I J R B A T, Issue (XII) Vol (III) Sept 2024: 116-125 OPEN ACCESS e-ISSN 2347 – 517X
A Double-Blind Peer Reviewed & Refereed Journal Original Article

INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
 www.ijrbat.in

STUDY OF SOME SOYBEAN VARIETIES CULTIVATED IN THE VIDARBHA REGION OF MAHARASHTRA STATE, WITH SPECIAL REFERENCE TO SOYBEAN TRYPSIN INHIBITOR (STI)

Nishikant B. Shiwankar[1,2]* Dayanand P. Gogle[1]

[1] *P. G. Department of Botany, University Campus, RTM Nagpur University, Nagpur-440033, India*

[2] *Department of Botany, D. D. Bhoyar College of Arts and Science, Mouda, Nagpur-441104, India.*

Corresponding author*: *nb.shiwankar.m@gmail.com*

Communicated : 28.05.2024 | Revision : 30.05.2024 & 30.06.2024 Accepted : 10.08.2024 | Published : 30.09.2024

ABSTRACT:

Proteins are the elemental components of the diet of all living organisms. The consumption of soy and other soy-based products is a major source of proteins in the daily diet globally. Soybean (*Glycine max* L.) is a rich source of low-cost proteins cultivated worldwide. The use of soy proteins has exponentially increased in the last few decades because of their potential as nutrients and their positive impact on health. However, despite their potential nutritional value, some anti-nutritional factors (ANFs) interfere with nutrient uptake, exert other physiological effects, and affect efficient nutrient assimilation. Soy proteins contain ANFs such as trypsin and chymotrypsin inhibitors, phytate, glycinin, beta-conglycinin, myo-inositol, allergens, and saponins. Soybean trypsin inhibitors which include, Kunitz trypsin inhibitors (KTI) and Bowman-Birk inhibitors (BBI) are the potential inhibitors of trypsin present in soybean seeds and soy-based products. In the present work, we investigated trypsin inhibitors from some soybean (*Glycine max* L.) varieties cultivated in the Vidarbha region of Maharashtra. study of soy and soy-based products were investigated for phytochemical study by Fourier transform infrared spectroscopy (FTIR), Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) for proteins and trypsin inhibitors, and trypsin inhibitors activity were carried out using substrate N-α-benzoyl-DL-arginine-p-nitroanilide hydrochloride (DL-BAPNA). The study also investigated the changes in the levels of soybean trypsin inhibitors due to soaking, roosting, boiling, microwave irradiation, and processed products like soy milk and soy tofu.

Keywords:- Anti-Nutritional Factors, *Glycine max*, Soybean trypsin inhibitor, SDS-PAGE, Trypsin inhibitor activity.

INTRODUCTION

Glycine max (L.) Merr. commonly known as Soybean, belongs to the family Leguminaceae, globally known as 'Golden Grain' It is widely grown in tropical, subtropical and temperate climates (Benedetti et al., 2016; Khojely et al., 2018). It might have originated in 7000 BCE in central China (Lee, Gyoung-Ah, et al., 2011). In India, besides being relatively a new crop, cultivation started in the early 1970s (Chauhan et al., 2005; Agarwal et al., 2013). Compared to most pulses soybean contains a double amount of protein with good quality essential amino acids. Nutritionally, soybeans contain protein (~35-40%), oil (~20%), carbohydrates (~30%) including dietary fiber, minerals (~5%) and other (7-8) based on the dry weight of mature raw seeds (Dixit et al., 2011; Medic et al., 2014; Xiao, 2008). Soybean is a rich source of low-cost proteins cultivated worldwide. Probably, there is no single protein source that is fascinating as much attention from so wide group of researchers and nutritionists as the soybean.

http://doi.org/10.29369/ijrbat.2024.010.3.00016

Besides possessing nutraceuticals soy has therapeutic properties. The abundance of bioactive phytochemicals found in soybeans, including isoflavones, phytic acids, tocopherols, phytosterols, and saponins, has drawn more attention to them. Consuming soybeans has been linked in recent research to a lower risk of osteoporosis, heart disease, and several types of cancer (Isanga & Zhang, 2008). Furthermore, it has been reported that bioactive peptides derived from soy improve several physiological processes, such as immunomodulatory, antibacterial, antihypertensive, anticancer, and antioxidant properties. Soybeans are regarded as a "functional food" with many health advantages because of their exceptional nutritional and therapeutic properties (Bilawal et al., 2022; Agyei, 2015). Despite having several potential nutritional factors such as carbohydrates, dietary fibre, fat and proteins, soybean seeds contain ANFs that interfere with the uptake of these nutrients and exert a negative impact on the nutritional quality of the soy and soy-based products (Liener,1994; Gilani, 2012).

