Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7006

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (5):** 1076-1081 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Effect of Soaking and Germination on Anti-Nutritional Factors of Garden Cress, Wheat and Finger Millet

Sumiran Patel and Samit Dutta*

Department of Food Business Management, College of Food Processing Technology and Bio-energy, Anand Agricultural University, Anand-388110, Gujarat, India

*Corresponding Author E-mail: sumiranpatel@gmail.com

Received: 20.09.2018 | Revised: 18.10.2018 | Accepted: 26.10.2018

ABSTRACT

***Objective**: The effects of soaking and germination on anti-nutrients of garden cress seeds, wheat and finger millet were estimated.*

***Methods**: The anti-nutrients determined were total cyanogen and oxalic acid for garden cress seeds, phytic acid and tannin for wheat and phytic acid and trypsin inhibitor activity for finger millet.*

***Results**: Total cyanogen and oxalic acid of soaked garden cress seeds decreased by 23.43 and 18.52 percent respectively whereas in germinated seeds, they were decreased by 46.85 and 54.15 percent respectively. Phytic acid of soaked and germinated wheat decreased by 12.09 and 48.94 percent respectively while tannin decreased by 19.26 and 50.78 percent in soaked and germinated wheat respectively. Phytic acid and trypsin inhibitor activity were decreased by 13.22 and 13.51 percent in soaked finger millet respectively whereas the same were decreased by 64.76 and 71.02 percent respectively for germinated finger millet.*

***Key words:** Garden cress, Wheat, Finger millet, Anti-nutritional factors, Soaking, Germination.*

INTRODUCTION

One of traditional medicinal plant loaded with nutrients is Garden cress (*Lepidiumsativum* L., Family: Brassicaceae), an annual erect herbaceous plant, cultivated all over India, North America and parts of Europe. In India, it is mainly cultivated in UP, Maharashtra, Gujarat, Rajasthan and Madhya Pradesh[1]. Cress is said to help regulate the menstrual cycle, and cress seeds help increase milk production and secretion in lactating mothers. Because of its high iron and protein content, it is good for post-partum and lactating mothers. Cress is also recommended in the treatment of iron-deficiency anaemia due to its iron content[12].

Wheat (Triticum spp.) is the main cereal crop in India. Wheat is the most important staple food crop for more than one third of the world population and contributes more calories and proteins to the world diet than any other cereal crops. The total area under the crop is about 29.8 million hectares in the country.

Cite this article: Patel, S. and Dutta, S., Effect of Soaking and Germination on Anti-Nutritional Factors of Garden Cress, Wheat and Finger Millet, *Int. J. Pure App. Biosci.* **6(5):** 1076-1081 (2018). doi: http://dx.doi.org/10.18782/2320-7051.7006

Wheat is considered as a good source of protein, minerals, B-group vitamins and dietary fiber although the environmental conditions can affect nutritional composition of wheat grains with its essential coating of bran, vitamins and minerals; it is an excellent health-building food[14]. Ragi or finger millet (*Eleusine coracana* L.) is one of the important millets grown in several regions of India. It is also commonly known as Koracan in Sri lanka and by different names in Africa and has traditionally been an important millet staple food in the parts of eastern and central Africa and India[7]. Finger millet is the richest source of calcium and iron Calcium deficiency leading to bone and teeth disorder, iron deficiency leading to anaemia can be overcome by introducing finger millet in our daily diet[20].

Anti-nutritional factors in seeds/cereals

Anti-nutritional factors are chemical compounds synthesized in natural foods and / or feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of food/feed) which exerts effect contrary to optimum nutrition. These anti-nutritional factors are also known as 'secondary metabolites' in plants and they have been shown to be highly biologically active. These secondary metabolites are secondary compound produced as side products of processes leading to the synthesis of primary metabolites[8].

Anti-nutritional factors are substances which reduce the nutritive value of foods by inhibiting digestibility and utilization of proteins. The nutritional value of pulses may be adversely affected by the presence of anti-physiological or toxic substances such as trypsin inhibitors, phytates, lectins, polyphenols, and flatulence causing agents, cyanogenic compounds, lathyrogens, esterogens, goiterogens, saponins, anti-vitamins and allergens[10].

Bioavailability is a general term that refers to how well a nutrient can be absorbed and used by the body. It can be affected by many factors such as the presence of anti-nutrients, for example, phytates, trypsin, tannin and polyphenols in foods, a person's need, fiber, competition with other nutrients and acidity of intestinal environment[18].

