J. Plantarum, 1(1): 17-23, 2019

EFFECT OF DIFFERENT PRE-TREATMENT METHODS ON TOTAL PROTEIN CONTENT AND PEROXIDASE ACTIVITY IN SELECTED PULSES

ZEB SADDIQE*, RAHEEMA RAHEEL, SABA JAVAID, RUKHSANA HUSSAIN

Department of Botany, Lahore College for Women University, Lahore, Pakistan
**Corresponding author's email: zeb_rukhsana@yahoo.com*

Abstract

The present work was carried out to determine the effect of extracting medium and different pretreatment methods on total soluble protein content and peroxidase activity in five pulses *i.e., Lens culinaris* Medik, *Cicer arietinum* L., *Vigna mungo* (L.) Hepper, *Vigna unguiculata* (L.) Walp. and *Vigna radiata* (L.) Wilczek most commonly used as source of protein in Pakistan. Under normal conditions the amount of total soluble protein content and peroxidase activity was highest in *V. radiata* and lowest in *V. mungo*. Phosphate buffer proved to be a better solvent for extraction of proteins and enzymes as compared to water in all the legumes except in *V. mungo*. Dry heating at 75°C decreased the protein content in all the samples except for *C. arietinum*. In case of peroxidase activity at 75°C the highest activity was observed for *C. arietinum* followed by *V. unguiculata* and *V. mungo* which indicated that peroxidases in these pulses are more resistant to dry heating at high temperature. Maximum protein content and peroxidase activity in case of *C. arietinum, V. unguiculata* and *V. mungo* was observed at 60°C while for *V. radiata* and *L. culinaris* the maximum protein content and enzyme activity was at 20°C. After inactivation at high temperature (between 60 - 80°C) reactivation of enzyme was observed in all the samples. Soaking in Sodium chloride (NaCl) solution caused an increase in content of total protein and peroxidase activity in all the pulses studied. The study concluded that all the pulses can be a good source of thermostable and salinity resistant peroxidases.

Key words: antioxidant enzymes, biocatalysts, disease prevention, free radicals, peroxidases

Introduction

Peroxidases are found in all the plants, microbes and animal tissues where they breakdown hydrogen peroxide (H_2O_2) (produced as a toxic product during cell metabolism) into oxygen gas and water (Petersen & Anderson, 2005). The multifunctional properties of these enzymes, broad substrate specificity and availability from different sources have led to the application of these enzymes in several biological and biochemical processes. The major roles of these enzymes in plants include suberization, lignifications, protection against pathogenic attack, wound healing and decomposition of H_2O_2 **(**Hiraga *et al*., 2001). Plant peroxidases can oxidize a variety of organic compounds including aromatic amines, phenols, sulfonates, and indoles using H_2O_2 as the oxidant (Van Deurzen *et al*., 1997; Adam *et al*., 1999; Veitch & Smith, 2001).

Peroxidases are versatile biocatalyst and are gaining popularity due to their biological properties and applications in biochemistry, biotechnology and related areas (Colonna *et al*., 1999, Veitch & Smith, 2001). The enzymes are used in food industry for optimizing blanching procedures due to their heat resistant properties (Reed, 1975) and are also widely used in enzyme immunoassays and in clinical biochemistry (Vamos-Vigiazo, 1981; Lin *et al*., 1996). The enzymes have the ability to generate free radicals and hence are used as a biocatalyst for the synthesis of several polymers (Kobayashi *et al*., 2001; Kobayashi *et al*., 2001a) for the biotransformation of a many organic molecules (Van Deurzen *et al*., 1997; Adam *et al*., 1999; Veitch and Smith, 2001), and in bioremediation (Klibanov *et al*., 1983; Wright & Nicell, 1999; Zhang & Nicell, 2000; Kinsley & Nicell, 2000; Wagner & Nicell, 2001). These are also used for the treatment of industrial wastewater containing phenolic compounds (Bódalo *et al*., 2006).

Legumes are widely grown throughout the world due to their dietary and economic importance. These food components adding variety to diet are a rich and economical source of proteins (Davis *et al*., 2010). Among pulses, lentils are of particular interest due to their unique nutritional and functional properties. In addition to proteins, lentils are also rich in important minerals, vitamins, and dietary fibers. Many reports are available regarding disease-preventing and health improving effects of lentils.

V. radiata grains are the most useful legumes because they are the major source of amino acids and protein (Imrie, 2005; Kulsum *et al*., 2007) are highly digestible and have less flatulence effect (Fery, 2002). The plant is also used for therapeutic purposes.

V. mungo beans are valued because of their high protein as well as amino acid content (Imrie, 2005; Kulsum *et al*., 2007) and are also highly digestible with less flatulence effects (Fery, 2002) and therapeutic properties. The antidote activity of the plant makes it a useful medicinal and cosmetic material (Jo *et al*., 2006; Sharma and Mishra, 2009).

V. mungo beans are valued because of their high protein as well as amino acid content (Imrie, 2005; Kulsum *et al*., 2007) and are also highly digestible with less flatulence effects (Fery, 2002) and therapeutic properties. The antidote activity of the plant makes it a useful medicinal and cosmetic material (Jo *et al*., 2006; Sharma and Mishra, 2009).

