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Effect of dehulling on nutritive value of quinoa seed

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In this era of ever-increasing world population, newer food and feed crops that have been hitherto neglected are gaining recognition. The rejection of such lesser-known food crops has been due not to any inferiority but to the lack of research resources in the place of origin and often to their being scorned as "poor people's plants." Quinoa whole and Quinoa dehulled was analyzed and reported that Depending on the chemical analysis of Quinoa whole, Quinoa dehulled, the Quinoa dehulled considered nutritionally dense due to its better nutritional composition and low anti-nutrients than Quinoa whole.

Key Words : *Chenopodium quinoa*, Pseudo cereals, Antioxident

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INTRODUCTION

The Indian subcontinent is a large land mass covering India, Pakistan, Nepal, Sri Lanka and Bangladesh and it sustains 20 per cent of the world' population. The area is prone to degradation of its natural resources due to intensive cultivation leading to declining soil fertility, changes in water table depth, deterioration in the quality of irrigation water, and rising salinity in the region. Much of the population has little access to a protein-rich diet, since wheat and rice are the principal food grains grown and consumed in the area. The growing population necessitates increased food production combined with a shift towards environmentally sound sustainable agriculture. It is therefore important to select crops requiring fewer inputs while able to respond to the nutritional deficiency prevalent in the region. Quinoa is still an "underutilized" crop, given its nutritional superiority over traditional crops and its wide adaptability to diverse agronomic conditions, and its commercial potential in South Asia has remained untapped. Quinoa, seed plant of *Chenopodium quinoa* is an annual broad-leaved plant, 1-2 m tall with deep penetrating roots which can be cultivated from sea level upto an altitude of 3800 m. It is a grain with intrinsic outstanding characteristics. Aspects like exceptional nutritional quality, genetic variability, adaptability to adverse climate and soil conditions, and low production cost constitutes quinoa as a strategic crop with potential contributor to food security and sovereignty. Quinoa adapts to desert, hot and dry climates. This crop can grow with relative humidity from 40% to 88% and survive with temperatures from -4°C to 38°C. It is resistant to low soil moisture, and can produce acceptable yields even with precipitations from 100 to 200 mm. Due

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to its ability to adapt to adverse climate and soil conditions where other crops are unable to grow, harvest can be obtained at altitudes from sea level to 4000 m. The cultivation of quinoa provides an alternative for countries with limited food production. The history of its human consumption reaches back 5000 years (Ando *et al.,* 2002 and Oelke *et al.,* 2012). Quinua (*Chenopodium quinoa*) has been cultivated in the Andean region for several thousand years, being one of the main grain crops supplying highly nutritious food.

Quinoa is an important food source for human consumption in the Andean region and has immense industrial value (Bhargava *et al.,* 2006 and Fuentes and Bhargava, 2011). The crop grows in different ecological zones, from sea level to 2000– 4000 m asl (Bazile *et al.,* 2013 and Fuentes and Bhargava, 2011). Quinoa has been selected by FAO (2014) as one of the crops destined to offer food security in the 21st century, because the quinoa plants are tolerant to salinity and drought stress,and can grow on marginal regions (Jacobsen *et al.,* 2003). The edible seeds of quinoa are small, round and flat. Seed colors can range from white to grey and black, or can be yellow and red. *Chenopodium quinoa* was considered as the mother of cereals. Today everyone knows that it is one of the oldest crop plants, included in the group of the so-called 'pseudocereals'. Seeds of this species are distinguished by high nutritive valuebecause of its very good chemical composition, high proportion of vitamins, microelements, fat, including essential unsaturated fatty acids (EFA), mainly linoleic and linolenic acids (Coulter and Lorenz, 1990). However, the greatest advantage of this plant is the content andquality of protein. Quinoa seed have a high protein content (about 15%), and its essential amino acid balance is excellent, because of a wider amino acid spectrum than cereals and legumes (Ruales and Nair, 1993), with higher lysine (5.1–6.4%) and methionine (0.4–1.0%) contents. Quinoa contains lysine, methionine and cysteine higher than common cereals and legumes making it complementary to these crops. Quinoa's protein quantity ranged from 10.4 per cent to 17.0 per cent depending on itsvariety.

