Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/2439
DOI URL: http://dx.doi.org/10.21474/IJAR01/2439
ISSN NO. 2320-5407

RESEARCH ARTICLE

VIRTUAL KEYBOARD.

HYGIENIC DETOXIFICATION OF BIOACTIVE AND FUNCTIONAL FOOD CONSTITUENTS OF SOYBEAN [*GLYCINE MAX* (L.)MERRILL] CULTIVARS.

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Manuscript Info

Manuscript History

Received: 20 October 2016
Final Accepted: 22 November 2016
Published: December 2016

Key words:- Soybean, trypsin inhibitor, lipoxygenase, peroxidaes, processing, detoxification.

Abstract

The agriculture produce soybean have phytochemically many constituents of nutraceuticals, and pharmaceutical herbal products. Soybean is known a golden bean because of its miraculous nutritive capacity it contains approximately 38-42 percent high valued proteins supplies sufficient amount of various kind of amino acids. Oil content ranges from 15-21 percent. Currently worldwide interest is increasing in a trend ready to eat food to achieve the goal for healthcare. The combination of food material constituents may be responsible for biological actions that have been eaten.

High temperature and pressure processing technique based only aqueous steam and time duration factors detoxification of various toxicants and anti- nutritional constituents. This processing technique no unhygienic chemical used or released that may be harmful for environmental pollution. Detoxification process parameters trypsin inhibitor found as specific activity ranged from 220-270 as compared to raw seeds were 930-110 (µM tyrosine/min/g protein), lipoxygenase ranged 4936-5502 as compared to raw seeds mean recorded 19053-24167(µM hydroperoxide/min/mg protein) and peroxidase enzyme also reduced the specific activity from 1.00-10.00 as compared to raw seeds have 18.249-20.807 (nM hydroperoxide/min/g protein) reduced highly significant as compare to raw seeds mean values of soybean. The processing technique can be established within specified parameters reduced toxic constituents variation as compare to raw material helps to increase consumer's confidence.

Introduction:-

Soybean [*Glycine max* (L.)Merrill] belongs to family fabaceae herbaceous annual of Asiatic origin. Its ranks first among the oil seed crops in the world and India both.Soybean has been adopted and commercially cultivated in Madhya Pradesh, Maharashtra, Rajasthan, Karnataka and Andhra Pradesh. At present, it has been established as a most important oilseed crop of M.P. and India. Gupta, et al. (2014). Soybean has unprecedented expansion in India by recording 15-20% annual growth rate. It has emerged very fast since early eighty's and occupied vital place in agriculture, edible oil economy, foreign exchange and up liftment of socio-economic status of soybean farmers. It

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contributes around 25% of total edible oil pool of the country. **The credit for such a big boost about 5.20 lacks MT production of soybean in the country goes to Madhya Pradesh popularly known as "Soya bowl" of the country.**

Soybeans are good source of vegetable protein used to supplemented animal protein in the vegetarian's diet. It is rich in low cholesterol lipids well known to researchers legumes are good source of fibre content. The process and technology are essential for improving the shelf life of soy products, enhancement of nutritional security almost its quality and safe for uses.

Anti-nutrients and phytochemicals:-

Soybeans contains various undesirable phytochemicals; anti-nutritional factor one of them principally is trypsin-inhibitors(TI),which limits straight utilization in food as whole uses of soybean seeds Verma et al. (2015) and Shivakumar et al. (2015) used KTI as genotypes morphomolecular identified for developing cultivars of soybean. Trypsin inhibitor is a antinutritional factor that affects protein digestibility Vineet et al., (2005). High level of trypsin inhibitors in a diet stimulates pancreatic juice secretion, causes pancreatic hypertrophy and poor growth performance in animals announced by Huisman & Tolman, (2001). Depresses the absorption of minerals, have a negative impact on utilization. The natural toxicant and anti-nutrients have been widely employed to describe plant defense metabolites are limiting factors in the food and nutrition Messina (2004).Seed contain several beneficial phytochemicals essential for growth, development and reproduction Peltier et al.(2009), Olorode et al.(2014). Soy flour, flour fortification, fortification of cereals products is more efficient means of incorporating soy protein in diet for human Liu, (1997), Young, (1997) consumption of soy has been linked to prevention and treatment of chronic disease.