Soybeans contain protease inhibitors, lectins, phytates, saponins, goitrogens, tannins, phytoestrogens, antivitamins, allergens, flatus-producing oligosaccharides etc. (Liener,1994; Gilani, 2012). These factors may differentiate as heat-labile and heat-stable ANFs. Protease inhibitors, lectins, goitrogens, and antivitamins come under heat-labile and saponins, phytates, tannins, phytoestrogens, allergens, and flatulence factors are heat-stable ANFs (Ali, 2022). Kunitz trypsin inhibitor (KTI) and Bowman-Birk inhibitor (BBI) are common protease inhibitors that have been well studied in soybean, which belong to two distinct families of serine protease (Birk, 1985; Laing, 2002). KTI is one of the ANFs that has gained significant importance for their potential inhibiting activity against trypsin-like serine proteases. It has about 21.5 kDa molecular weight, comprising 181 amino acids (lee, 2012; Oliveira et al., 2012; Kumar et al., 2019). BBI is a protein with an approximate molecular weight of 8000 and comprises 71 amino acid residues, notable for its high cysteine content, which enables the formation of seven disulfide bridges. The three-dimensional structure of soybean-derived BBI has been well-established and confirmed (Werner & Wemmer, 1991).

In the present work, we investigated trypsin inhibitors, protein profile and phytochemical investigation of some soybean (*Glycine max* L.) varieties cultivated in the Vidarbha region of Maharashtra. study of soy and soy-based products were investigated by SDS-PAGE, FTIR, soybean trypsin inhibitors activity was carried out using substrate DL-BAPNA. The study also investigated the efficacy in term of inactivation of the soybean trypsin inhibitors on soaking, roosting, boiling, microwave irradiation, and processed products like soymilk and soy tofu.

MATERIAL AND METHODS

Study ANFs with special reference to STI were carried out for soy-based commercial products and different soybean varieties using SDS-PAGE profile, phytochemical study by FTIR, and spectrophotometer-based estimation as shown in Figure 1. The study also proposed to investigate changes in the levels of KTI due to soaking, roosting, boiling, microwave irradiation, and processed food products like soy milk and soy tofu.

Materials:

Glycine max (L) Merr. commonly called soybeans. *Glycine max (*seeds) are collected from various fields in the Vidarbha region of Maharashtra, India. The classification and identification were done using the Ugemuge, N.R. Flora of Nagpur District, 91,1986.; Freshly harvested seeds of five genotypes (MAUS 158, JS 93-05, JS 335, JS 72-44, and JS 95-60) were dried, cleaned with dry cloths and stored in a

http://doi.org/10.29369/ijrbat.2024.010.3.00016

glass container for further use. seeds were used within three to four months of harvesting. The soy tofu and soy milk samples were purchased from the market of Nagpur, Maharashtra (India).

Chemicals:

Kunitz trypsin inhibitor (KTI) from soybean, trypsin from bovine pancreas, phosphate buffer saline (PBS) purchased from Himedia, N-α-benzoyl-DL-arginine-p-nitroanilide hydrochloride (DL-BAPNA), dimethyl sulfoxide (DMSO), purchased from Merck, Mumbai. Bradford reagent from SRL, India, calcium chloride dihydrate ($CaCl_2.2H_2O$), acrylamide, bis-acrylamide (N, N′-Methylenebisacrylamide), Tris-HCl (Tris(hydroxymethyl) aminomethane hydrochloride), sodium dodecyl sulfate (SDS), ammonium persulfate (APS), N,N,N',N'-Tetramethylethylenediamine (TEMED), glycerol, bromophenol blue, β-mercaptoethanol, dithiothreitol (DTT), prestained protein ladder (marker), Coomassie brilliant blue R-250, methanol, acetic acid, formaldehyde, sodium carbonate, sodium thiosulphate, silver nitrate, Citric acid, Acetic acid, n-Hexane and double distilled water etc.

