International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org || Volume 3 Issue 12 || December 2014 || PP.07-10

Evaluation Of Genotypic Variation And Soaking Induced Changes In Certain Anti- Nutritional Factors And Antioxidants In Chickpea (*Cicer Arietinum* L).

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ABSTRACT: *Influence of applied water soaking on raw and treated chickpea seeds were investigated for their antinutritional content viz trypsin inhibitor, tannins, phytic acid and total phenolics. The water soaking treatments caused significant decrease in tannin, phytic acid and trypsin inhibitor activity however, total phenolic contents exhibited a conspicuous increase may be as a result of soaking as compared to raw seeds. Based on these results water soaking is recommended for chickpea preparation not only for improved nutritional quality but also for higher absorption rates in the body.*

KEY WORDS: *Chickpea seeds; Soaking; Anti-nutritional factors Send correspondence to Department of Biosciences, Christian Eminent College Indore, India.*

I. INTRODUCTION

Though legume seeds contain a moderately high amount of proteins, certain minerals, vitamins and their subsequent use in food; is still limited due to presence of certain antinutritional factors (ANFs). These include tannins [1], phytic acid [2], trypsin inhibitors [3,4] and flatulence-causing oligosaccharides [5]. Tannins inhibit the digestive enzymes and thus lower the digestibility of most nutrients, especially protein and carbohydrates [1]. Phytic acid was considered as one of the antinutrients mainly because of its ability to bind essential dietary minerals as well as proteins and starch, and thereby reducing bioavailability for humans [6]. However trypsin inhibitors strongly inhibit trypsin activity reducing the digestion and absorption of dietary protein [7]. These compounds have alternative beneficial roles in the plant defense mechanism thus enhancing productivity [8,9,10]. Agronomic trait, such ANFs increases the crop productivity and thus influences market value. It is also important to know the nutritional status of seeds and their subsequent biochemical changes after soaking. Some of the adequate and economic technologies aimed at reducing or removing ANFs of legume treatment prior to their human and animal consumption. Hence, the present study was undertaken to study the soaking effects on the seeds of chickpea cultivars grown in Madhya Pradesh region for varying time duration and analyzing its effect on anti nutritional contents.

II. MATERIALS & METHOD

2.1 Materials : Total eight chickpea (*Cicer arietinum* L.) cultivars were selected for analysis. The mature and dry seed material was obtained from R.A. K. College of Agriculture Sehore, Madhya Pradesh.

2.2 Methods : Soaking: A sample (100 g) of seeds was soaked in distilled water at a ratio of 1:4 (w/v) for 12 hours at room temperature.

2.3 Chemical analysis : Trypsin inhibitor (TI) activity was determined following the standard procedures as described by Kakade *et al*. [11] as modified by Hammerstrand *et al.* [12]. The total phenolic content (TPC) of each sample was estimated using the Folin– Ciocalteu colorimetric method according to Mallick and Singh [13]. Phytic acid was estimated following Wilcox *et al.* [14]. Tannins were measured as tannic acid equivalents Swain *et al.* [15]. DPPH radical scavenging activity was determined according to the methods described by Liangi *et al.* [16]. and Hitoshi *et al.* [17]. Methanolic extraction was done as per the procedures described by Tomoyuki *et al.* [18] and Chidambara *et al.* [19].

III. STATISTICAL ANALYSIS

All work was done in triplicates and the data presented are means ±standard deviation on the dry weight basis. Data were analyzed using a paired t- test. Significance was accepted at $p \leq 0.05$

IV. RESULTS & DISCUSSION

The effect of various treatments on chickpea seed under investigation for trypsin inhibitor, phytic acid, tannic acid and total phenolics are presented in the table1. The significant (($p \leq 0.05$) differences in the protein and non protein composition between dry and water soaked of chickpea seeds except for treatments of phenol composition. These results are in agreement with [20] and further in accordance with [21].Trypsin inhibitor is said to be one of the major antinutritional factor of raw and soaked seed. The results obtained by Khattab *et al.* [21] included water soaking; boiling, roasting and microwave cooking affected the nutritional quality of such legume seeds by these physical treatments. They observed that cooking generally inactivates heat sensitive factor such as trypsin inhibitor as a result of denaturation of these heat labile proteins caused up to 88.80 to 94.35% trypsin inhibitor activity reduction in different sample. The earlier reports of Alaa *et al*. [22] reported that the inhibitory activity of some of the pulses like chickpea, lentil and mung bean decreased in most seeds after soaking in water at room temperature for 24 hours and that heating the soaked legume seeds at 121º C for 30 min brought complete inactivation of trypsin inhibitors unit in these pulses. Our results exhibited the same pattern of reduction in protein based antinutritional factors in the chickpea genotypes that are selected for present investigation. Alajaji and EI- Adawy [23] have studied the effects of nutrition composition of chickpea as affected by microwave cooking and other traditional cooking methods on nutritional and antinutritional composition of chickpea seeds. They observed that cooking chickpea by microwave not only save the time but also retains the most nutritive value. In our present investigation the soaking treatments have declined the antinutritive factors thus maintaining the nutritive value of seed intact in terms of trypsin inhibitor.

