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Effect of soaking on anti-nutritional factors in the sun-dried seeds of hybrid pigeon pea to enhance their nutrients bioavailability

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Abstract

Certain anti-nutritional factors (ANFs) interact with proteins and form insoluble complexes hence, decreases bioavailability of proteins. Twenty four different hybrid varieties of pigeon pea [*Cajanus cajan* (L.) Millsp.] were analysed for reduction in ANFs on different soaking treatments. Soaking in distilled water and different salt solutions viz. 1% NaHCO₃, mixed salt solutions (MSS) (1.5% NaHCO₃+0.5% Na₂CO₃+0.75% citric acid) led to a significant (P<0.05) reduction in total phenols, ortho-dihydroxyphenols, tannins and phytates. Phenols and tannins are water soluble and present in seed coat hence, a large reduction observed after simple soaking in distilled water i.e 33.07% and 40.9% respectively. Soaking in distilled water, 1% NaHCO₃ and MSS reduced ortho-dihydroxyphenols by 17.39%, 34.78% and 47.83% whereas phytate by 14.9%, 18.3%, and 21% respectively. Amongst various methods studied, soaking in MSS found to be the most effective in reducing levels of ANFs in pigeon pea hybrids.

Keywords: pigeon pea, hybrids, anti-nutritional factors (ANFs), soaking, salt solutions, MSS

Introduction

Pulses constitute one of the richest sources of including valuable but incompletely balance protein, particularly in vegetarian's diet (Ghadge *et al.*, 2008) [20] and are consequently an important part of the people's diet in many parts of the world (Arinathan *et al.*, 2003; 2009) [4, 5]. Among the pulses crops, pigeon pea [*Cajanus cajan* (L.) millsp.] is the most widely produced and consumed food legume worldwide belongs to family *Leguminosae*, also known as red gram, arhar, tur *dhal*. The fruit of the pigeon pea is classified as a pod and each pod contains 3 to 5 seeds which are round or lens in shape. Pigeon pea originated from India and Asia where it travelled to African countries (Onyebuashi, 1986) [33]. India known as the largest producer and consumer of pigeon pea worldwide due to its predominantly grown and consumption. In pigeon pea production, India has highest production of 265000 MT in the world followed by Myanmar (900000 MT), Malawi (237210 MT), United Republic of Tanzania (206057 MT), Kenya (89390 MT), Uganda (84200 MT), Dominican Republic (27998 MT) and Nepal (14082 MT) (FAOSTAT, 2012) [19]. The India's 1st ranks in pulse production have become constant since history of long many back decades (FAOSTAT, 2012) [19]. Pigeon pea is economically and nutritionally important legume as major source of proteins in poor communities of many tropical and subtropical regions of the world viz. India, Myanmar, Malawi, United Republic of Tanzania, Kenya, Uganda, Dominican Republic, Nepal etc. (Singh *et al.*, 1984; ICRISAT 1986; FAOSTAT, 2012) [19, 22]. Inadequate availability and consumption of protein foods in developing countries are a major concern as large segments of population of these countries suffer from protein malnutrition (Arinathan *et al.*, 2009; Soris and Mohan, 2011) [5, 41]. It has been estimated that the total production of legumes provide almost as much protein (20-30%) to the world as wheat and over 50 % more than rice or corn (Rockland *et al.*, 1981; Gopalan *et al.*, 1985) [21, 37].

Nutritionists look for available substitute of protein, which satisfy nutrient requirements of pigeon pea seeds used in daily diet, because of protein possess immense health-related benefits and also contribute the best alternative due to their high nutritive value. They contain a high level of crude protein ranges from 21-30% (Udedibie and Igwe, 1989; Amaefule and Onwudike, 2000) [43, 3]. The composition of matured and dry pigeon pea supply significant amount of energy through water (11.5%), carbohydrates (63.4 %), lipids (1.2 %), dietary fibres (4.4%) and minerals (3.5%), starch also the major constituents of pigeon pea (Ihekoronye and Ngoddy, 1985). In addition, pigeon pea contains reasonable levels of thiamine, riboflavin and niacin (Bressani *et al.*, 1974; Arora, 1977) [18, 6]. In spite of the nutritional potential of the pigeon pea, they are underutilized as food.