Method:

Method applied for study ANFs with special reference to STI studied in the soy-based commercial products and different soybean varieties that are cultivated in the Vidarbha region of Maharashtra.

Processing of sample and Extraction

Soybean seeds were processed and prepared by soaking overnight (about 15 h) in Milli-Q water; soy flour was boiled for 30 min at 120 ^{0}C; soy flour was roosted at 90-110 ^{0}C about 5-8 min till the flour changed to brownish; soy flour was kept under microwave irradiation for 30 min. A Commercially made product like soymilk and soy tofu was purchased from the market and freeze-dried by lyophilizer till the complete removal of water traces. All these previously proceeded and the ground powder was used for the extraction of protein.

Finely ground soy flour of all the soy varieties and commercial products (500 mg) was homogenized in 15 ml of Milli-Q water. The homogenized preparation was extracted for 3 h in a rotational shaker incubator at room temperature, followed by centrifugation at 12,000 rpm for 15 min at 7 ^{0}C. Collected supernatant from all the samples after centrifugation was further processed for different analyses.

Proteins Profile (SDS-PAGE):

Prepared samples were used after dilution with PBS and combined with loading dye (Laemmli, U.K., 1970) in a 10:3 (v/v) ratio and then heated in a water bath at 95 °C for 5-7 minutes. A stacking gel (5%, pH 6.8) and a separating gel (12 &15%, pH 8.8) were prepared between the plates and allowed to polymerize completely for 30-40 minutes. The electrophoresis tank was filled with Tris-glycine running buffer (pH 8.3). Afterwards, the cooled samples were loaded 10 µl (containing about 50 µg of protein), KTI as standard and prestained protein ladder (5 µl) into wells created by the stacking gel. The gel was run initially by applying a voltage of 50-60 V. Once the samples passed through the stacking gel, the voltage was increased to 120 V until they reached just above the bottom of the plates. Finally, the gel underwent silver staining as shown in figure 2. (Oakley et al.,1980). A similar process was repeated for the second set of samples, where the JS 72-44 samples processed different methods of inactivation of STI [seeds soaked overnight (about 15 h) in Milli-Q water; soy flour was boiled for 30 min at 120 ^{0}C; soy flour was roosted at 90-100 ^{0}C about 5-8 min)].

FTIR Analysis:

Fourier transform infrared spectroscopy (FTIR) was performed on Shimadzu spectrometers (IRAffinity-1/ MIRacle10) within a range of

http://doi.org/10.29369/ijrbat.2024.010.3.00016

wavenumber between 400-4000 cm^{-1} to investigate the chemical composition of defatted soy flour, commercial soy milk and soy tofu.

Trypsin inhibitor activity:

The trypsin inhibitor activity (TIA) of the extract was assessed by a previously reported and described by Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G., 1974. on a double-beam spectrophotometer (SHIMANDZU, UV-1800) by utilising DL-BAPNA as the substrate. The activity is quantified in terms of the number of trypsin units inhibited. A single trypsin inhibitory unit (TIU) corresponds to an increase of 0.01 in absorbance at 410 nm for every 10 ml of the reaction mixture. Each analysis was conducted in triplicate.