Preoxidase (EC.1.11.1.X) occurs in plants mainly in soybean seed. It is ubiquitous in nature. It catalyze the oxidation of many organic compounds by hydrogen peroxide: animes (o-phenylendiaminne,p-phenlendediamine, benzidine), phenol (pyrogallol, guaicol, o-cresol) hydorquinones, etc. The soybean seed have oxidoreductive peroxidase enzyme belong to class III of the super family phytochemically seed coat content highest activity Ghaemmaghami,et al.(2010).

Peroxidase reaction:[$AH_2 + H_2O$+ peroxidase$\rightarrow 2H_2O + A$] here, AH_2 is a hydrogen donor and A is its oxidized form. Increases in peroxidase activity have been reported in a number of host-parasitic interactions. The peroxidase activity is the reasably stable to heat that have destroyed all peroxidase activity and usually considered to be more adequate to destroy other enzymes and most microbs.In the processing therefore, the adequacy of the blanching process can be monitored by disappearance of peroxidase activity of seeds USDA,(1975).The oxidation of guaicol (colourless) to forms tetraguaicol and water. Reaction: H_2O_2+ Guaicol+Peroxidase$\rightarrow$ Tetra-guaicol (colored)+ Water.

The enzyme lipoxygenase (linoleate oxygen oxidoreductase, (EC 1.13.11.12) are dioxigenase that catalyze the hydroperoxidation of polyunsaturated fatty acids containing cis-cis pentadienes [conjugated hydroperoxide derivatives] moieties both linoleic linolenic acids are substrate of lypoxygenase may be a more appropriate enzyme to measure the adequacy of blanching of vegetables than peroxidase of soybeans seed (Marenco et al. (1995), Meriles et al (2000). The enzyme in oil-bearing seeds of soybeans, can be an important source of hydroperoxides formed in the oil during extraction. Lipoxygenase from soybean seed is the best characterized among plant. Lypoxygenase reaction : [-CH=CH-CH_2-CH=CH-)+O_2+ lypoxygenae $\rightarrow$ [-COOH-CH=CH-CH=CH-] **(**Williams et al. 1986). Soybean seed lipoxygenase catalyses the hydroperoxidation such as linoleic and linolenic acids and enhance the peroxidation of polyunsaturated fatty acids, leading to the production of several reactive molecules that account for the undesirable grassy beany flavor and taste in soybean processed products (Igor, and Svetlana,2008). Biochemically the increasing of oleic acid in oil content also reduced the trypsin inhibitor palmitic, linoleic, and linolenic acid as well as lipoxygenase in soybean seeds Gupta et al. (2014). Its enzymys lypoxygenase produce beany or unacceptable odors, develops the off flavour and which limited their utilization when seeds come contact the water during process.

Materials and methods:-

The experimental materials are most popular five cultivars of soybean namely as,JS-20-29(V_1),JS-20-34(V_2),JS-97-52(V_3),JS-93-05(V_4) and JS-95-60(V_5) were purchased from soybean research unit (BSP),JNKVV, Jabalpur(M.P)

India. Thermal processing by autoclave cooking for $T_{1\ for}$ 10 min., $T_{2\ for}$ 20min. and $T_{3\ for}$ 30 minutes. The raw seeds of each cultivars were also hydroponically germinated T_4 in seed germinator at 25-26 ^{0} C temperature carefully.
For the analysis bioactive , functional phytochemical constituents of each cultivars were dried in hot air oven at 55 ^{0}C for 6-8 hours till the range 5-6% equilibrium moisture content AOAC (1984) then ground with laboratory hand grinder pack in to plastic air tight container the chemical analysis were carried both raw and processed seeds flour.

Trypsin inhibitor:-
The trypsin inhibitors activity in soybeans was determined by Keshun Liu and Pericles Markakis, (1989).slightly modified method by Kakade et al (1974), Rackis,(1980), AACC(2000). Protein concentration was determined by modified using standard BSA solution for calibration by Temel (2003).

Lipoxygenase:-
The method of Axelrod et al. (1981) was followed with a slight modification. The activity of LOX isozyme was determined via the increase in absorbance at 234 nm after addition of linoleic acid in 0.1 M phosphate buffer (pH 9.0). Lypoxygenase activity was expressed as an optical density increase per mg protein/ min.