On the other hand, pigeon pea contain some anti-nutritional factors (ANFs) in their seeds and meal which decrease the availability of these nutrients (Ahmed *et al.*, 2006) [2] and may have adverse effects on human nutrition. These ANFs are mainly tannins, phenols, phytic acids, phytates and trypsin inhibitors. It was reported that those ANFs might reduce the nutritional quality of dry beans even if they are present in low concentrations (Abd El-Hady and Habiba, 2003) [1]. Therefore, pigeon peas need to be nutritionally improved and remove or reduce the amount of these compounds. Several treatments such as fermentation, germination, thermal treatments (boiling, cooking and autoclaving), dehulling and soaking procedures have been applied to remove or reduce the amount of these compounds from pigeon peas (Abd El-Hady and Habiba, 2003; Martin-Cabrejas *et al.*, 2004) [1, 27].

In general, these processing techniques improve not only the flavour and palatability of legumes but also increase the protein digestibility, bioavailability of nutrients, by destroying the ANFs (Chau and Cheung, 1997; Martin-Cabrejas *et al.*, 2004; Khandelwal *et al.*, 2010) [27, 9, 25]. As a matter of fact, there is very few published research found on the content and alterations of ANFs of hybrid pigeon pea varieties.

The present investigation was carried out with an attempt to gain an insight into the effect of soaking on anti-nutritional factors (ANFs) (*viz.* phenols, ortho-dihydroxyphenols, tannins and phytates) of pigeon pea [*Cajanuscajan* (L.) millsp.] to enhance their nutrients bioavailability with the removal of the anti-nutritional factors (ANFs) and make it edible safe protein sources for global consumption.

Materials and Methods

Seed Samples

The laboratory experiments were conducted in the Laboratories of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (Punjab) - 141004, India. For this purpose, twenty four hybrids pigeon pea genotypes *viz.* PHP-23, PHP-24, PHP-25, PHP-26, PHP-27, PHP-28, PHP-30, PHP-32, PHP-33, PHP-34, PHP-35, PHP-36, PHP-37, PHP-38, PHP-39, PHP-40, PHP-41, PHP-42, PHP-44, PHP-45, PHP-47, PHP-48, PHP-49, and PHP-50 were procured from Department of Plant Breeding and Genetics of the university. The samples were sun-dried, cleaned to free them from extraneous matter and ground in powder form. The powdered samples were stored in air tight bottles at room temperature (25 °C).

Sample Preparation (Soaking)

The sample preparation depicted in Figure-1. The soaking of seeds of each genotype was done separately in distilled water, 1% sodium bicarbonate solutions and mixed salt solution (MSS) (1.5% NaHCO₃+0.5% Na₂CO₃+0.75% citric acid) for 18 hours. After soaking, the excess of water in the seeds was drained off. The seeds were then dried in an oven at 55°C and dried sample was finely ground into flour to pass through a 50 mesh screen for further analysis.

Determination of Total Phenol Content

Total phenols were estimated using Folin Ciocalteu reagent (Swain and Hillis 1959) [42]. Methanol extract (0.5 ml) was evaporated to dryness and the residue was redissolved in distilled water and mixed thoroughly. To this 0.5 ml of Folin-Ciocalteu reagent was added and shaken thoroughly. After 5 min, 1 ml of saturated sodium carbonate solution was added and rested for 1 hour. Absorbance of the blue colour was recorded at 725 nm. Concentration of total phenols was

determined from the standard curve which was prepared using 10-60 µg gallic acid.