V. unguiculata commonly known as cowpea is a less common pulse with high nutritional values (Arinathan *et al*., 2003). *V. unguiculata* is an important food legume, and its use as a leafy vegetable is essential in many countries. The plant is heat and drought tolerant (Sellschop, 1962). The plant can grow under drought and at high temperatures compared to

other crops (Hall & Patel, 1985; Hall et al., 2002; Hall, 2004). The crop grows well in low fertile soils due to its symbiotic association with mycorrhizae (Kwapata & Hall, 1985), ability to fix nitrogen at high rates (Elawad & Hall, 1987) and its tolerance against a range of soil pH as compared to other legumes (Fery, 1990).

C. arietinum commonly known as Chickpea is among the most widely grown and consumed legumes in the world particularly in tropical and subtropical countries due to its high carbohydrate and protein content. Proteins present in this plant are of better quality than other legumes e.g., pigeon pea, green gram and black gram (Kaur & Singh, 2005). Attia *et al*. (1994) reported the effect of cooking on the chemical constituents of chickpea seeds. Increase in the temperature and time of processing generally reduces its nutritive value and availability of lysine (Kon & Sanshuck, 1981; Chau *et al*., 1997).

The present study was designed to check the effect of different conditions such as high temperature, dry heating, solvent used for extraction, and NaCl on total soluble protein and peroxidase contents in five different legumes.

Materials and methods

Plant material and extract preparation: The legumes used in the present study were purchased from a local market of Lahore, Pakistan. For extraction, 3 g of each legume were grinded with 9 ml of phosphate buffer (0.1 M, pH 7.2) and 0.1 g of polyvinyl polypyrrolidone (PVP). The contents were centrifuged at 6,000 rpm for 20 min at 4°C and the supernatant obtained was filtered through three layers of muslin cloth and stored at 4°C.

Protein determination: Total soluble protein content was estimated using the Biuret method (Racusen and Johnstone, 1961). For analysis, 0.1 mL of supernatant and 1.0 mL of Biuret reagent were mixed and optical density was measured at 545 nm. In control 0.1 mL of distilled water replaced supernatant. For calculating protein content standard protein curve was prepared from bovine serum albumin and protein content was expressed as mg/g of tissue (Figure 1).

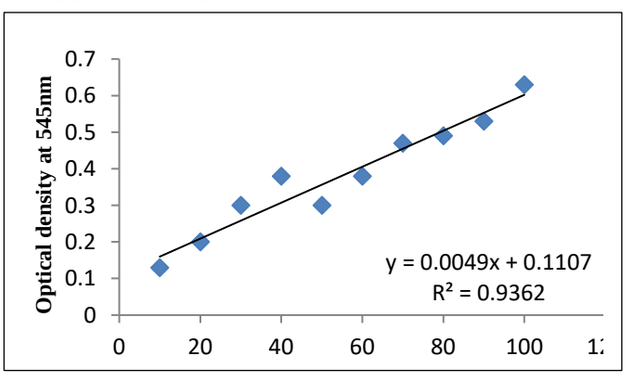

Figure 1 Standard calibration curve for protein

Enzyme assay: For peroxidase activity, method of Racusen and Foote (1965) was followed. The reaction mixture was prepared by mixing crude enzyme extract (0.1 mL), 1% guaiacol solution (0.2 mL), 0.2 mL of H2O2 solution and 2.5 mL of phosphate buffer (0.1 M, pH 7.2). In control distilled water was used in place of enzyme extract. The enzyme activity was determined by measuring absorbance of reaction mixture at 470 nm. Enzyme activity was expressed as mg/g of tissue. The specific activity was calculated by dividing the total activity units by milligrams of proteins.

Effect of extracting solvent: To determine the effect of extracting solvent on extraction of protein and enzyme all the five legumes samples were soaked and extracted in distilled water in place of phosphate buffer to find the difference in enzyme activity and protein content of legumes.

Effect of temperature on enzyme activity: To determine the effect of temperature as pre-treatment on the enzymatic activity and protein content of different legume samples, 3 g of each legume was taken, wrapped in a piece of cloth, and incubated for 20 minutes in a water bath at 20, 40, 60, 80 and 100°C respectively.

Effect of NaCl on enzyme activity: To observe the effect of different concentrations of NaCl as pre-treatment on the enzymatic activity and protein content of legumes, 3 g of each legume was incubated for 15 minutes in 2, 4, 6, 8 and 10% solutions of NaCl.

Effect of dry heating on enzyme activity All 5 samples of legumes were incubated for 5 hours at 75°C in an electric oven to find out its effect on enzymatic activity and protein content of all legumes.

Statistical Analysis All the treatments were repeated in triplicates and the data was expressed as mean ± standard deviation. The graphs were constructed using the average mean for each sample. Results were considered significant at $p < 0.05$.

Results and discussion

The good thing about enzymes is that they can be isolated from living cells and can function in a test tube under lab environment. Peroxidases are the most studied enzymes in plants and their universal occurrence in plants indicates their functional importance. Peroxidases are found in many plant-based food products and are involved in the oxidation of organic compounds causing deterioration in color, flavour and nutritional level. These changes in quality of food are more pronounced in frozen and canned fruits and vegetables (Nebesky *et al*., 1950; Bruemmer *et al*., 1976; Kampis *et al*., 1984). In the present study five pulses mostly used as a source of protein replacing animal proteins in many developing countries were evaluated for their total soluble protein and peroxidase content under normal and pre-treatment conditions and results were compared.