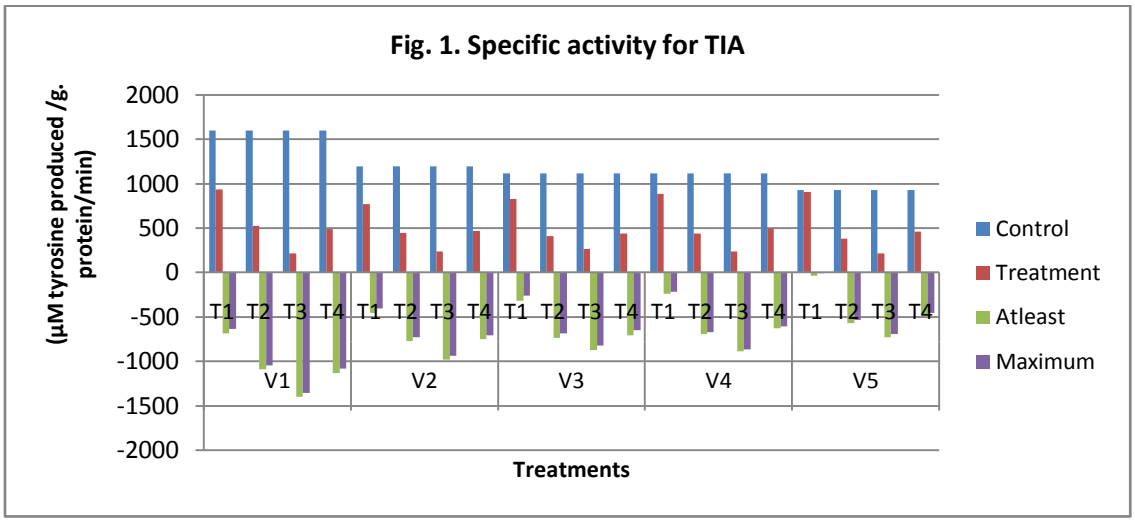
Peroxidase:-
Defatted soybean seed flour prepared and used to prepare buffers in accordance with the methods of Gomori,G(1955).The activity of SBP was calculated by Chance and Maehly, (1955).Protein concentration was measured by the modified Lowry method for the protein determination using standard BSA solution for calibration by Temel,(2003).Specific activity by Silva et al.(2000) Ghaemmaghami et al.(2010).

Results and Discussion:-

Table.1and fig.1 revealed about the effect autoclaved as well as germinated processing techniques on the specific activity for trypsin inhibitor as amount of tyrosine produce per gram protein per minute of each five improved cultivars of soybean seed. The Dunnett's t test for multifactor's comparison was used to identify the significant difference and level of confidence among five cultivars with their (raw seed) control mean.

Table 1:- Specific activity for TIA (µM tyrosine produced /g. protein/min)

Treatments	Control	Processed	d value	Confidence level		
V1T1	1600	940	-660.000	-683.417	to	-636.583
V1T2	1600	530	-1070.000	-1093.417	to	-1046.583
V1T3	1600	220	-1380.000	-1403.417	to	-1356.583
V1T4	1600	490	-1110.000	-1133.417	to	-1086.583
V2T1	1200	770	-430.000	-453.024	to	-406.976
V2T2	1200	450	-750.000	-773.024	to	-726.976
V2T3	1200	240	-960.000	-983.024	to	-936.976
V2T4	1200	470	-730.000	-753.024	to	-706.976
V3T1	1120	830	-290.000	-316.920	to	-263.080
V3T2	1120	410	-710.000	-736.920	to	-683.080
V3T3	1120	270	-850.000	-876.920	to	-823.080
V3T4	1120	440	-680.000	-706.920	to	-653.080
V4T1	1120	890	-230.000	-240.833	to	-219.167
V4T2	1120	440	-680.000	-690.833	to	-669.167
V4T3	1120	240	-880.000	-890.833	to	-869.167
V4T4	1120	500	-620.000	-630.833	to	-609.167
V5T1	930	910	-20.000	-38.730	to	-1.270
V5T2	930	380	-550.000	-568.730	to	-531.270
V5T3	930	220	-710.000	-728.730	to	-691.270
V5T4	930	460	-470.000	-488.730	to	-451.270