The maximum reduction in the level of phytic acid content was observed (JGK1) when the seeds of *Cicer arietinum* were soaked in water for 12 hours at room temperature. This could be due to the fact that phytic acid in dried legumes exists wholly as a water soluble salt (probably potassium phytate) [24]. Udensi *et al.* [5] have studied the thermal processing effect on antinutritional factors of vegetable cowpea. They observed prediction in phytic acid contents of legume seeds during heat treatments attributed may be partly due to the heat labile nature of the phytic acid and in the formation of insoluble complex between phytate and other components. In a similar study Cream and Haisman [24] reported that in cooking phytic acid contributes with Ca and Mg in the seeds to form insoluble Ca and Mg phytates. Further they concluded that regardless of pulse type different physical treatments drastically reduce the antinutritional load of the seed with moist heating being the most effective to ensure their safety and quality in the food and feed using combination of techniques. Influence of soaking was found to be minor effects on reducing up to the levels of tannin 4.10 %. The lowest reduction in the tannin contents were observed in JGK2. This may be due to the fact that this compounds show their predominance on the seed coats [25] are water soluble [26] and consequently leach into the liquid medium .Such consequences of decrease in the tannin content could be related to the fact that these compounds are heat labile. Mubarak [27] have studied the nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus* L) as affected by some home traditional processes. They found that the tannin contents of mung bean seed was reduced after water soaking for 12 hours, boiling in tape water for 90 minutes, autoclaving at 121°C for 30 minutes respectively. Our results are in agreement with the observation of Mubarak [27] having minor reduction in tannin contents in chickpea seeds. However, the results stated by Rehman and Shah [28] for the tannin content of black gram, red kidney bean, and white kidney bean was significantly reduced after ordinary cooking and pressure cooking respectively for which our results deviate.

The total phenolic contents estimated in various chickpea seeds exhibited that the highest amount of total phenolics were elevated up to 3 times in JGG1 in water soaked condition. In similar study however, Paramjyothi and Anjali [29] reported significant decrease in polyphenols content of chickpea soaking. However a significant increase in methanol extract of germinated seeds after 72 hours renewed the synthesis of polyphenols or degradation of high molecular weight insoluble polymer into smaller molecular weight that might have reacted with the reagents [30]. In similar study Amal khatak [31] have studied the influence of germination techniques on phytic acid and polyphenols contents of chickpea sprouts. Using time and type of illumination techniques they observed that green light can be used for producing sprouts with high polyphenols (and consequently high antioxidative potential) while sprouts with low polyphenols and low phytic acid contents (and hence higher protein digestibility) can be produced under red and blue light for 48 hours. This may be attributed to their shorter wavelength and higher energy ranges established a core relationship between germination and light illumination. The invitro free radical scavenging activity in chickpea was done using DPPH method. Such studied were also taken by Rai *et al.* [32] in the *Nelumbo nucifera* seeds (HANN) to wistar rats and caused significant dose dependent increase in the level of SOD. In our studies however quantitative enhancement vis-avis total antioxidants levels were elevated and their correlation with antinutritional factors could be linked.

	Cultivars	Trypsin inhibitor (TIUmg/gm)		Phyticacid (mg/gm)		Tannin (mg/gm)		Phenolics (mg/100gm)		Antioxidant (% inhibition)	
		Before soaking	**After soaking**	**Before soaking**	**After soaking**	**Before soaking**	**After soaking**	**Before soaking**	**After soaking**	**Before soaking**	**After soaking**
1	JGK1	0.146±0.02	0.141±0.05	1.06±0.01	0.425±0.02	4.16±0.04	4.03±0.01	15.43±0.03	27.16±0.04	12.52	8.29
2	JGK2	0.196±0.04	0.189±0.01	0.65±0.01	0.53±0.01	6.12±0.04	6.10±0.04	19.2±0.01	25.6±0.04	9.48	7.08
3	JGK3	0.184±0.02	0.179±0.03	0.715±0.02	0.292±0.04	4.15±0.03	4.08±0.03	5.65±0.04	7.5±0.05	14.56	10.48
4	JG11	0.151±0.01	0.132±0.05	1.04±0.01	0.58±0.01	6.10±0.04	5.98±0.01	50.39±0.04	55.19±0.01	19.37	16.06
5	JG412	0.121±0.04	0.118±0.04	0.682±0.01	0.346±0.02	2.63±0.05	2.55±0.04	10.69±0.03	16.66±0.04	13.56	12.08
6	JG6	0.169±0.05	0.165±0.05	0.78±0.05	0.32±0.02	5.49±0.02	5.38±0.03	20.87±0.03	24.6±0.04	13.24	13.09
7	JGG1	0.216±0.02	0.208±0.04	1.07±0.05	0.892±0.02	3.29±0.03	3.18±0.04	18.12±0.01	54.41±0.03	16.76	13.63
8	JG14	0.143±0.05	0.123±0.03	0.73±0.05	0.543±0.01	3.9±0.02	3.74±0.03	10.34±0.04	22.12±0.05	9.13	7.41

Fig 1. Effect of soaking on antinutritional factors of *Cicer arietinum* L.

V. CONCLUSION

Chickpea is the alternative protein after milk in the large population country like India. The processing methods for *Cicer arietinum* L. is requisite due to its high content of antinutrients and hence their digestion. A simple and inexpensive processing technique like water soaking, keeping the nutrients improves its acceptability. Our results revealed that soaking significantly reduces certain antinutritional factors in chickpea as phytates, tannins, trypsin inhibitor and increases antioxidants after 12 hours soaking thus, enhancing its absorption in the body.

ACKNOWLEDGEMENT

The author is thankful to Dr. Rajesh Vyas, Principal of Christian Eminent College Indore and Prof. Y.S. Chouhan, Vice Principal of Christian Eminent College Indore India for providing all facilities to carry out the research work.

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