Total soluble protein content: The results of total soluble protein content in the five legumes are summarized in Figure 2. The highest protein content at room temperature was determined for *V. radiata* (8.856 mg/g tissue) while lowest content was observed in *V. mungo* (5.44 mg/g tissue). Except for *V. mungo* the difference in protein content among the pulses was not significant ($p > 0.05$).

Peroxidase content: Peroxidase content in the five legumes in terms of enzyme activity was determined using guaiacol as substrate. Highest enzyme content was determined for *V. radiata* (0.036 mg/g tissue) for which maximum protein content was also determined while lowest content was determined in *V. mungo* (0.0039 mg/g tissue) for which the protein content was also lowest (Figure 2). Statistically

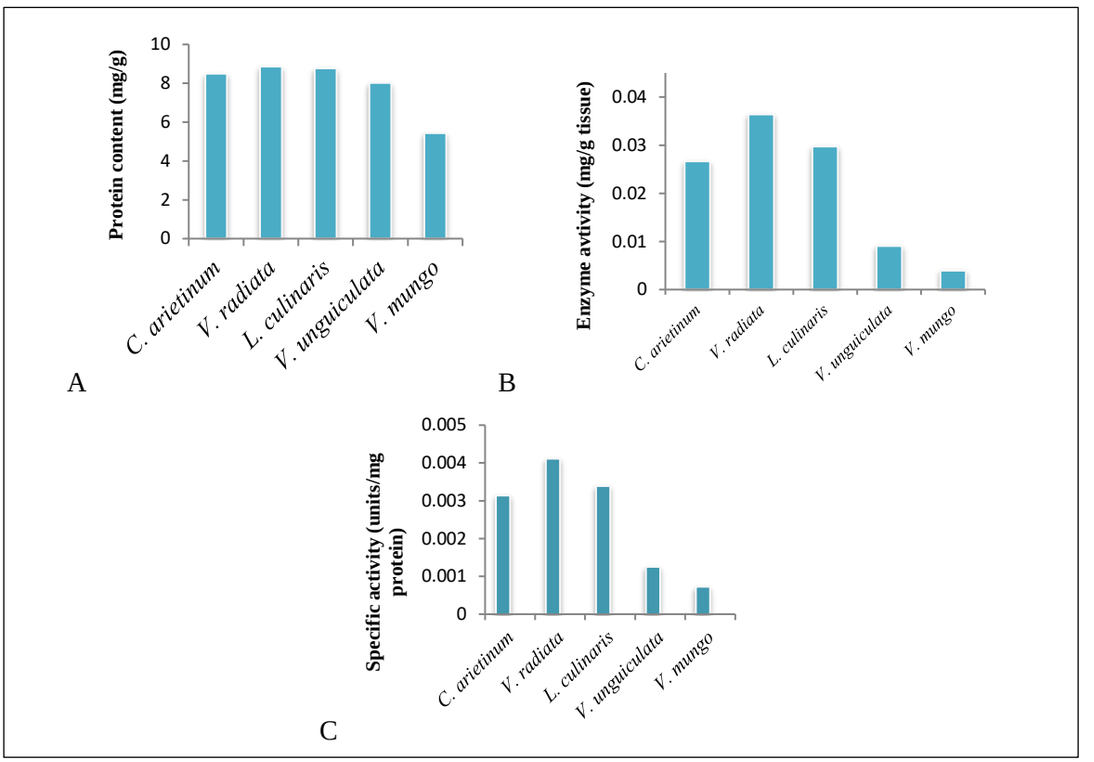

Figure 2 Total soluble protein content (A), peroxidase content (B) and specific activity (C) in five pulses at room temperature