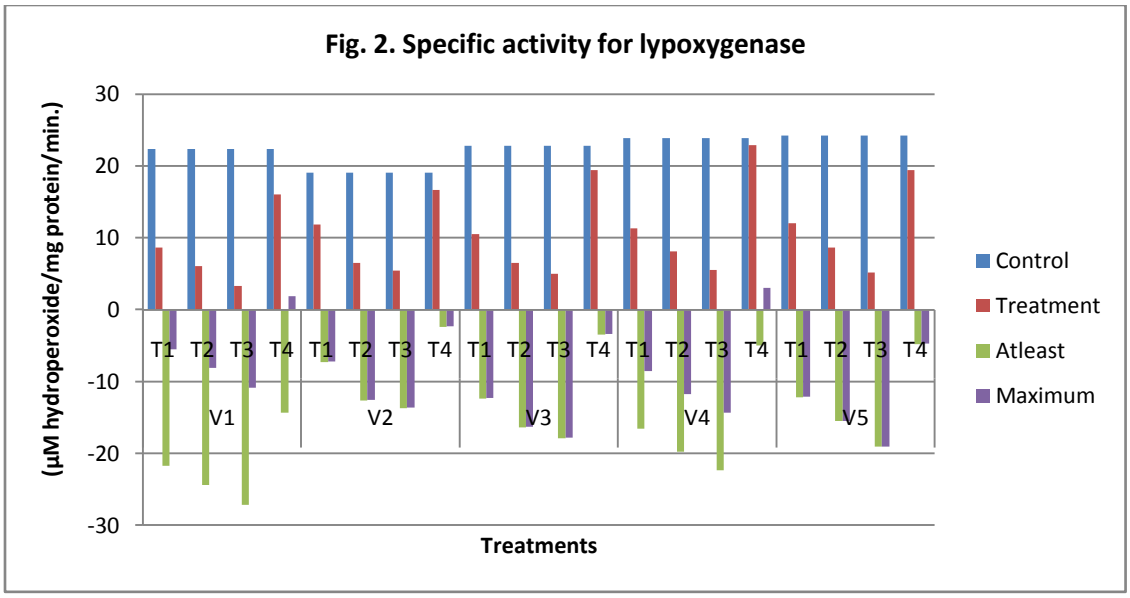
The raw seed of variety V_1 recorded significant superior as 1600 µM tyrosine produced per gram protein per minute, while V_5 showed lowest 930 µM tyrosine per gram protein per min. The result indicate that all five varieties differ significantly reduced the specific activity of trypsin inhibitor by the processing on T_1, T_2, T_3, and T_4, as compared with their raw seeds was control. The confidence interval level for treatment T_1, T_2 and T_3 autoclaved among the variety V_1 differ significantly reduced (d value) is highest than V_2, V_3, V_4, and V_5 differ significant with their control. Hence we can say with 95 percent confidence the variety V_1 loosed significantly much more activity of trypsin inhibitory on cooking at T_1,T_2, and T_3 with their control by at least -683.41 to -636.58,-1093.41 to -1046.58 and -14.41 to -13.58 respectively, followed by V_2,V_4 and V_3 ranged at least -453.02 to -936.97 respectively. The variety V_5T_3 also reduced significant and maintain the quality by at least -728.73 to -691.27, Messina, M. (2004).

Table.2 and fig.2 revealed about the effect autoclaved as well as germinated processing techniques on the specific activity for lypoxygenase as amount of hydroperoxide produce per mg.protein per minute of each five improved cultivars of soybean seed. The raw seed of variety V_5 recorded significant superior as 24.17 µM hydroperoxide produced per mg. protein per minute, while V_2 showed lowest 19.05 µM hydroperoxide produced per mg. protein per min. The result indicate that all five varieties differ significantly reduced the specific activity of lypoxygenase by the processing on T_1, T_2 and T_3, as compare to raw seeds. The confidence interval level for treatment T_3 among the variety V_5 differ significantly highest reduced (d value) than V_1, V_4, V_3 and V_2 differ significant with their control mean Marenco, et al.(1995).

Table 2:- Specific activity for lypoxygenase (µM hydroperoxide/mg protein/min.)