Determination of Tannins Content

Tannins were determined by Folin Denis reagent (Swain and Hillis 1959) [42]. An aliquot of 1 ml was taken and the volume was made to 8.5 ml with distilled water. To this, 0.5 ml of Folin-Denis reagent was added. After 3 min, 1 ml of saturated sodium carbonate solution was added. Mixed well and absorbance was measured at 760 nm. Standard curve was prepared using different concentrations of tannic acid.

Determination of Ortho-dihydroxy Phenols Content

Ortho-dihydroxyphenols were assessed by the method of Nair and Vaidyanathan (1964) [31]. Methanol extract (5 ml) evaporated to dryness. The residue left behind was dissolved in 1 ml of distilled water and shook well. Added 0.5 ml of 10% TCA, 1 ml of 10% sodium tungstate, 0.5 ml of 0.5 N HCl and 1 ml of 0.5 % sodium nitrite and mixed thoroughly. A yellow colour developed. After 5 min, 2 ml of 0.5 N NaOH was added. The colour changes to light cherry colour which was read at 540 nm. Standard curve was prepared using the range of 10-70 µg of catechol.

Extraction and Determination of Phytic Acid Content

Extraction for phytic acid was done by refluxing 0.5 g sample with 20 ml of 0.5M nitric acid for 3-4 hrs with continuous shaking followed by centrifugation. The supernatant was used for the estimation. Phytic acid was analysed by the method of Davies and Reid (1971) [11]. 0.2-1.0 ml of the filtrate was diluted with distilled water to a final volume of 1.4 ml to which 1.0 ml of a solution of ammonium ferric sulphate containing 50 µg iron was added. After thorough mixing, were placed in boiling water bath for 20 min. When cooled to room temperature, 5 ml amyl alcohol and 0.1 ml ammonium thiocyanate solution was added to each test tube. The contents of the test tubes were immediately mixed by inversion and shaking. After centrifugation for a short time (5 min) at low speed, the intensity of the color in the amyl layer was determined at 465 nm against an amyl alcohol blank exactly 15 min after addition of the ammonium thiocyanate solution.

Statistical Analysis

Experiments were conducted in triplicates. Data were analyzed on the basis of statistical analysis. The mean, standard error, critical difference and their statistical significance were ascertained with the help of software CPCS1 and GSTAT.

Results and Discussion

The nutritional importance of a given feedstuff in a diet is well evident, depends not only on the nutrient composition, but also on the presence of anti-nutritional factors (ANFs). Hence, elimination or inactivation of ANFs is absolutely necessary to improve the nutritional quality of pigeon pea and effectively utilize their full potential as human food. Total phenolic, tannins, ortho-dihydroxy phenol and phytate content of pigeon pea hybrids is depicted in Table-1. Soaking of pigeon pea seeds is the most simple and inexpensive method for bringing reductions in polyphenols, tannins and other water soluble ANFs. The significant (P<0.05) percent reductions in ANFs of pigeon pea following different soaking treatments is observed as shown in Figure-2. Chopra and Sankhala (2004) [10] stated that soaking is effective in significantly reducing ANFs and concomitantly increasing

nutrients bioavailability of legumes. During soaking, legumes come in contact with water which causes leaching of water soluble ANFs into water.