The seeds are an excellent example of functional food, defined as lowering the risk of various diseases and exerting health-promoting effects (Repo Carrasco-Valencia and Serna, 2011 and Vega-Galvez *et al.,* 2010). Besides nutrients, quinoa contains bitter and toxic compounds (saponins) especially in the hull. Therefore, quinoa in most cases is dehulled/polished and washed (Lopez Garcia, 2007). Research is focusing on developing effective dehulling methods to remove saponins and on cultivating new 'sweet' cultivars that contain less saponins (Galwey *et al.,* 1990; Koziol, 1992 and Reichert *et al.,* 1986).

Quinoa farming and consumption in India is still at a nascent stage however recent impetus in this direction has already been taken. One of recent project "project Anantha" by Andhra Pradesh was sought to push quinoa, with its lower water intake, as an alternative crop in the dry terrain of Anantapur district. The United Nations has declared 2013 the International Year of Quinoa, which aims at focusing global attention on the role it can play in contributing to food security, nutrition and poverty eradication and policies (Burlingame *et al.,* 2012 and FAO, 2013). The worldwide popularity of quinoa and initial promising reports from Asia make it an important candidate as an alternative crop in this region. And this could be achieved only by an integrated effort at all levels: information, awareness, popularization, research and marketing.

METHODOLOGY

Locale of the study:

The present study was conducted at Department of Food and Nutrition, College of Home science, Maharana Pratap University of Agriculture and Technology, Udaipur, (Rajasthan).

Collection of samples:

Quinoa sample as whole (QW) and Dehulled (QD) were purchased from local market of Udaipur (Rajasthan) in a single lot to avoid varietal difference.

Chemical properties:

Nutritional components:

Quinoa whole (QW), Quinoa dehulled (QD) were analyzed for nutritional content. Nutritional evaluation of the Quinoa whole (QW), Quinoa dehulled (QD) was done for their proximate composition and mineral estimation (calcium, iron, zinc, potassium, phosphorus) were analyzed (Plate A). Standard procedures were used for the estimations. Percentage carbohydrate and energy contents were determined by calculation using difference method, respectively. The procedures have been described as under:

Proximate composition:

It is the determination of a group of closely related compounds together. It includes determination of amount of moisture, protein, fat (ether extract), ash and fibre with nitrogen free extract and carbohydrates being estimated by subtracting the sum of these five percentages from 100.

Moisture:

Moisture is the major component of food. The moisture content of any food is determined not only to analyze the chemical composition of food material on moisture free basis but also to assess the shelf life of the products.

Moisture content of samples was analyzed by the method described by NIN (1983). Ten gram sample was weighed in a dried and weighed petri dish. The weight of the sample along with the petri dish was taken at regular intervals until a constant weight was obtained. The moisture percentage was calculated using following formula:

$$\text{Moisture (g/100g)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of sample (g)}} \times 100$$

Crude protein:

The protein nitrogen is converted into ammonium sulphate by boiling with concentrated sulphuric acid. It is subsequently decomposed by the addition of excess alkali and the liberated ammonia is absorbed into boric acid solution containing an indicator by steam distillation. Ammonia forms a loose compound, ammonium borate with boric acid, which is titrated directly against standard HCl. The protein content of food stuff is obtained by estimating the nitrogen content of the material and multiplying the nitrogen content by the factor 6.25 (NIN, 1983). Kjel plus nitrogen estimation system was used to estimate the amount of nitrogen in the samples. 0.2 g moisture free sample was transferred to the digestion tube. Ten ml of concentrated sulphuric acid and 3 g catalyst mixture (5 parts of K_2SO_4 + 1 part of $CuSO_4$) was added and was left overnight. The tubes were then placed in a pre-heated digestion block. The digestion block was pre heated to 60°C for 10 minutes. Once the digestion tubes were placed, temperature was further increased to 100°C and samples were kept until the colour of the samples turned bluish green or colorless. Digested samples were taken for distillation where the ammonium radicals were converted to ammonia under excess alkali