Treatments	Control	Processed	d value	Confidence level		
V1T1	22.3	8.66	-13.640	-21.755	to	-5.525
V1T2	22.3	6.05	-16.250	-24.365	to	-8.135
V1T3	22.3	3.29	-19.010	-27.125	to	-10.895
V1T4	22.3	16.06	-6.240	-14.355	to	1.875
V2T1	19.05	11.81	-7.240	-7.300	to	-7.180
V2T2	19.05	6.47	-12.580	-12.640	to	-12.520
V2T3	19.05	5.39	-13.660	-13.720	to	-13.600
V2T4	19.05	16.67	-2.380	-2.440	to	-2.320
V3T1	22.83	10.48	-12.350	-12.401	to	-12.299
V3T2	22.83	6.49	-16.340	-16.391	to	-16.289
V3T3	22.83	4.99	-17.840	-17.891	to	-17.789
V3T4	22.83	19.39	-3.440	-3.491	to	-3.389
V4T1	23.87	11.28	-12.590	-16.601	to	-8.579

V4T2	23.87	8.1	-15.770	-19.781	to	-11.759
V4T3	23.87	5.5	-18.370	-22.381	to	-14.359
V4T4	23.87	22.89	-0.980	-4.991	to	3.031
V5T1	24.17	12.01	-12.160	-12.169	to	-12.151
V5T2	24.17	8.65	-15.520	-15.529	to	-15.511
V5T3	24.17	5.14	-19.030	-19.039	to	-19.021
V5T4	24.17	19.41	-4.760	-4.769	to	-4.751



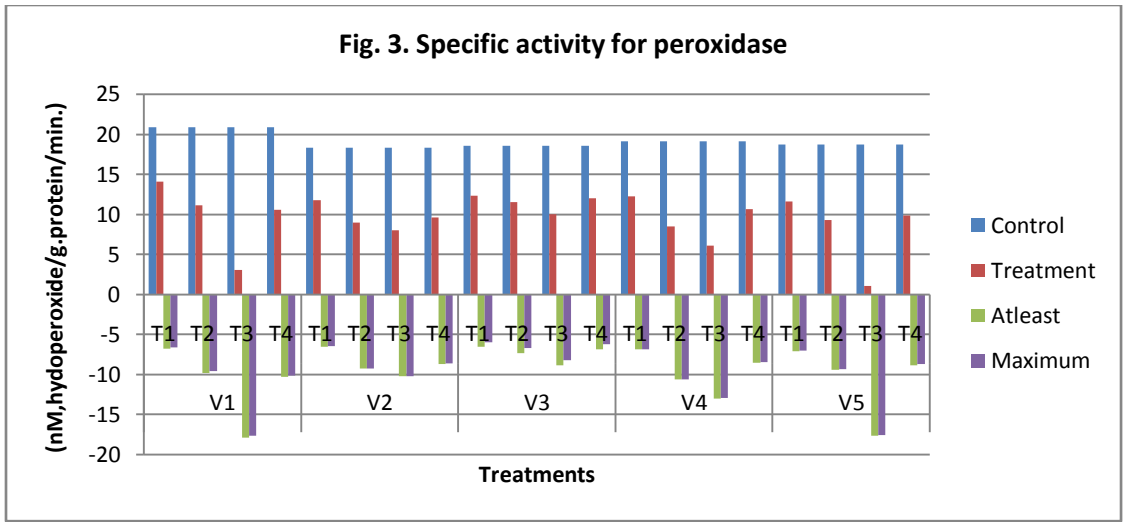
Hence we can say with 95 percent confidence the variety V_5T_3 loosed significantly much more activity of lypoxygenase by at least -19.03 to -19.02 followed by V_1T_3 -27.12 to -10.89,V_4T_3 -22.38 to -14.35, V_3T_3 -17.89 to-17.78 and V_2T_3 -13.72 to -13.60 with their control respectively. We conclude that variety V_5 more affected by duration of high temperature and pressure were found to be superior rather than V_2,V_4,V_3 and V_2 respectively with respect losses of lypoxygenase activity as compare with five varieties of raw soybean seeds.