The phenolic compounds or their oxidized products form complexes with essential amino acids, enzymes and other proteins, thus lowering their protein digestibility and nutritional values (Shahidi and Naczki 1992) [38]. Total phenol content varied widely from 3.09 mg/g to 12.32 mg/g with a mean value of 6.56 mg/g in pigeon pea hybrids as given in Table-1. PHP-35 had the lowest phenol content among all hybrids tested followed by PHP-28 and PHP-26. Hence, these genotypes are promising with respect to phenolic content. Figure-3 shows that mean phenolics content decreased from 6.56 mg/g to 4.39 mg/g (percent reduction of 33.07%) by soaking in distilled water. More or less the same percentage losses of phenolics have been observed earlier during soaking in *C. cajan* (Igbiedioh *et al.*, 1995) [23]. Reductions in total phenolic content were 42.8% after soaking in 1% NaHCO₃ (Figure-2). The contents of phenolics in pigeon pea were reduced to the maximum extent when soaked in salt water as compared to distilled water. This accords with an earlier report in *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al.*, 1995) [44]. The marked loss of phenolics when subjected to soaking in salt solution may be attributed to its effect in creating an ionic environment. The changed ionic environment in turn might change the seed coat permeability. This enables greater and rapid solid losses (Deshpande, 2002, Mulimani and Supriya, 1994) [8]. The most pronounced effect amongst the salt solutions was registered by soaking in MSS which reduced mean phenolics upto 2.56 mg/g (60.9% reduction). Significant differences (P<0.5) were observed within processed pigeon pea seed samples. Soaking in salt solution showed an improvement to the protein quality (DeLeon *et al.*, 1992) [12].

The tannin causes a decrease in the digestibility of protein and carbohydrates as a result of the formation of insoluble enzyme-resistant complexes (Reddy *et al.*, 1985). Deshpande *et al.* (1986) [13] reported that polyphenols could react with proteins and enzymes so they could also act as trypsin inhibitors and amylase inhibitors. Tannin content was ranged between 0.57 to 2.87 mg/g in pigeon pea hybrids. PHP-38 had the lowest tannin content among all hybrids. This accords with an earlier study of Ene-Obong (1995) in pigeon pea but the levels of tannin of pigeon pea hybrids in the present study (Table-1) seem to be slightly higher as compared with that observed by Onweluzo and Nwabugwu (2009). This variation in results may be due to varietal differences. Tannins are located in seed coat and being water soluble, simple soaking can reduce large content of tannins (Figure-2 and 4). Reduction in tannin content was 40.9% due to soaking in distilled water. This was slight similar to an earlier investigation in different varieties of *Pisum sativum* (Bishnoi *et al.*, 1994) and *Vicia faba* (Sharma and Sehgal, 1992) [39]. Reduction was not much pronounced after soaking in NaHCO₃ and MSS over soaking in distilled water on the tannin content of the seed samples. Ortho-dihydroxy phenol content varied from 0.11 mg/g to 0.45 mg/g and PHP-38 was found to have the lowest content among all hybrids tested, followed by PHP-47 and PHP-45. Figure-5 shows a very small amount of ortho-dihydroxy phenols was found in hybrids. Soaking in distilled water reduced total ortho-dihydroxy phenol content upto 17.39%. Percent reduction further increased to 34.78% by soaking in 1% NaHCO₃ and 47.83% by soaking in MSS (Figure-2).

Among all the ANFs, phytic acid is one of prime concern for human nutrition and health management (Kumar *et al.*, 2010). The phytate molecule is negatively charged at physiological pH and is reported to bind nutritionally important essential divalent cations, such as iron, zinc, magnesium and calcium. This forms insoluble complexes, thereby making minerals unavailable for absorption (Rimbach *et al.*, 1994). Phytic acid content was exhibited in range of 6.24 mg/g to 10.08 mg/g (mean 8.62 mg/g). PHP-33, PHP-34 and PHP-35 had the lower content of phytic acid, hence, promising with respect to phytate content. Egli *et al.* (2002) and Mulimani *et al.* (2003) [29] reported higher values of phytate in millet and pigeon pea. However the disparity may be expected since according to Reddy *et al.* (1982), phytate level varies in legumes and cereals with variety, cultivar type and soil type among other factors. Effect of soaking on phytate content in pigeon pea hybrids is shown in Figure-6. Phytate is water-soluble, thus a considerable amount of phytate is removed into the water on soaking (14.9%). In addition, this process also enhances the action of naturally occurring phytase in legumes (Kumar *et al.*, 2010). There was 18.3% reduction after soaking in 1% NaHCO₃ and 21% reduction after soaking in MSS for phytic acid (Figure-2). Similar results for reduction in phytic acid in soaked legumes have been reported earlier by Deshpande and Cheryan (1983) [14]. El-Hady and Habiba (2003) [1] observed a 36% reduction in phytic acid in kidney beans after an overnight soaking in water at room temperature. The loss in phytates during soaking of the tested samples may be due to leaching of phytate ions into the soaking water under the influence of a concentration of gradient (difference in chemical potential), which governs the rate of diffusion.