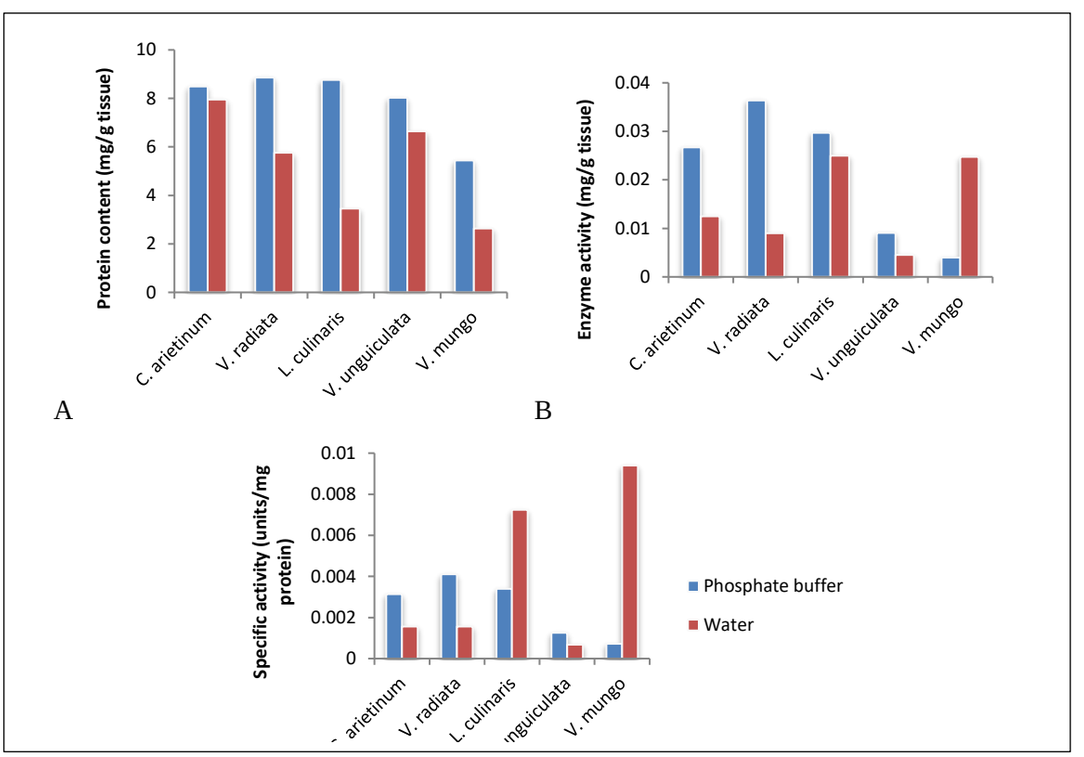

Figure 3 Total soluble protein content (A), peroxidase content (B) and specific enzyme activity (C)

significant difference in peroxidase content was observed between *C. arietinum* and *V. radiata* and between *V. radiata* and *V. unguiculata* ($p < 0.05$). The results of specific activity followed the same pattern with highest specific activity in *V. radiata* (0.004 units/mg protein) and lowest in *V. mungo* (0.0007 units/mg protein) (Figure 2). The results indicated that *V. radiata* can be a good source of peroxidases to be used for commercial applications.

Extraction in water: Generally for biochemical analysis the proteins and enzymes are extracted in phosphate buffer. Since the pulses are normally soaked in water before cooking, distilled water was used as solvent to extract plant proteins and the results were compared with those of phosphate buffer. In all the samples protein content was higher in phosphate buffer than water indicating high potential of phosphate buffer to be used for extraction of proteins from the plant material. Statistically significant difference was observed between protein content extracted in phosphate buffer and water for *V. radiata*, *L. culinaris* and *V. mungo* (Figure 3).

Peroxidase content extracted in water: The results of peroxidase content followed the same pattern as proteins with high enzyme content extracted in phosphate buffer in all the samples except *V. mungo* which gave highest enzyme content in water indicating high solubility of *V. mungo* peroxidases in water than phosphate buffer (Figure 3). The difference between peroxidase content extracted in two solvents was statistically significant in case of *C. arietinum*, *V. radiata* and *V. mungo*. The specific activity of the enzyme was highest for *V. mungo* in water followed by *L. culinaris* while in the other three samples, the specific activity was high in phosphate buffer (Figure 3).

Effect of dry heating on protein and peroxidase content: The results of total protein content and enzyme activity under dry heating treatment were compared with those obtained at room temperature and are summarized in Figure 4. After heating, a decrease in total protein content was observed in all the samples except *C. arietinum* for which the protein content was higher as compared to that at room temperature (Figure 8). Among the five samples, highest protein content at 25°C was determined for *V. radiata* (8.856 mg/g tissue) and lowest for *V. mungo* (5.4403 mg/g tissue). At 75°C highest protein content was in *C. arietinum* (9.481 mg/g tissue) and lowest in *V. mungo* (4.996 mg/g tissue).

In the present study higher enzyme content was observed for *C. arietinum*, *V. unguiculata* and *L. culinaris* at 75°C as compared to 25°C indicating that the peroxidases of these three pulses are highly thermostable. Increase in enzyme activity after heating at high temperature may indicate reactivation of the enzyme which has been associated with peroxidases. At room temperature *V. radiata* (0.036 mg/g tissue) showed highest enzyme activity while lowest activity was observed in *V. mungo* (0.003 mg/g tissue). At 75°C *C. arietinum* had highest activity (0.039 mg/g tissue) and *L. culinaris* had the lowest (0.011 mg/g tissue).

The specific activity of the enzyme followed the same pattern as that of the enzyme activity with high specific activity for *C. arietinum*, *V. unguiculata* and *L. culinaris*. The highest specific activity was observed for *V. mungo*.

Effect of temperature: The variations in temperature differently affected total protein and peroxidase content in all the samples. The protein content of different legumes was determined at different temperatures. A decrease in protein content of legumes with increase in temperature was observed till 40°C and then there was an increase in protein content up to 60°C and again then there is decrease in protein content until temperature reading of 80°C with exception to *V. unguiculata* that showed increase in protein content. Then with increase in temperature till 100°C, the protein content of legumes increased. Initially the maximum protein content at 60°C is an indication of the stability of proteins and then increase in protein content at 100°C is due to the reactivation of proteins (enzymes) at high temperature (Figure 5). No significant correlation was observed between temperature and total protein content in the tested legumes.

High temperature is a common treatment method used to inactivate enzymes. However, peroxidase can recover its activity after heating (Lu and Whitaker, 1974; Anthon and Barrett, 2002; Schwimmer, 1944). Studies have shown that reactivated peroxidases can cause significant deterioration in the quality of various high-temperature-short-time (HTST) processed foods (Lu and Whitaker, 1974, Schwimmer, 1944; Adams, 1978). Reactivation of an enzyme is a complex process controlled by many factors including time taken to attain the required temperature. If the time is short, reactivation can occur more easily (Lu and Whitaker, 1974, Schwimmer, 1944; Adams, 1978). In horseradish peroxidase, for example, reactivation takes place after inactivation at 70°C, 90°C, or 110°C (Tamura and Morita, 1975).