Table.3 and fig.3 revealed about the effect autoclaved as well as germinated processing techniques on the specific activity for peroxidase enzyme as amount of hydroperoxide produce per g protein per minute of each five improved cultivars of soybean seed. The raw seed of variety V_1 recorded significant superior as 20.81 nM hydroperoxide produced per mg. protein per minute, while V_2 showed lowest 18.25 nM hydroperoxide produced per mg. protein per min. The result indicate that all five varieties differ significantly reduced the specific activity of peroxidase enzyme by the processing on T_1, T_2, T_3 and T_4 as compare to raw seeds. The confidence interval level for treatment T_3 among the variety V_1 differ significantly highest reduced (d value) than V_5, V_4, V_2 and V_3 differ significant with their control. Hence we can say with 95 percent level of confidence the variety V_1T_3 loosed significantly much more activity of peroxidase enzyme by at least -17.89to -17.68 followed by V_5T_3 -17.70 to -17.59 however, V_3T_3 recorded at least -8.88 to- 8.25 lowest but significant with their control respectively. The variety V_1 more affected by time of high temperature with pressure were found to be superior rather than V_5, V_4, V_2 and V_3 respectively with respect losses of activity of peroxidase enzyme as compare with five varieties of raw soybean seeds.

Table 3:- Specific activity for peroxidase (nM,hydoperoxide/g.protein/min.)

Treatments	Control	Processed	d value	Confidence level		
V1T1	20.81	14.07	-6.740	-6.843	to	-6.637
V1T2	20.81	11.07	-9.740	-9.843	to	-9.637
V1T3	20.81	3.02	-17.790	-17.893	to	-17.687
V1T4	20.81	10.55	-10.260	-10.363	to	-10.157

V2T1	18.25	11.71	-6.540	-6.550	to	-6.530
V2T2	18.25	8.96	-9.290	-9.300	to	-9.280
V2T3	18.25	8.01	-10.240	-10.250	to	-10.230
V2T4	18.25	9.55	-8.700	-8.710	to	-8.690
V3T1	18.56	12.27	-6.290	-6.607	to	-5.973
V3T2	18.56	11.53	-7.030	-7.347	to	-6.713
V3T3	18.56	9.99	-8.570	-8.887	to	-8.253
V3T4	18.56	11.98	-6.580	-6.897	to	-6.263
V4T1	19.09	12.2	-6.890	-6.920	to	-6.860
V4T2	19.09	8.44	-10.650	-10.680	to	-10.620
V4T3	19.09	6.06	-13.030	-13.060	to	-13.000
V4T4	19.09	10.58	-8.510	-8.540	to	-8.480
V5T1	18.65	11.54	-7.110	-7.161	to	-7.059
V5T2	18.65	9.26	-9.390	-9.441	to	-9.339
V5T3	18.65	1	-17.650	-17.701	to	-17.599
V5T4	18.65	9.85	-8.800	-8.851	to	-8.749



Conclusion:-

We conclude that variety V_1 more effect for trypsin inhibitory activity (TIA) (µM tyrosine produced gram protein/min) at high temperature rather than V_2, V_3, V_4 ad V_5 with respect to the losses of trypsin inhibitory activity as compare to raw seeds of five varieties. Germination or sprouting process was found better than cooking for 10 min by autoclaved.

The inactivation of lypoxygenase activity show very clearly with 95 percent confidence interval label the thermal processing much more better than germinating process for production of good quality and long storage stability for full fat soy flour, hence variety V_1 was found highly sensitive for specific activity for lypoxygease enzyme at high temperature among the others as compare to raw seeds.

Inactivation of peroxidase activity required more than 20 min processing at high temperature. The results are concluded activity of peroxidase enzyme is more stable till the 20 min autoclaved process as compare trypsin inhibitory activity then lypoxygenase activity reduced proportionally positive to time of autoclaved processing.

Acknowledgement:-

The authors highly acknowledged to Dr. O.P. Sharma (HOD) Department of chemistry and Dr.(Smt.) Anjali Bajpai, Dr.S.K. Bajpai, and Dr A.K. Bajpai , Govt. Model Science College(Auto) Jabalpur(MP).