Table 1: Anti-nutritional factors (ANFs) content (mg/g) in Pigeon pea [*Cajanus cajan* (L.) Millsp.] hybrids

S. N.	Pigeon pea Hybrids	Total Phenol	Tannin	Ortho-dihydroxy Phenol	Phytate
1.	PHP-23	6.07±0.02	1.51±0.02	0.20±0.007	7.31±0.03
2.	PHP-24	5.01±0.03	1.43±0.02	0.25±0.021	8.41±0.04
3.	PHP-25	3.84±0.04	1.32±0.01	0.31±0.011	8.68±0.06
4.	PHP-26	3.43±0.02	1.45±0.03	0.45±0.009	8.46±0.05
5.	PHP-27	8.75±0.03	2.87±0.05	0.32±0.013	7.37±0.05
6.	PHP-28	3.32±0.01	1.59±0.04	0.20±0.015	9.01±0.06
7.	PHP-30	3.78±0.06	2.27±0.04	0.23±0.005	9.30±0.04
8.	PHP-32	4.02±0.02	1.77±0.05	0.27±0.013	8.21±0.03
9.	PHP-33	5.51±0.03	1.70±0.03	0.23±0.015	6.24±0.03
10.	PHP-34	4.08±0.01	1.40±0.03	0.19±0.021	6.78±0.02
11.	PHP-35	3.09±0.02	1.20±0.02	0.17±0.023	7.21±0.05
12.	PHP-36	4.93±0.03	1.57±0.04	0.21±0.015	9.58±0.06
13.	PHP-37	4.76±0.02	1.25±0.03	0.25±0.017	9.43±0.05
14.	PHP-38	3.86±0.03	0.57±0.01	0.11±0.022	9.41±0.04
15.	PHP-39	6.37±0.02	1.70±0.01	0.30±0.023	8.69±0.08
16.	PHP-40	9.74±0.04	2.08±0.02	0.35±0.021	10.06±0.03
17.	PHP-41	8.38±0.02	1.50±0.05	0.22±0.020	9.26±0.02
18.	PHP-42	6.77±0.05	1.22±0.03	0.20±0.019	9.31±0.04
19.	PHP-44	9.16±0.04	1.49±0.03	0.12±0.011	7.36±0.02
20.	PHP-45	11.33±0.08	2.12±0.02	0.15±0.016	9.35±0.07
21.	PHP-47	10.21±0.03	1.88±0.02	0.12±0.024	8.38±0.05
22.	PHP-48	8.43±0.04	1.71±0.03	0.21±0.008	10.08±0.17
23.	PHP-49	12.32±0.15	2.38±0.04	0.19±0.012	9.71±0.03
24.	PHP-50	10.28±0.03	1.76±0.02	0.23±0.007	9.32±0.01
	MEAN	6.56	1.66	0.23	8.62
	CD(P<0.05)	0.0494	0.0328	0.0328	0.065