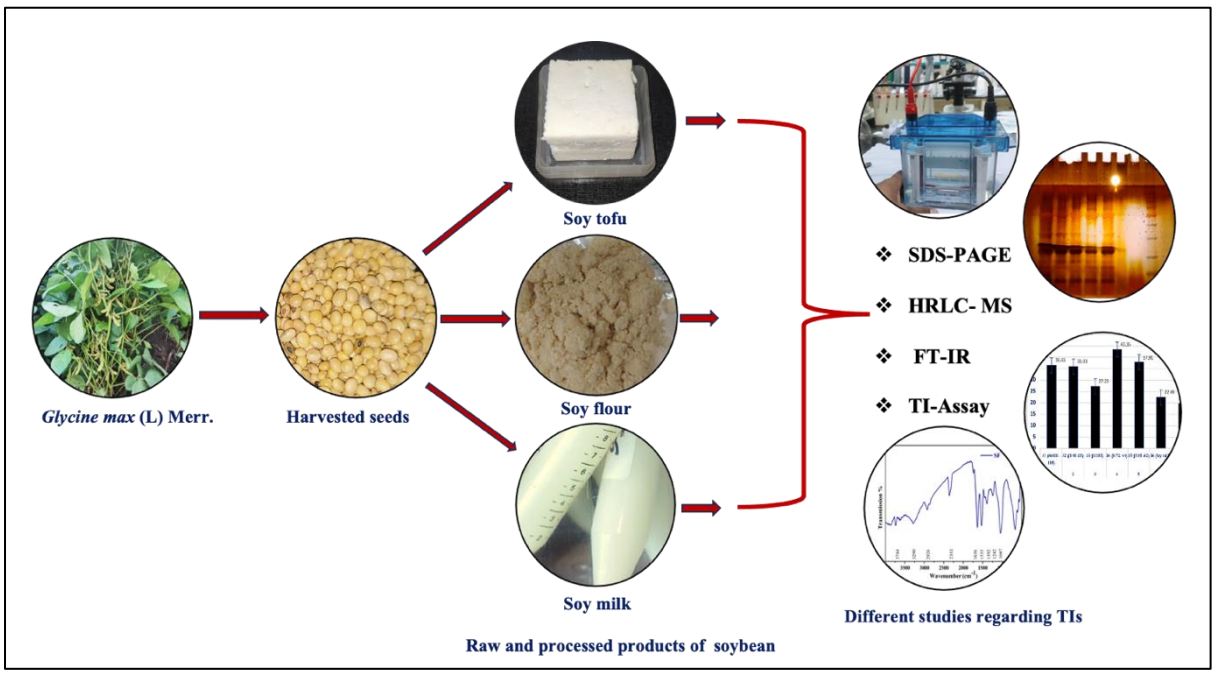

Figure 1: Schematic representation of different studies in soybean and derived products.

RESULTS AND DISCUSSION

Study ANFs with special reference to STI were successfully studied in the soy-based commercial products and different soybean varieties that are cultivated in the Vidarbha region of Maharashtra. The study focused on the KTI profile along with other proteins by SDS-PAGE, FTIR and estimation of trypsin inhibitor activity (TIA) of cultivated soybean varieties and commercially available products in the local market. We also investigate changes in the levels of KTI due to soaking, roosting, boiling, microwave irradiation, and processed products like soy milk and soy tofu.

Proteins Profile (SDS-PAGE):

Soy seeds of five different genotypes (MAUS 158, JS 93-05, JS 335, JS 72-44, and JS 95-60), soy milk and soy tofu were extracted in BPS added with loading dye (Laemmli, U.K., 1970) and a sample were loaded on the gel (10 µL each). along with standard (KTI) and prestained protein ladder.

The SDS-PAGE profile of different samples shows the density band of KTI protein for MAUS 158, JS 93-05, JS 335, JS 72-44, JS 95-60, soy milk and soy tofu shown in Figure 2, (a). The band density concerning KTI was observed to be highest in lane 4. (JS 72-44) and lowest concentration in lanes 1. (MAUS 158), 2. (JS 93-05) among all the studied genotypes and

http://doi.org/10.29369/ijrbat.2024.010.3.00016

commercial products. The SDS-PAGE profile shown in Figure 2, (b) depicted a change in the density of KTI among all the differently processed soy seeds and flour. The highest density band was recorded in lane-1 for raw soy flour (JS 72-44), which is the highest concentration among all the studied genotypes and commercial products. The concentration in the processed soy flour (JS 72-44) was observed to decrease in order of lane 1. Control (raw) 2. roosted, 5. microwave irradiated, 3. boiled, 4. Soaked (highest to lowest). There is no complete inactivation of KTI observed with soaking overnight (about 15 h); boiled for 30 min at 120 ^{0}C; roosted at 90-110 ^{0}C for about 5-8 min till the flour changed to brownish. To achieve the highest degree of inactivation, further processing may be required concerning time, and change or modification in the methodology of KTI inactivation. To calculate the exact concertation of KTI and BBI from Total TIA densitometric study needs to be performed. Furthermore, the depicted observation from the protein profile was compared with TIA activity and discussed (Yalcin & Basman, 2015; Vagadia et al., 2017; Kumar et al., 2019).