Variations in enzyme activity were observed in all the samples and peroxidases extracted from different pulses gave difference in activity depending upon temperature. The optimum and maximum temperatures varied among the tested materials. Thus the enzyme activity decreased in *V. radiata*, *C. arietinum* and *L. culinaris* with increase in temperature till 40°C while in *V. mungo* and *V. unguiculata* the activity increased with increase in temperature. Initially there was a gradual increase in enzyme activity followed by a rapid increase till 60°C and then again decrease in enzyme activity till 80°C. *V. mungo* showed increase in enzyme activity till temperature of 100°C while *V. unguiculata* showed increased enzyme activity with increase in temperature till 100°C. *L. culinaris* and *V. radiata* showed decreased enzyme activity with increase in temperature till 60°C then both show increase in enzyme activity till 80°C, *L. culinaris* showing rapid increase and *V. radiata* showing gradual increase. *V. radiata* then showed further rapid increase till 100°C and *L. culinaris* showing decrease in enzyme activity. *C. arietinum* after increase till 40°C show decrease in enzyme activity till 60°C and show again increase till 80°C and decrease in enzyme activity till 100°C (Figure 5). The highest specific activity was observed for *L. culinaris* at 80°C (Figure 5). No significant correlation was observed between temperature and enzyme activity and between temperature and specific activity in the selected legumes.

Effect of NaCl on protein content and enzyme activity: Inhibitors like NaCl can denature the three dimensional structure of proteins rendering them non-functional. To determine the effect of NaCl, the plant extract was incubated at different NaCl concentrations at room temperature for 15 min. Difference in activity was determined by difference in absorbance.

A regular change in protein content was determined

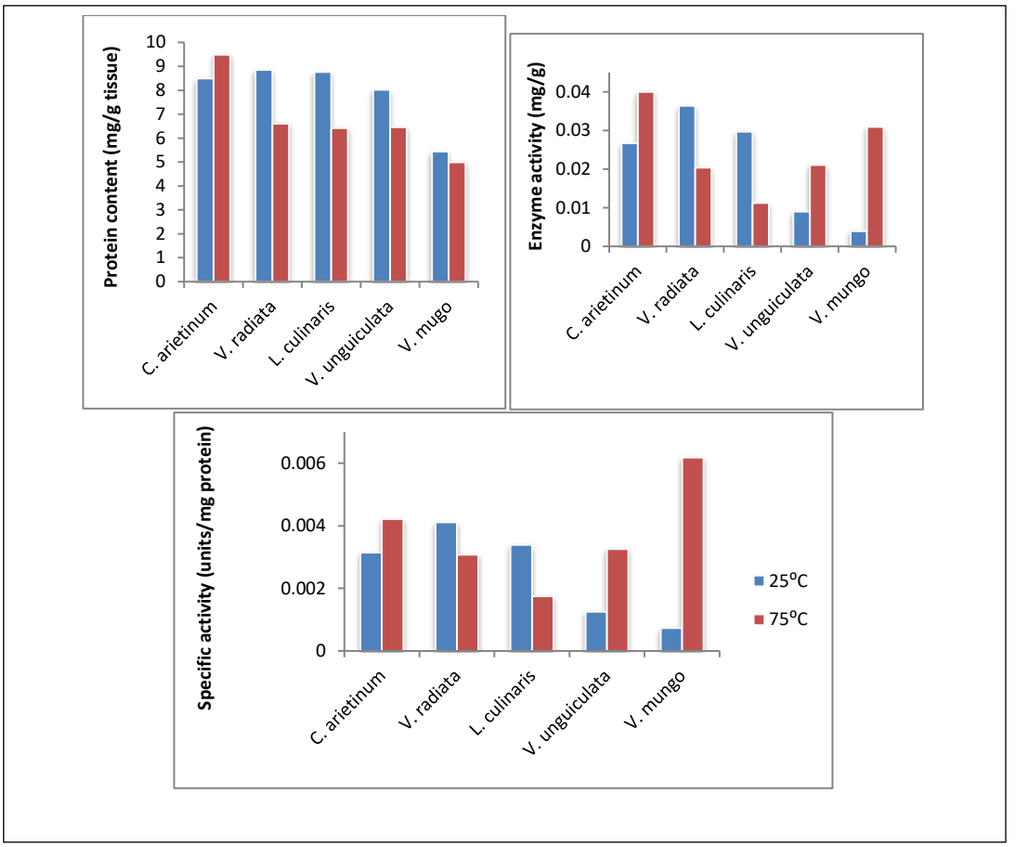

Figure 4 Total soluble protein content (A), peroxidase content (B) and specific enzyme activity (C) of legumes at room temperature and at 75°C