Values are mean of three replicates ± Standard error

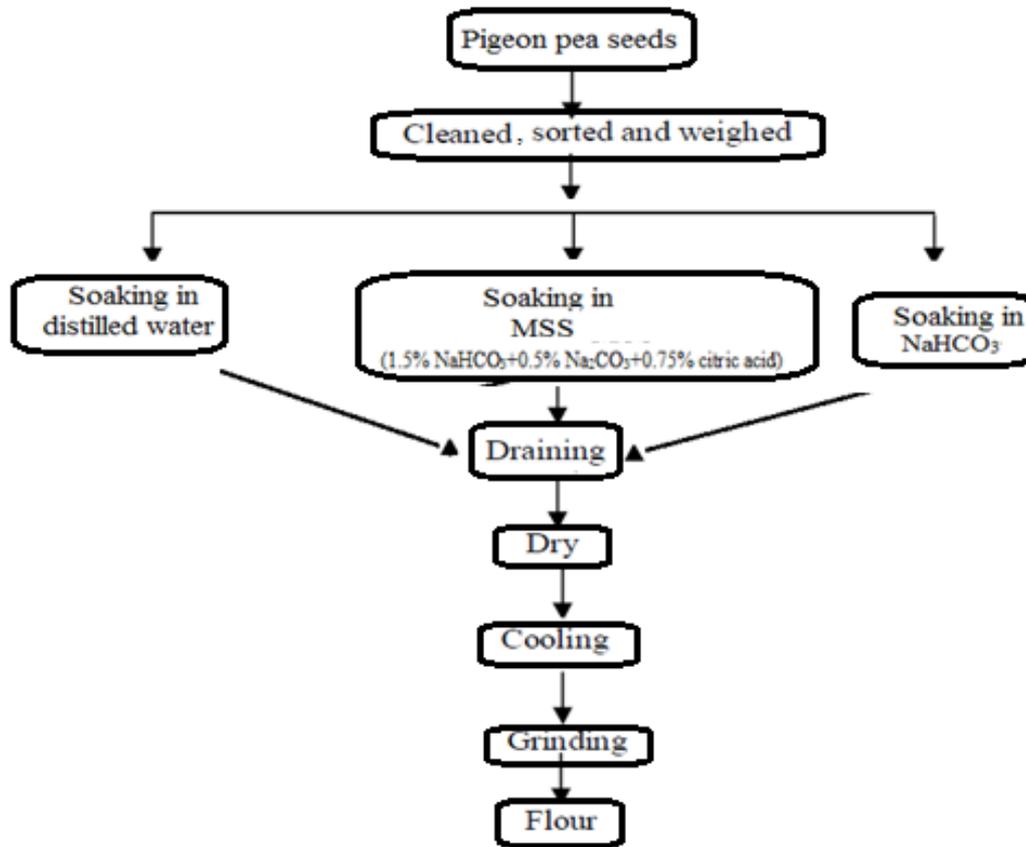


Fig 1: Flow diagram for sample preparation

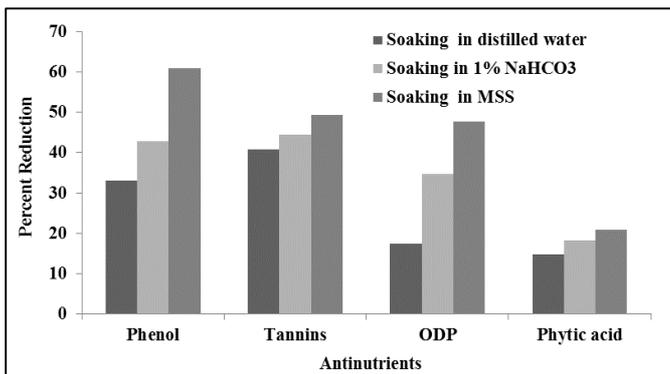


Fig 2: Percent reduction in ANFs of pigeon pea hybrids by different soaking treatments. Data are expressed as mean \pm SE of twenty four pigeon pea hybrids. Significant reductions were observed ($P \leq 0.05$). ODP, ortho-dihydroxyphenol