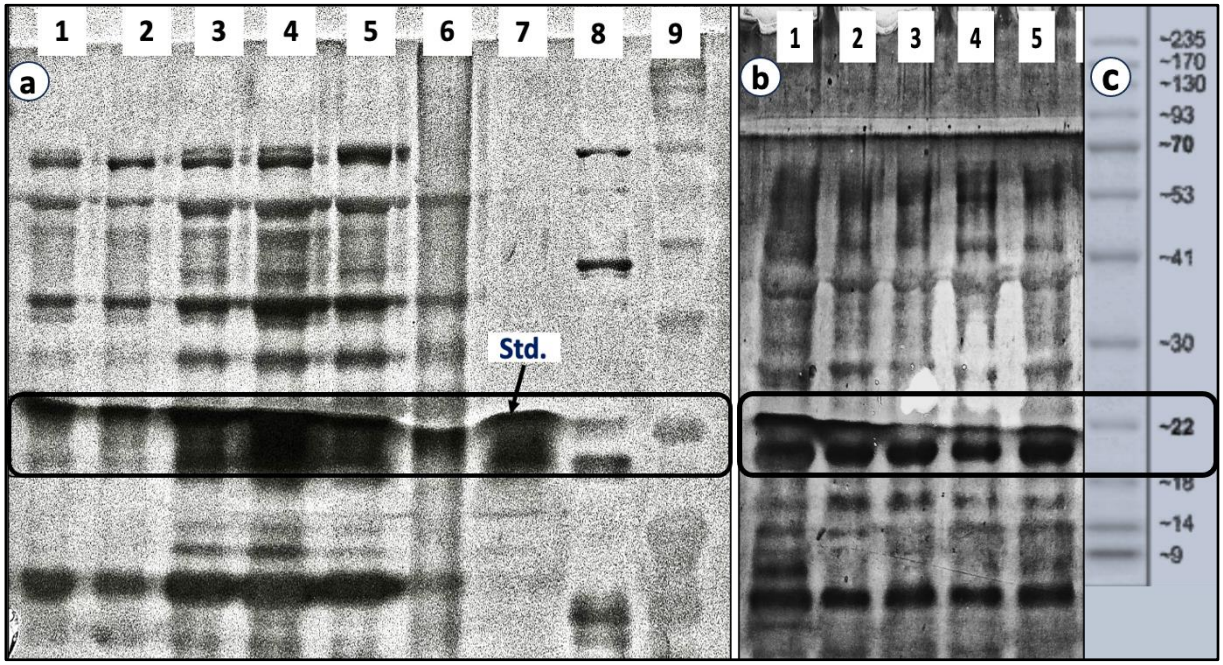

Figure 2: **a.** Freshly extracted seeds of five genotypes (**lane 1.** MAUS 158**, 2.** JS 93-05, **3.** JS -335, **4.** JS 72-44, **5.** JS-95-60), commercial products (**6.** soy milk **8.** soy tofu.) and standard (**lane 7.** KTI, **9.** prestained protein ladder); **b.** Changes in the density of KTI polypeptide after processing of JS 72-44 (**lane 1.** Control (raw) **2.** roosted, **3.** boiled, **4.** soaked, **5.** microwave irradiated) and commercial product **6.** soymilk **7.** soy tofu.); **c.** Prestained protein ladder labelling as a marker (kDa).