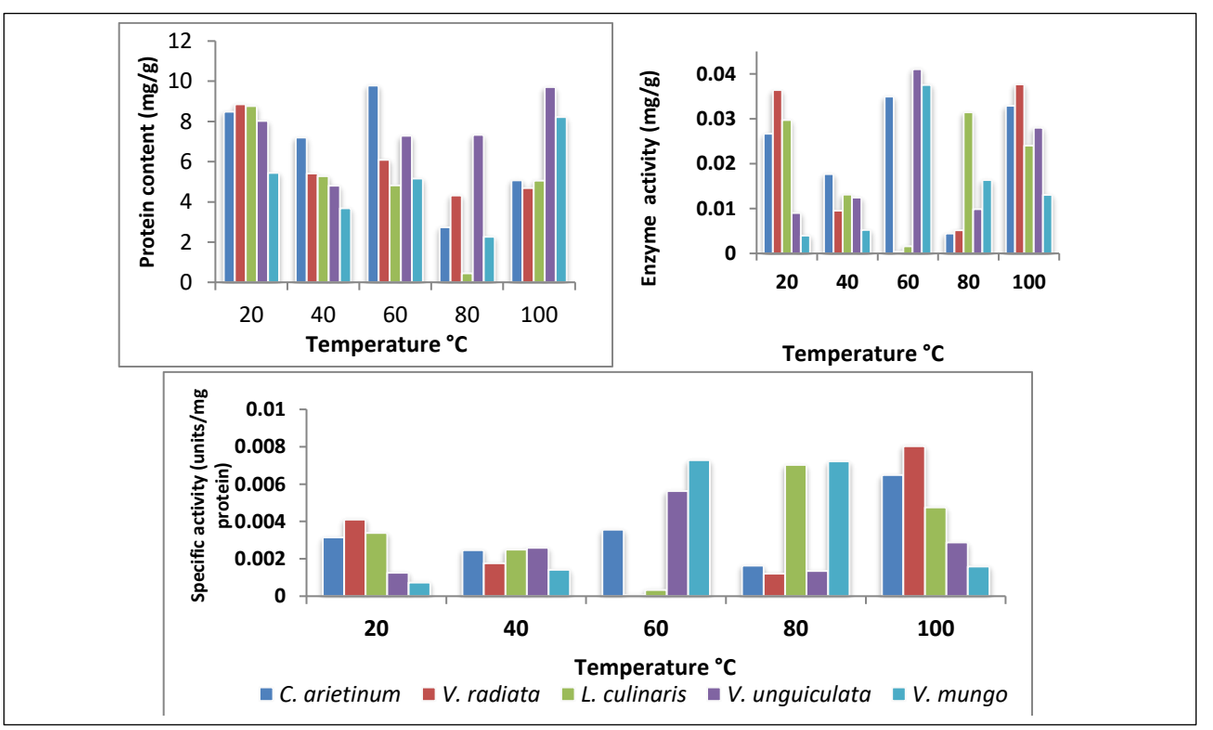

Figure 5 Effect of temperature on total protein content (A), peroxidase content (B) and specific enzyme activity (C) in five legumes

which followed the same basic pattern in all the samples An increase in protein content was observed in all the samples at 2% NaCl that decreased in all the samples at 4% with further increase at 6 and 8% and then a decrease at 10%. The results indicated that NaCl may have a positive effect on proteins. No significant difference in protein content of different samples was observed at all the tested concentrations (Figure 6). No significant correlation existed between NaCl concentration and total protein content in the tested legumes. The inhibitory effect of NaCl on activity of certain enzymes in plants has been reported (Evans & Sorger, 1966; Hansen-Porath & Poljakoff-Mayber, 1968, 1969). Though the actual effect of salts on enzyme activity is not known, it is assumed to be related to the lyotropic effect of ions on 3-D
structure of enzyme.

In the present study highly variable effect of NaCl was observed on peroxidase activity extracted from different samples. In case of *C. arietinum* increase in enzyme activity was observed with addition of NaCl till 6% with a gradual decrease on further increase in NaCl concentration. For *V. radiata* a smooth curve was obtained with gradual increase in enzyme activity up to 4% and then decrease in activity till 10%. For *L. culinaris* the activity gradually increased with addition of NaCl with a decrease observed only at 8%. For *V. unguiculata* no regular pattern in enzyme activity was observed while for *V. mungo* highest enzyme activity was observed at 10% NaCl (Figure 6). For specific activity all the samples showed highest specific activity of enzyme at 4% NaCl (Figure 6). No significant correlation was observed between NaCl concentration and enzyme activity and between NaCl concentration and specific activity in the selected legumes ($p > 0.05$).

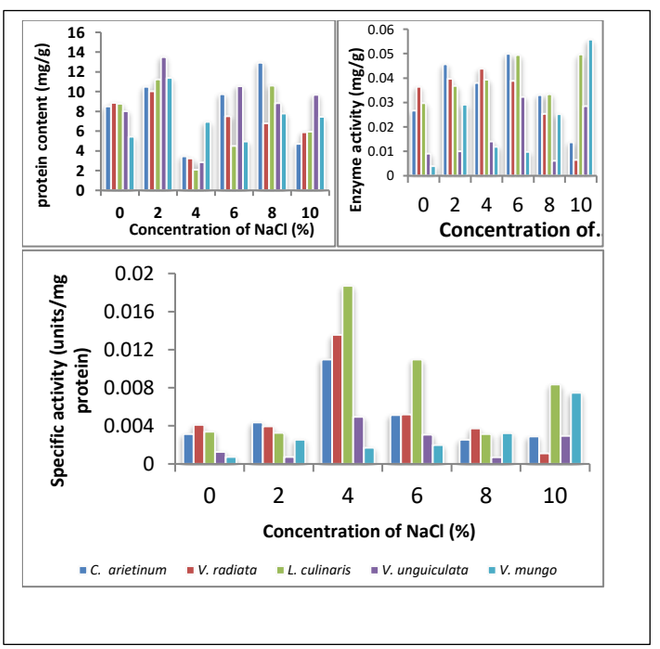

Figure 6 Effect of different concentrations of NaCl on total soluble protein content (A), peroxidase content (B) and specific enzyme activity (C) in five legumes