Crude protein estimation

Estimation of crude fat

Atomic absorption spectrophotometer

Saponin estimation estimation of phytic acid

Plate A : Chemical analysis of Quinoa

post neutralization of acid in the digested samples with 40 per cent sodium hydroxide. Mixed indicator (methyl red + methyl blue) was added to the solution and titrated with the standardized N/10 HCl. The titration value was determined and the following formula was used to estimate the amount of nitrogen liberated:

$$\text{Nitrogen (g/100g)} = \frac{\text{14.01 x Normality of HCl (0.1) x (TV – BV)}}{\text{SW (g)}} \text{ x 100}$$

where, 14.01= Ammonia's molecular weight
0.1N= Titration solution (HCl) normality
TV= Titer value
BV= Blank value
SW= Sample weight
Protein % = % N × 6.25 (For food samples)

The protein content of the sample was obtained by multiplying the nitrogen with a factor 6.25.

Crude fat:

Fat was estimated as crude ether extract of moisture free sample by the method given by Jain and Mogra (2006). Fat content of the sample was estimated on Soxhlet Plus system, which works on the principle of improved soxhlet method. Weighed amount of moisture free sample (5 g) was placed in a thimble. The thimble was inserted in the thimble holder to be kept in an already weighed beaker and 80 ml petroleum ether (60-80^0 C) was poured in the beaker. The beakers were loaded in the system and temperature was set at 100^0 C. The process was left to operate for 120 minutes and the temperature was increased to the recovery temperature, which was twice the initial boiling temperature. Rinsing was thus done twice in order to collect the remaining fat in the sample. Beakers were taken out and put in a hot air oven. Thimble holders were removed from the beakers and the beakers were weighed. The amount of fat present in the sample was calculated using the following formula:

$$\text{Fat (g/100g)} = \frac{\text{Weight of either extract fat (B – A)}}{\text{Weight of sample (g)}} \text{ x 100}$$

where, A= Weight of empty flask (g)
B= Weight of flask+ fat (g)
B-A = Weight of fat (g)

Ash:

Ash was estimated by the method given by Jain and Mogra (2006). Five grams of moisture free sample was weighed in previously heated, cooled and weighed crucible. Sample was then completely charred on the hot plate, followed by heating in muffle furnace at 600^{0}C for 5 hours. The crucible was cooled in desiccators and weighed. The process was repeated till constant weights were obtained and the ash was almost white or grayish in color. Ash content of samples was calculated using following formula:

$$\text{Ash (g/100g)} = \frac{\text{Weight of ash (g)}}{\text{Wight of sample taken (g)}} \text{ x 100}$$

Crude fibre:

Fibre is an insoluble vegetable matter indigestible by proteolytic and diastatic enzymes and cannot be utilized except by microbial fermentation. It is usually composed of cellulose, hemicelluloses and lignin. Crude fibre estimation was done as per the method given by 3 gram of moisture and fat free sample was placed in 500 ml beaker and boiled with 200 ml of 1.25 per cent sulphuric acid for thirty minutes. The volume was kept constant during boiling by adding hot distilled water. This was filtered through muslin cloth and the residue was washed with hot distilled water till free from acid. The residue was then transferred to same beaker and boiled for 30 minute with 200 ml of 1.25 per cent sodium hydroxide solution. After boiling, mixture was filtered through muslin cloth and the residue was washed again with hot distilled water till free from alkali followed by washing with 50 ml alcohol and ether. Then it was taken into a crucible (it was weighed before as (W_1) and residue was dried in an oven at 130^{0}C for 2-3 hours, cooled and weighed (W_2). Heat in muffle furnace at 600^{0}C for 2-3 hours, then cool and weigh again (W_3). Crude fiber was determined using following formula:

$$\text{Per cent crude fibre} = \frac{(W - W_1) - (W_3 - W_1)}{\text{Weight of sample}} \text{ x 100}$$

where, W_1= Weight of empty crucible
W_2 =Weight of crucible with dry residue
W_3 = Weight of crucible with heated residue

Carbohydrate:

The carbohydrate content of the sample on dry weight basis was calculated by difference method (Jain and Mogra, 2006) as given below:

Carbohydrate (g/100g) = 100 – (moisture + crude fibre + ash + protein + fat)

Energy:

The energy value of sample was calculated using physiological fuel value *i.e.* 4, 9, 4 kcal per gram of protein, fat and carbohydrate, respectively.

