.60 ^a	394.65 ^a	4.56 ^a
KS3	163.30 ^a	0.67 ^a	2.07 ^c	384.50 ^d	117.56 ^b	1.59 ^b	37.44 ^a	396.95 ^a	3.92 ^b
KS4	108.52 ^c	0.66 ^a	1.98 ^c	543.73 ^b	109.51 ^d	1.63 ^a	23.86 ^c	379.76 ^b	3.59 ^c
KS5	126.94 ^b	0.56 ^c	2.11 ^{bc}	508.46 ^c	118.23 ^b	1.58 ^{bc}	30.00 ^b	361.89 ^c	3.88 ^b

Note: Means on the same column with different superscripts are significantly different ($p < 0.05$). K stands for kabuli cultivar and D for desi. S2 = dehulled, soaked and later boiled; S3 = Dehulled and boiled; S4 = boiled and dehulled; S5 = soaked, germinated, boiled and dehulled

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