Conclusion

Under normal conditions highest total soluble protein content and peroxidase activity was observed in *V. radiata* and lowest in *V. mungo*. Phosphate buffer was a better choice for extraction of proteins and enzymes as compared to water in all the legumes except in *V. mungo*. Proteins and peroxidases found in *C. arietinum* were stable after dry heating at 75°C followed by *V. unguiculata* and *V. mungo* which indicated that peroxidases in these pulses are more resistant to dry heating at high temperature. Peroxidases in *C. arietinum*, *V. unguiculata* and *V. mungo* were quite stable at 60°C while inactivation at high temperature was not permanent and reactivation was observed in all the samples. Saline conditions were found to have stimulating effect on total protein and peroxidase content in all the legumes. Thus it can be concluded that all these pulses can be used for extraction of thermostable and salinity resistant peroxidases.

References

Adam, W., M. Lazarus, C.R. Saha-Moller, O. Weichold, U.H oh, D . Haring and P. Schreier . 1999 . Biotransformations with peroxidases. *Adv. Biochem. Eng. Biotechnol*., 63: 73-108.

Adams, J.B. 1978. The inactivation and regeneration of peroxidase in relation to the high temperature-short time processing of vegetables. *J. Food Technol*., *13*: 281-297.

Anthon, G.E. and D.M. Barrett, 2002**.** Kinetic parameters for the thermal inactivation of quality-related enzymes in carrots and potatoes. *J. Agric. Food Chem.*, 50: 4119-4125.

Arinathan, V., V. R. Mohan, A. John De Britto and C. Murugan. 2003. Chemical composition of certain tribal pulses in South India. *Int. J. Food Sci. Nutr*., 54: 209-217.

Attia, R.S., El-Tabey, A.M. Shehata, M.E. Aman and M.A. Hamza 1994. Effect of cooking and decortication on the physical properties, the chemical composition and the nutritive value of chickpea (*Cicer arietinum* L.). *Food Chem.*, 50: 125–131.

Bódalo, A., J.L. Gómez, E. Gómez, A.M. Hidalgo, M. Gómez and A.M. Yelo. 2006. Removal of 4-chlorophenol by soybean peroxidase and hydrogen peroxide in a discontinuous tank reactor. *Desalinatio.*, 195: 51.

Bruemmer, J.H., B. Roe and E.R. Bowen. 1976. Peroxidase reactions and orange juice quality. *J. Food Sci.*, 41, 186.

Chau, C.F., P.C. Cheung and Y.S. Wong. 1997. Effect of cooking on content of amino acids and antinutrients in three Chinese indigenous legume seeds. *J. Sci. Food Agr*., 75: 447–452.

Colonna, S., N. Gaggero and P.P. Richelmic. 1999. Recent biotechnological developments in the use of peroxidases. *Trends Biotechnol*., 17: 163-168.

Davis, J., U. Sonesson, D.U. Baumgartner and T. Nemecek. 2010. Environmental impact of four meals with different protein sources: Case studies in Spain and Sweden. *Food Res. Int.*, 43: 1874-1884.

Elawad, H.O.A. and A.E. Hall. 1987. Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. *Field Crops Res.*, 15: 229–244.

Evans, H.J. and G.J. Sorger. 1966, Role of mineral elements with emphasis on univalent cations. *Ann. Rev. Pl.Physiol*.,17: 74-76.

Fery, F.L. 2002. New opportunities in *Vigna*. pp. 424-428. In: Trends in new crops and new uses. Janick, J. and Whipkey, A. Edition. ASHS Press, Alexandria, VA.

Fery, R.L. 1990. The cowpea: production, utilization, and research in the United States. *Hort. Rev.*, 12:197–222

Hall, A.E. 2004. Breeding for adaptation to drought and heat in cowpea. *Eur. J. Agron.*, 21:447–454.

Hall, A.E. and P.N. Patel. 1985. Breeding for resistance to drought and heat. In: Cowpea Research, Production and Utilization. Singh, S. R. and Rachie, K. O. Edition. Wiley, New York, pp 137–151

Hall, A.E., A.M. Ismail, J.D. Ehlers, K.O. Marfo, N. Cisse S. Thiaw and T.J. Close. 2002. Breeding cowpeas for tolerance to temperature extremes and adaptation to drought. In: *Challenges and Opportunities for Enhancing Sustainable Cowpea Production*. Fatokun, C. A., Tarawali, S.A., Singh, B.B., Kormawa, P.M. and Tamo, M. Edition. *International Institute of Tropical Agriculture, Ibadan, Nigeria*, pp 14–21.

Hansen-Porath, E.H. and Poljakoff-Mayber, A. 1968. The effect of salinity in the growth medium on carbohydrate metabolism in pea root tips. *Plant & Cell Physiol*., 9: 195-203.

Hansen-Porath, E.H. and Poljakoff-Mayber, A. 1969. The effect salinity on the malic dehydrogenase of pea roots. *Pl. Physiol*., 44: 1031 – 1034.

Hiraga, S., K. Sasaki, H. Ito, Y. Ohashi and H. Matsui. 2001. A large family of class III plant peroxidases. *Plant Cell Physiol*., 42: 462-468.