Energy (kcal/100g) = [(% protein x 4) + (% carbohydrate x 4) + (% fat x 9)]

Mineral profile:

Mineral solutions of selected samples were prepared by wet ashing method compiled by Jain and Mogra (2006). The plant material was digested with a mixture of acids to form a clear white precipitate which was then dissolved in water and made upto a definite volume. An aliquot from this was used for determination of selected minerals.

Wet ashing:

One gram moisture free sample was taken in a digestion tube and 5 ml of concentrated HNO_3 was added to it and was left overnight. It was then heated slowly for 30 minutes and cooled. Five ml of perchloric acid (70%) was added and heated over digestion block until the particles were completely digested and the solution became clear. After digestion, volume of digested matter was made upto 50 ml with double distilled water. Prepared mineral solution was stored in makeup bottles and mineral analysis was done by atomic absorption spectrophotometer (AAS4141)

Total antioxidant activity:

Principle:

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Various antioxidant activity methods have been used to monitor and compare the antioxidant activity of foods. These analytical methods measure the radical scavenging activity of antioxidants against free radicals like the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical.

Reagents required:

The reagents used for the study were 2,2- diphenyl-1-picryl-hydrazyl(DPPH), methanol, obtained from Merck or sigma. All reagents used were of analytical grade.

Extraction method:

The dried powder of sample was extracted individually by cold percolation method (Parekh and Chanda, 2007) using methanol to determine the antioxidant activity. 10g of dried powder was taken with 100ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120rpm for 24 hrs. After 24 hrs the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. Supernatant was collected and the solvent was evaporated and the dry extract was stored at 4^0 C in air tight bottles.

Procedure:

The reaction mixture consisted of DPPH in methanol (0.3mM, 1 ml) 1 ml methanol and the solvent extracts (1ml) was incubated for 30 min. in dark, after triplicate and expressed in mean average. Control solution was also prepared and zero was set using solvent methanol. The free radical scavenging activity was calculated according to the following equation:

$$\text{Scavenging (\%)} = \frac{(A_0 - A_1) * 100}{A_0}$$

where, A_0 – Absorbance of the control

A_1 – Absorbance of sample

The DPPH scavenging activity was determined using the method followed by Ranilla *et al.* (2010) with slight modifications. Summarily, 250 µL of quinoa beverage was added to 4ml of 60 µM DPPH solution prepared in 95 per cent ethanol. The reaction mixture was placed in a dark environment for about 20 minutes and absorbance was read at 517 nm. For comparison, 250 µL of 95 per cent ethanol was used as control. Percentage inhibition was calculated according to the formula:

$$\text{\% inhibition} = \frac{\text{DPPH}_{\text{control}} \; \text{DPPH}_{\text{test sample}}}{\text{DPPH}_{\text{control}}} * 100$$

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation as well as relevant discussion have been summarized

Table 1 : Proximate analysis of whole Quinoa seed flour (QW), Dehulled Quinoa seed flour (QD)

Sr. No.	Treatment	Nutrients g/100g													
		Moisture		Fat		Ash		Protein		Fibre		CHO		Energy(Kcal)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	QW	4.09	0.610	4.6	0.156	3.6	1.67	12.52	0.73	8.98	8.84	65.25	1.45	361.49	7.11
2.	QD	2.89	0.455	3.88	0.735	2.88	1.81	12.23	3.09	7.99	12.385	63.56	1.99	377.04	3.59

under following heads :

Proximate analysis:

Moisture, crude fat, ash, crude protein, crude fibre, carbohydrates and energy contents of QW, QD are depicted in Table 1and discussed below:

The chemical analysis of Quinoa seed for proximate composition for moisture, fat, ash, protein, fibre and energy. Moisture content was higher in QW (4.09g/100g) followed by QD (2.89g/100g). Highest amount of crude fat content was exhibited in QW (4.6g/100g) followed by QW (3.88g/100g). Ruales and Nair (1993) reported that Quinoa seeds have approximately 9 per cent fat on a dry weight basis. Quinoa fat has a high content of oleic acid (24%) and linoleic acid (52%). Whole seed or dehulled seeds of Quinoa seed contain 5-6 per cent total lipids. Protein, the body building nutrient, According to results protein was 12.23g/100g in QD and 12.52g/100g in QW. Gonzalez *et al.* (1989) conducted a study and result revealed that the seeds have a higher nutritive value than most cereal grains. Quinoa also contains all ten essential amino acids, and its protein content ranges from 12.9 to 16.5 per cent. Of primary interest is the high lysine value, an essential amino acid that is deficient in many grains. The protein content of about 15% in quinoa is much higher than that found in cereals such as wheat, barley, oats, rice, and sorghum. The soluble protein contents in quinoa are similar to those in barley and higher than those in wheat and maize. Total ash was found in QW and QD (3g/100g) and (2.88g/100g). Ogungbenle (2009) studied the Nutritional evaluation and Nutritional properties of quinoa (*Chenopodium quinoa*) flour the content of ash was found between the range of 1.2%-4.08% among flour.

QW and QD showed higher content of crude fibre (8.98g/100g and 7.99g/100g). Lamothe (2015) Greater consumption of fibre-rich whole grains is associated with a lower risk of type 2 diabetes and cardiovascular disease. Quinoa is an excellent source of dietary fibre, comprising about 2.6%-10% of the total weight of the grain; about 78% of its fibre content is insoluble and 22% soluble. It was observed that all two variations of Quinoa seed exhibited carbohydrate content of QW and QD which ranged from 63.56 g to 65.25/100g. Yao (2014) found that Starch, as a carbohydrate, provides the major source of physiological energy in the human diet. The content of starch in quinoa ranges from 58.1% to 64.2% of dry matter, of which 11% is amylose. The energy values can also be seen to be varying possibly due to protein and carbohydrate content among QW and QD. The values ranged from 361.49 kcal in QW to 377.04 kcal in QD. The total content of components depends on the variety or environmental factors Meneguetti *et al.* (2011).

Mineral profile:

Quinoa seed are also rich in micronutrients such as minerals and vitamins. Table 2 shows the mineral content of QS and quinoa flour. The main minerals are calcium, iron, zinc potassium, phosphorus (Table 2).

The major mineral contents for QW, QD are presented in Table 2. The difference was found between flours for calcium, Iron, Zinc, potassium, phosphorus. In case of calcium, QW recorded higher value 86.3 ppm than QD (55.1). Abdelazim Sayed and Abdelazim Abdellatif (2018) Quinoa flour and quinoa flat bread had the balanced minerals content as (mg/100 g) Magnesium 502 and 560, Potassium 732 and 755, Manganese 444 and 489, Copper 0.75 and 0.88, Iron 10.5 and 15.56, Phosphorus 411 and 487, Zinc 4.1 and 5.66, calcium 86.3 and 89.56 and Sodium 2.44 and 1130.55 mg/100 g,

Table 2 : Mineral composition of Quinoa seed whole (QW), Quinoa seed dehulled (QD)

Sr. No.	Treatment	Calcium(mg)		Iron(mg)		Zinc(mg)		Potassium (mg)		Phosphorus (mg)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1.	QW	86.3	0.6	15.0	0.1	4.0	0.1	732.0	5.5	411.0	4.1
2.	QD	55.1	0.4	14.2	0.1	4.0	0.1	656.0	4.3	404.9	3.0

Table 3 : Anti- nutritional analysis of Quinoa seed whole (QW), Quinoa dehulled (QD)

Sr. No.	Treatment	Saponin%		Phytic acid%	
		Mean	SD	Mean	SD
1.	QW	9.13	0.80	10.36	1.90
2.	QD	4.16	1.00	6.23	2.40