MATERIAL AND METHODS

Garden cress seeds used in the present study were procured from Medicinal and Aromatic Plant Research Station, Anand Agricultural University, Anand whereas wheat and finger millet were procured from the local market of Anand. After cleaning, the seeds/grains were subjected to different processing such as soaking and germination. The chemicals used for the analysis were of analytical grade (Lobachemie Pvt. Ltd). Double distilled water was used for the analysis.

Soaking

The raw, clean seeds and grains were soaked in water (1:3) with 0.1 per cent formaldehyde solution (to prevent mould growth during germination)[5]. The garden cress seeds were soaked for 12 h[17], wheat grains were soaked for 10 h[9] and finger millet were soaked for 12 h[13] at room temperature. The soaked grains were stirred periodically in order to remove the gases accumulated around the seeds and the steep water was changed after every 4 h interval.

Germination

The soaked garden cress seeds were washed, spread over the muslin cloth and then placed into seed germinator. The seeds were allowed to germinate for 8, 12 and 15 h at 15, 25 and 35 °C temperature and 90 per cent relative humidity. The wheat grains were allowed to germinate for 36, 48 and 72 h at 30, 34 and 37 °C temperature and 85 per cent relative humidity[9]. The soaked finger millet grains were allowed to germinate for 12, 24 and 36 h at 30, 34 and 37 °C and 80 per cent relative humidity[2]. The grains were removed and washed after every 12 h interval in order to prevent development of off smell.

Sample preparation

The germinated seeds/grains were then dried under vacuum tray dryer by layering them uniformly at 700 mm of Hg vacuum. Garden

cress seeds were dried at 50 °C for 4 h[19], Wheat grains were dried at 60 °C for 8 h[9] and Finger millet grains were dried at 60 °C for 6 h. After 2 h, the surface was scrapped for uniform drying and to prevent sticking of the grains to the tray surface. Germinated and dried grains were milled to form fine flour using a stone flour mill. Flour was sieved through 100 mesh sieve which passed flour with particle size of around 0.1 mm or lesser than that.

Determination of tannin

2 g of ground sample was taken in a soxhlet flask and refluxed with 25 ml methanol for 3-4 h. The extract was filtered with Whatman No. 1 filter paper and the volume was made to 25 ml with methanol. 1 ml of filtrate was taken into the test tube, 5 ml of HCl – Vanillin reagent was added and kept for 20 min at room temperature. The color intensity was measured at 525 nm in UV spectrophotometer. For establishing standard curve, 0.1 – 0.5 ml of standard catechin solution was taken and the total volume was made to 1 ml with methanol and followed the similar procedure as per the sample. The result was expressed as mg catechin equivalents per 100 g of sample[21].

Determination of total cyanogen

Weigh 20 mg of ground sample, transfer to 1 litre distillation flask or 800 ml Kjeldahl flask, add 200 ml water and let stand for 2 hours. Autolysis should be conducted with apparatus completely connected for distillation. Steam distill, collect 150 – 160 ml distill in NaOH solution (0.50 gm in 20 ml water) and dilute to definite volume i.e. 250 ml. Take 100 ml, add 8 ml of 6 M NaOH and 2 ml of Potassium iodide solution (5 per cent) and titrate with 0.02 M $AgNO_3$ until permanent turbidity appears. For easy recognition of the end point of titration, it is recommended that a black back ground be used.1 ml of 0.02 M silver nitrate contains 1.08 mg of Hydrocyanic acid[3].

Determination of oxalate content

1 g of the sample was weighed into 100 ml conical flask. A portion of 75 ml of 3 M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then filtered using Whatman No. 1 filter paper. The sample filtrate [extract] (25 ml) was collected and titrated against hot [80 to 90 °C] 0.1 N $KMnO_4$ solution to the point when a faint pink color appeared that persisted for at least 30 s. the concentration of oxalate in each sample was obtained from the calculation. 1 ml of 0.1 N $KMnO_4$ contains 0.006303 g oxalate[4].

Phytic acid estimation

The soaked, germinated and dried samples of wheat and finger millet were estimated for phytic acid content using the Phytic acid estimation kit (Megazyme International Limited, Ireland).

Determination of Trypsin inhibitor activity

A method developed by Kakade*et al*. 1974 was used to determine trypsin inhibitor activity. One gram of finely ground millet flour was extracted with 50 mL of 0.01 mol/L NaOH for 1.5 h. The pH of the suspension was between 8.4 and 10.

Portions (0, 0.6, 1.0, 1.4 and 1.8 mL) of millet suspension were pipetted into duplicate sets of test tubes and adjusted to 2.0 mL with water. After 2 mL of trypsin solution (4 mg trypsin in 200 mL 0.001 mol/L HCl) was added, each tube was placed in a water bath at 37 °C. To each tube, 5 mL of BAPA solution (40 mg of benzoyl- DL-arginine-p-nitroaniline [BAPA] in 100 mL water with 1 mL dimethyl sulphoxide) previously warmed to 37 °C was added. Exactly 10 min later the reaction was terminated by adding 1 mL of acetic acid (30 mL acetic acid in 100 mL distilled water). After thorough mixing, the contents of each tube were filtered (Whatman No. 3) and the absorbance of the solution measured at 410 nm wavelength against a reagent blank.