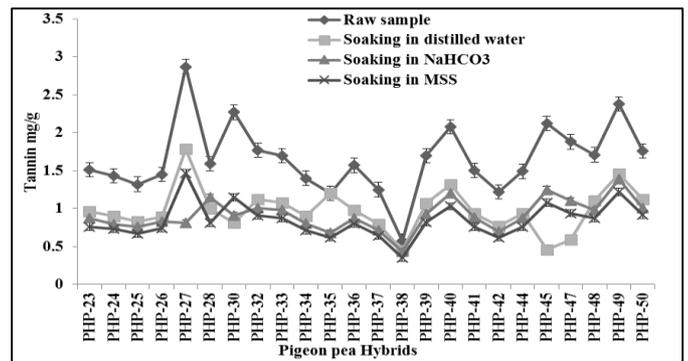


Fig 4: Effect of different soaking treatment on tannin content (mg/g) in pigeon pea hybrids. Significant differences were observed within processed pigeon pea seed samples w.r.t. total tannin content ($P \leq 0.05$)

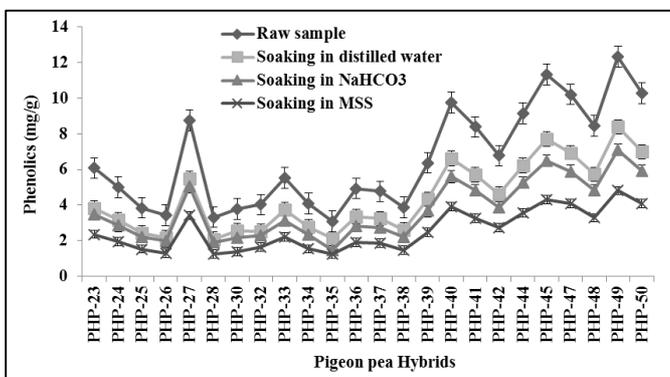


Fig 3: Effect of different soaking treatment on phenolics content (mg/g) in pigeon pea hybrids. Significant differences were observed within processed pigeon pea seed samples w.r.t. total phenolics content ($P \leq 0.05$)

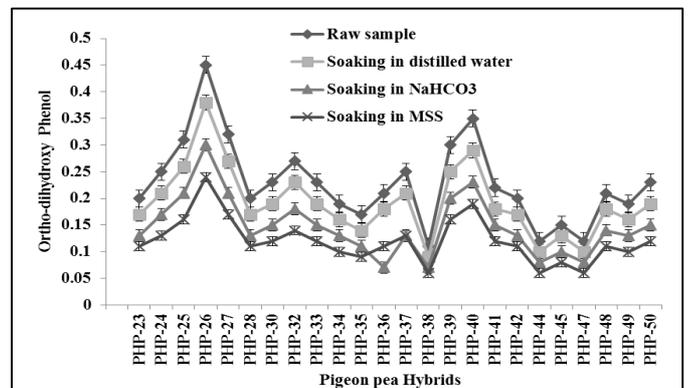


Fig 5: Effect of different soaking treatment on ortho-dihydroxyphenol content (mg/g) in pigeon pea hybrids. Significant differences were observed within processed pigeon pea seed samples w.r.t. ortho-dihydroxyphenol content ($P \leq 0.05$)

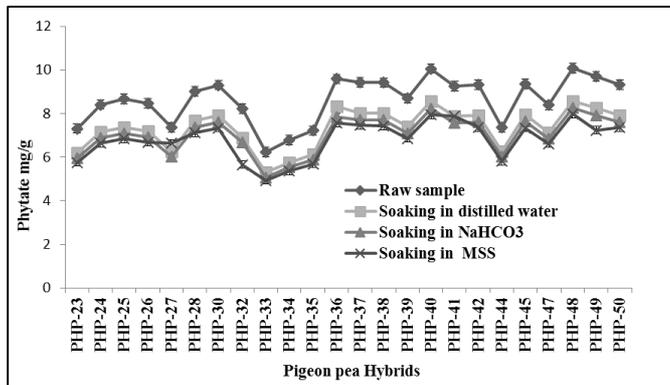


Fig 6: Effect of different soaking treatment on phytate content (mg/g) in pigeon pea hybrids. Significant differences were observed within processed pigeon pea seed samples w.r.t. total phytate content ($P \leq 0.05$)

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