Imrie, B. 2005. The New Rural Industries, A handbook for farmers and investors, Black gram, http://www.rirdc.gov.au/pub/handbook/blackgram.

Jo, B.K., G.W. Ahn, J.H. Jeong and Y.I. Hwang. 2006. Clinical studies on the anti-irritation effects of mung bean (*Phaseolus aureus*) extract in cosmetics. *SOFW-J*., 132(1/2): 8-16.

Kampis, A., O. Bartuczkovacs, A. Hoschke and V. Aosvigyazo. 1984. Changes in peroxidase-activity of broccoli during processing and frozen storage. *Lebensm. Wiss. Technol*., 17: 293-295.

Kaur, M. and N. Singh. 2005. Studies on functional, thermal and pasting properties of flours from different chickpea (*Cicer arietinum* L.) cultivars. *Food Chem.*, 91: 403-411.

Kinsley, C. and J.A. Nicell. 2000. Treatment of aqueous with soybean peroxidase in the presence of polyethylene glycol. *Bioresour. Technol*., 73: 139-146.

Klibanov, A.M., T.M. Tu and K.P. Scott. 1983 . Peroxidase-catalysed removal of phenols from coal- conversion wastewaters. *Science*, 221: 259-260.

Kobayashi, S., H. Uyama and S. Kimura. 2001. Enzymatic polymerization. *Chem. Rev*., 101: 3793-3818.

Kobayashi, S., H. Uyama, H. Tonami, T. Oguchi, H. Higashimura, R. Ikeda and M. Kubota, 2001a. Regio- and chemo-selective polymerization of phenols catalyzed by oxidoreductase enzyme and its model complexes. *Macromol. Symp.*, 175: 1-10.

Kon, S. and D.W. Sanshuck. 1981. Phytate content and its effect on cooking quality of beans. *J. of Food Process. Preservation*, 5: 169–178.

Kulsum, M.U., M.A. Baque and M.A. Karium. 2007. Effects of different nitrogen levels on the morphology and yield of blackgram. *J. Agron*., 6: 125-130.

Kwapata, M.B. and A.E. Hall. 1985. Effects of moisture regime and phosphorus on mycorrhizal infection, nutrient uptake, and growth of cowpeas [*Vigna unguiculata* (L.) Walp.]. *Field Crops Res.*, 12: 241–250.

Lin, Z., L. Chen and W. Zhang. 1996. Peroxidase from *I. cairicia* (L) SW. Isolation, purification and some properties, *Process Biochem*., 5, 443.

Lu, A.T. and Whitaker, J.R. 1974**.** Some factors affecting rates of heat inactivation and reactivation of horseradish peroxidase. *J. Food Sci., 39*: 1173-1178.

Nebesky, E.A., Esselen, W.B., Jr.; Kaplan A. M.; Fellers, C. R. 1950**.** Thermal destruction and stability of peroxidase in acid foods. *Food Res*., 15, 114.

Petersen, C. E. and B. J. Anderson. 2005. *Investigations in the Biology 1151 Laboratory*, Stipes Publishing L.L.C. Champaign, IL, USA, pp: 45.

Racusen, D. and D.B. Johnstone. 1961. Estimation of protein in cellular material. *Nature*, 191: 492-493.

Racusen, D. and M. Foote. 1965. Protein synthesis in dark grown bean leaves. *Can. J. Bot.*, 43: 817-824.

Reed, G. 1975. *Oxidoreductases: Enzymes in Food Processing*. Academic Press, New York, p. 216.

Schwimmer, S. 1944. Regeneration of heat inactivated peroxidase. *J. Biol. Chem*., *154*: 487.

Sellschop, J. P. F. 1962. Cowpeas, *Vigna unguiculata* (L.) Walp. Review article. *Field Crop Abstracts*, 15, 1.

Sharma, P. and N.K. Mishra. 2009. Ethnomedicinal uses and agro- biodiversity of Barmana region in Bilaspur District of Himachal Pradesh, Northwestern Himalaya. *Ethanobotanical Leaflets.*, 13: 709- 721.

Tamura, Y. and Morita, Y. 1975. Thermal denaturation and regeneration of Japanese-radish peroxidase. *J. Biochem. (Tokyo), 78*: 561-571.

Vamos-Vigiazo, L. 1981. Polyphenol oxidase and peroxidase in fruits and vegetables, CRCCrit Rev. *Food Sci. Nutr.*, 84.

Van Deurzen, M.P. J., V.F. Rantwijk and R.A. Sheldon. 1997. Selective oxidations catalyzed by peroxidases. *Tetrahedron*., 53: 13183-13220.

Veitch, N.C and A.T. Smith. 2001. Horseradish peroxidase. *Adv. Inorg. Chem.*, 51: 107-162.

Wagner, M. and J.A. Nicell. 2001. Treatment of a foul condensate from kraft pulping with horseradish peroxidase and hydrogen peroxide. *Water Res.*, 35: 485-495.

Wright, H. and J.A. Nicell. 1999. Characterization of soybean peroxidase for the treatment of aqueous phenols. *Bioresour. Technol.*, 70: 69-79.

Zhang, G.P. and J.A. Nicell. 2000. Treatment of aqueous pentachlorophenol by horseradish peroxidase and hydrogen peroxide. *Water Res.*, 34: 1629-1637.