The reagent blank was prepared by adding 1 mL of the acetic acid to a test tube containing trypsin and water (2 mL each) before the 5 mL of BAPA solution was added. As the millet samples showed no detectable absorption at 410 nm, no sample blank had to be prepared. One trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 mL of the reaction mixture under the conditions used

herein. Trypsin inhibitor activity was expressed in terms of trypsin units inhibited (TIU). The experiment was performed in duplicate.

RESULTS AND DISCUSSION

The standardization of germination of garden cress seeds was based on reduction in the total cyanogen content and oxalic acid content. The total cyanogen in raw garden cress was 6.83 mg/ 100 g which was decreased upto 5.23 mg/ 100 g after 12 h of soaking and it was further decreased upto 3.63 mg/ 100 g after 16 h of germination at 25 °C. The increase in total cyanogen at 35 °C was due to there was no germination at that temperature because the seeds of garden cress became dry at that particular temperature. Decrease in total cyanogen during germination could be a possible explanation of the removal of free cyanides during germination which are responsible for the loss in nutritional value of the garden cress.

Oxalic acid content was reduced from 118.43 mg/100 g to 96.15 mg/100 g during the 12 h soaking. It was further decreased upto 53.85 mg/ 100 g after 16 h of germination at 25 °C. The reduction in oxalic acid during soaking and germination may be due to leaching of oxalate oxidase and oxalate decarboxylase. Similar results for reduction in oxalic acid content of soaked and germinated grains were reported by Brudzynski and Salamon[6].

Phytic acid in wheat was decreased from 744 mg/100 g to 654 mg/100 g during 10 h soaking. It was further reduced upto 381.32 mg/100 g after germination for 72 h at 37 °C. The reduction in phytic acid during germination could be attributed to leaching out during hydration as well as activation of phytase during germination[16,22].

Tannin content of raw wheat was in the range of 598.2 mg/100 g to 605.4 mg/100 g with mean value of 602.3 mg/100 g. It was reduced to 486.07 mg/100 g during 10 h soaking and further reduced upto296.30 mg/100 g after 72 h of germination at 37 °C. The reduction in tannin during germination could be attributed to the increased activity of polyphenol oxidase and other catabolic enzymes. During germination, these enzymes are activated, resulting in the hydrolysis of various compounds including carbohydrate, protein, fiber and lipid as well as phenolic compounds.

Phytic acid content of raw, 10 h soaked and germinated finger millet were estimated. Phytic acid level in raw finger millet was in the range of 674.30 mg/100 g to 678.61 mg/100 g with mean value of 676.77 mg/100 g. It was decreased upto 587.20 mg/100 g during 12 h of soaking and further reduced to 238.46 mg/100 g after 36 h of germination at 37 °C. The reason could be the same as described for the wheat above.

Trypsin inhibitor activity of raw finger millet was in the range of 6.37 TUI/mg to 6.87 TUI/mg with mean value of 6.59 TUI/mg. During the soaking, trypsin units were reduced from 6.37 TUI/mg to 5.70 TUI/mg which were further reduced to 1.91 TUI/mg after 36 h of germination at 37 °C. Reduction in the level of trypsin inhibitor activity in finger millet may be due to changes in both endosperm and axis of finger millet during soaking and germination. Since the TIA in the endosperm was very low compared with the high levels in the axis, it would appear that TIA was more degraded in the endosperm as reported by Mbithimwikya *et al*.[15].

CONCLUSION

The minimum total cyanogen and oxalic acid content in garden cress were found at 16 h of germination period at 25 °C temperature. There was no germination at 35 °C temperature because the seeds of garden cress became dry at that particular temperature. The minimum phytic acid and tannin content in wheat were found at 37 °C temperature after 72 h of germination. In finger millet, the maximum reduction in phytic acid and trypsin inhibitor activity were found after 36 h of germination at 37 °C temperature. The present study showed that soaking and germination improved the nutraceutical properties of garden cress, wheat and finger millet by

reducing the anti-nutritional factors which are responsible for reduction in the nutritive value of food by inhibiting digestibility and utilization of proteins. Therefore, these germinated seeds/cereals can be used in health foods formulations.