References:-

1. AACC (2000). Approved methods of the AACC. 10th edition. St. Paul, USA, American Association of Cereal Chemists.
2. AOAC, (2000). Official methods of analysis (17th ed.) Volume I. Association of Official Analytical Chemists, Inc. Maryland, USA. .
3. Axelrod, B., Cheesebrough, T.M. and Laakso, S.(1981). Lipoxygenase from soybean. Methos in Enzymology, 71 : 441-451.
4. Chance, B. and Maehly, A.C. (1955). Enzymatic Assay of Peroxidase (EC 1.11.1.7) from soybean, Methods in enzymology, 2 : 773.
5. Silva, E.D., Teixeira, M. and Franco, T. (2000). Purification of soybean peroxidase (Glycine max L.) by metal affinity partitioning in aqueous two-phase system. J. Chromatog. B-743, 287.
6. Ghaemmaghami, F., Alemzadeh, I. and Motamed, S. (2010). Seed coat soybean peroxidase : Extration and Biocatalytic properties determination. Indian Journal of Chemical engineering, 7(2) : 28-38.
7. Gomori, G. (1955). Methods in enzymology. In : Colowick SP, Kaplan No, editors. Vol. I. New York : Academic Press, p. 138.
8. Gupta,G.K., Sharma S.K., Kumar V. and Ramteke, R. (2014). Effect of purple seed stain disease on physical and biochemical traits of soybean. Soybean Res.12 (1): 77-84.
9. Huisman, J. and Tolman, G.H.(2001). Antinutritional factors in the plant proteins of diets for non-ruminants. Recent Developments in Pig Nutrition, 3, 261-291, Netherlands
10. Igor, A. Butovich and Svetlana, M. Lukyanova (2008). Inhibition of lipoxygenase by linolyl hydroxamic acid: comparative in vitro studies. J. Lipid Res. 49: 1284- 1294.
11. Meriles J.M., Guzman C.A. and Maestri, D.M.(2000) Lypoxygenase activity of soybean genotypes grown in Argentina. Molecules, 5:475- 478.
12. Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G. (1974) Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. American Association of Cereal Chemists, 51, 376-377.
13. Verma K.,Talukdar, A., Shivkumar, M., Kumar B., Lal S.K., Srivastava N., Sapra R.L., Harish G.D. and Girmila, V.(2015). Biochemical screening for trypsin inhibitor factors and morphomolecular characterization of soybean (*Glycune max* L merr.).Indian J.Gene., 75 (4): 490-495.
14. ley AF, K ühn K, Schewe T,editors (1998). Plant lipoxygenase: structure and mechanism in eicosanoids and related compounds in plants and animals.Portland Press; 1998. p. 69-95.
15. Liener, I.E.(1980). Antinutritional factors as determinants of soybeans quality. In: Frederick, T.C., World Soybean Research Conference II. Westview Press Boulder, Colorado. 703-712.
16. Messina, M. (2004). Nutritional value of soyfood. Ph.D. Thesis Department of Nutrition, Michigam State University, United State of Maerica, pp. 6.
17. Olorode, Omobolanle, O. Idowa Michael, A., Bamgbose, A. and Ayano Adeola, E. (2014). Chemical, phytochemical and functional properties of selected seeds flours. Inter. J. Nutr. Food Sci., 3(6) : 572-578.
18. Rackis, J.J. (1980) Biological active components. Ch. 6, In soybeans chemistry and technology. Volume-1Proteins, A.K. Smith and S.J. Circle (ed), p. 158-202. AVI Publishing Co., Inc, Westport, CT.
19. Marenco, R.A., Lopes, N.F. and Moreira, M.A.(1995). Photosynthesis ad leaf lipoxygenase activity in soybean genotypes lacking seeds lipoxygenase isozymes. R. Bras.Fisiol.Veg. 7(1): 21-25.
20. Shivakumar, M.,Verma, K., Talukdar, A.,Srivastava, N., Lal, S.K., Sapra, R.L. and Singh, K.P. (2015). Genetic variability and effect of heat treatment on trypsin inhibitor content in soybean (*Glycune max* (L) merrill.). Legume Res.,38: 60-65.
21. Temel, R.E. (2003). Lowry protein assay, J. Biol. Chem. 278 : 4792.
22. USDA (1975). Enzyme inactivation tests (frozen vegetables) inspection procedure for the use of USDA inspection. Agril. Mar. Ser.US,Dep.of Ag.,Washington DC.
23. Vineet, K., Anita, R. Vimal, P. and Chauhan, G.S.(2005). Changes in lipoxygenase isozymes and trypsin inhibitor activity in soybean during germination at different temperatures, Indore, India.
24. Williams, D.C., Lim, M.H., Chem, A.O., Pongborn, R.M. and Whitaker, J. (1986). Blanching of vegetables for freezing-which indicator enzyme to use. Food Technol. 40(6) : 130-137.