Table 4 : Total Anti- oxidant activity analysis of Quinoa seed whole (QW), Quinoa dehulled (QD)

Sr. No.	Treatment	Total antioxidant activity	
		Mean	SD
1.	QW	44.34	2.19
2.	QD	32.54	0.94

respectively. The distribution of minerals in quinoa seeds revealed that phosphorus and magnesium were localized in embryonic tissue, while calcium and potassium were present in the pericarp (Kiaus *et al.*, 2012; Konishi *et al.*, 2004 and Mohammad *et al.,* 2017) found that abrasion of quinoa seeds (for saponin elimination) caused specifically a decrease in calcium content. Calcium (83.33 mg/100g), magnesium (202.17 mg/100g), zinc (4.23 mg/100g) and acid were also higher in raw flour. The total content of minerals in amaranth, quinoa and oats is about twice as high as in other cereals (Dyner *et al*., 2007 and Sadiq *et al.,* 2008).

Iron content was higher in QW (15.0 ppm) followed by QD (14.2 ppm). Among two flours zinc content was found no difference in QW and QD (4.0 ppm). Potassium was higher in QW (732.0 ppm) than QD (656.0 ppm). Phosphorus was also higher in QW (411.0 ppm). Koziol (1992) has summarized that the contents of K (927 mg/100 g), Ca (149 mg/100 g), Mg (250 mg/100 g), P (384 mg/100 g), S (150–220 mg/100 g), Fe (13.2 mg/100 g), and Zn (4.4 mg/100 g) in quinoa seeds are much higher than those of cereals such as wheat and rice. Konishi *et. al.* (2004) studied the content of Ca, K, Mg, Fe, Zn, P were analyzed in the whole Quinoa seed and Dehulled Quiona seed. There is relatively small difference in the content.

Anti-nutritional analysis:

The anti-nutritional factors *viz.,* saponin and phytic acid was analyzed in QW and QD. The results obtained are presented in Table 3 and discussed below:

The saponin content in quinoa seed was 0.14% to 2.3%. These values are higher than those in soybean and oat, but lower than in green pea (Mastebroek *et al.,* 2000; Guclu-Ustundag and Mazza, 2007). Saponin content was found to be highest in QW (9.13) than QD (4.16). Ridout (1991) reported that Quinoa contains about 1.0% to 1.2% saponins, which are bitter and have anti nutritional effects. To be edible, quinoa grains must have the saponins removed, since they affect the colour and palatability of the products. The phytic acid content was lower in QD (6.23 %) than QW (10.36%). Phytic acid is not only present in the outer layers of Quinoa seeds, as in the case of rye and wheat, but is also evenly distributed in the endosperm. Ranges of 10.5 to 13.5 mg/g of phytic acid for five different varieties of quinoa were reported by Koziol, similar to the range of 7.6 to 14.7 mg/g for other cereals. Depending on chemical analysis of Quinoa whole (QW) and Quinoab dehulled (QD), the Quinoa dehulled considered nutritionally dense due to its better macro and micronutrient and low anti-nutritional content than Quinoa whole. According to Vega-Galvez (2010) the general content of phytic acid in quinoa is low and ranges from 10.5 mg to 13.5 mg, in comparison with corn that contains 720 mg, wheat 390 mg and rice 60 mg.

Total antioxidant activity:

DPPH is a free radical generating compound and has been widely used to evaluate the free radical scavenging ability of various antioxidants. Antioxidant activity was evaluated by measuring the DPPH radical scavenging activity of Quinoa whole (QW) and Quinoa dehulled (QD).

Bhaduri (2016) conducted a study on Antioxidant and Anti proliferative Activities of Quinoa and result revealed the antioxidant activity (1586 ± 41.42) and DPPH scavenging capacities (82.71 ± 0.03) of quinoa seed. The anti oxidant activity in quinoa seed whole and dehulled was 44.34 and 32.54. Anti oxidant activity was found to be highest in QW than QD and there difference was found in the anti oxidant activity.

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