REFERENCES

1. Agarwal, N. and Sharma, S., Garden cress (*Lepidium sativum* L.) – A non-conventional traditional plant item for food product. *Indian Journal of Traditional Knowledge*, **12(4):** 699-706 (2013).
2. Ajay, P., Process Development of Nutri-rich Food for Pregnant and Lactating Women, M.Tech. Thesis. Submitted to College of Food Processing Technology and Bio-energy, Anand Agricultural University, Anand (2014).
3. AOAC, Official methods of analysis. Association of Official Analytical Chemists. Washington D. C., USA (1986).
4. AOAC, Official methods of analysis. Association of Official Analytical Chemists. Washington D. C., USA (2005).
5. Archan Sehgal, S. and Kawatra, A., Reduction of polyphenol and phytic acid content of pearl millet grains by malting and blanching. Department of food and nutrition, CCS Harayana Agricultural University, Hisar, Harayana, India (1998).
6. Brudzyński, A. and Salamon, A., The oxalic acid content in selected barley varieties grown in Poland, as well as in their malts and worts. *Journal of the Institute of Brewing*, **117(1):** 67-73 (2011).
7. FAOSTAT, Statistics, Food and Agriculture Organization, Rome. http://www.faostat.fao.org.Accessed on 29.02.2016 (1995).
8. Gemede, H. F. and Ratta, N., Antinutritionalfactores in plant foods: Potential health benefits and adverse effects. *International Journal of Nutrition and Food Sciences*, **3(4):** 284-289 (2014).
9. Hussain, I., Uddin, M. and Aziz, M., Optimization of antinutritional factors from germinated wheat and mungbean by Response Surface Methodology, *International Food Research Journal*, **18(3):** 957-963 (2011).
10. Jain, A., Kumar, S. and Panwar, J., Antinutritional factors and their detoxification in Pulses- A review. *Agriculture Review*, **30(1):** 64-70 (2009).
11. Kakade, M. L., Rackis, J. J., Mcghee, J. E. and Puski, G., Determination of trypsin inhibitor activity of soya products. *Cereal Chemistry*, **51:** 376 (1974).
12. Kasabe, P., Patil, P., Kamble, D. and Dandge, P., Nutritional, elemental analysis and antioxident activity of garden cress (*Lepidium sativum* L.) seeds. *International Journal of Pharmacy and Pharmaceutical Sciences*, **4(3):** 392-395 (2012).
13. Khokhar, S. and Chauhan, B., Effect of domestic processing and cooking on in-vitro protein digestibility of moth bean. *Journal of Food Science*, **51(4):** 1083 (1986).
14. Kumar, P., Yadava, R. K., Gollen, B., Kumar, S., Verma, R. K. and Yadav, S., Nutritional Contents and Medicinal Properties of Wheat: A Review. Life Sciences and Medicinal Research, 1-10 (2011).
15. Mbithimwikya, S., Ooghe, W., Vancamp, J., Nagundi, D. and Huyghebaert, A., Amino acid profile after sprouting, Autoclaving and lactic acid fermentation of finger millet (*Elusine coracana*) and kidney beans (Phaseolus vulgaris L.). *Journal of Agriculture Food Chemistry*, **48(8):** 3081-3085 (2000).
16. Michael, E. N. A. and Wiebs, S., Changes in phytic acid and phytate during germination of two faba bean cultivars. *Journal of Food Science*, **48:** 270-271 (1983).
17. Mukhopadhyay, D., Parihar, S., Chauhan, S., Preeti and Joshi, S., Effect of temperature desiccation on seed viability of Lepidiumsativum L., *New York Science Journal*, **3(5):** 34 (2010).
18. Norhaizan, M. E. and Faizadatul, A. W., Determination of phytate, iron, zinc, calcium contents and their molar ratios in

commonly consumed raw and prepared food in Malaysia. *Malaysia Journal of Nutrition*, **15(2):** 213-2222 (2009).

19. Panwar, H. and Guha, M., Effect of processing on nutraceutical properties of Garden cress (*Lepidium sativum* L.) seeds. *International Journal of Pharmacy and Pharmaceutical Sciences*, **6(7):** 315-318 (2014).
20. Singh, P. and Singh, R., Finger millet for food and nutritional security. *African Journal of Food Science*, **6(4):** 77-84 (2012).
21. Steve, I., Oluwole Influence of germination and fermentation on chemical composition, protein quality and physical properties of wheat flour (*Triticum aestivum*). *Journal of Cereals and Oilseeds*, **3(3):** 34-47 (2012).
22. Tizazu, S., Urga, K., Abuye, C. and Retta, N., Improvement of energy and nutrient density of sorghum based complementary foods using germination. *African Journal of Food Agriculture Nutrition and Development*, **10(8):** 2928-2942 (2010).