FTIR Analysis:

FTIR spectra of soy and soy-based products reveal the chemical composition of defatted soy flour, commercial soy milk and soy tofu shown in figure 3. (a), (b) and (c), respectively. The spectra supervised between the range of 4000-400 cm-1 show a distinct peak at 2926 cm-1 and 2852 cm-1, depicting asymmetrical stretching of CH3 and asymmetrical stretching C-H for the presence of lipids, proteins and carbohydrates. The peaks between 2224-2210

http://doi.org/10.29369/ijrbat.2024.010.3.00016

cm-1 shows O-C-O stretching, and O-C-O bending at 675-685 cm-1 corresponds to the carbon dioxide. A peak significantly observed at 1636 cm-1 is due to C-N, C-O protein stretching representing amide-I, and a strong transmittance peak at 1520 cm-1 suggested protein amide-II corresponds to N-H bend, C-N stretch (Chen et al., 2013; Lee et al., 1018). The weak transmittance peak at 1242 cm-1 advocates amide-III which corresponds to C-N stretching. (Chen et al., 2013; Ghahri et al., 2018; Lee et al., 1018; Lumakso et al., 2015). A transmittance peak at 1392 cm-1 corresponds to CH3 bending vibration, which again confirms the existence of proteins. Stretching corresponding to C-O at 1047 cm-1 confirms the presence of starch. The strong and distinct peak observed at 1744 cm-1 corresponds to C=O stretching, and the small transmittance peak at 1155 cm-1 CO-O-C asymmetrical stretching, C-O stretching suggests the presence of lipids in soy milk and soy tofu, which is missing at in defatted soy flour. Variable and small bands between 4000 -3500 cm-1 correspond to water-vapour O-H stretching (Chen et al., 2013; Ghahri et al., 2018; Lee et al., 1018; Lumakso et al., 2015; B aker & Must akas, 1973).

The trypsin inhibitor activity (TIA) of the extract was assessed by a previously reported method (described by Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G., 1974; Liu, 2019) by utilizing Nα-benzoyl-DL-arginine p-nitroanilide hydrochloride (BAPNA) as substrate. The activity is quantified in terms of the number of trypsin units inhibited. A single trypsin inhibitory unit (TIU) corresponds to an increase of 0.01 in absorbance at 410 nm for every 10 ml of the reaction mixture.

The five different soy genotypes (MAUS 158, JS 93-05, JS 335, JS 72-44, and JS 95-60), soy milk and soy tofu were analysed for TIA assay (Kakade et al. 1974; Liu, 2019). The maximum inhibition observed was 43.35 TIA (%) in soy variety JS 72-44 TIA (%) and the minimum was about 35.93 TIA (%) for variety JS 93-05, while the soy milk and soy tofu processed from unknown genotypes gave 22.48 and 19.77 TIA (%) soy flour respectively. The range of TIA activity of other genotypes is shown in Figure 4. However, furthermore, the genotype JS 72-44 showed maximum TI activity proceeds for processing using a few methods of TIs inactivation which was used conventionally (Kumar et al., 2019; Liu, 2019). The seeds and flour were used raw (control), roasted, boiled, soaked and microwave irradiated along with processed soy milk and tofu. Processing includes soaking overnight (about 15 h); boiling for 30 min at 120 ^{0}C; roosted at 90-110 ^{0}C for about 5-8 min till the flour changes to brownish. Furthermore, processed samples were assessed for change in TIA (Kakade et al. 1974) shown in Figure 5. The decrease in TIA was observed due to processing, however, the complete inactivation was not achieved by a conventional method of inactivation if considered economical and nutritional feasibility. The maximum inactivation was achieved by soaking for 15 hrs. where it comes to 26.11 TIA (%). In previous it was found that 35% of the TI was inactivated after 96 hours of soaking (Mumba et al., 2014). *Lactobacillus species*-mediated inactivation achieved about an 85% decrease in TIs after 5 days of fermentation at 37 °C (Gao et al., 2013). To achieve about 80-85% TI inactivation, thermal treatment needs temperatures between 100 and 200 °C for 15 to 30 minutes (Westfall and Hauge, 1948; Egounlety and Aworh, 2003; Andrade et al., 2016). Many conventional methods like prolonged heating at high temperatures can degrade heat-sensitive nutrients, amino acids, vitamins, ascorbic acid etc. (Hamid et al., 2017). So adaptation to new methods and optimization of processing conditions is essential to secure the nutritional

http://doi.org/10.29369/ijrbat.2024.010.3.00016

values of soy-based and other legume-based food by enhancing efficacy for real-time analysis, removal and inactivation of different ANFs.

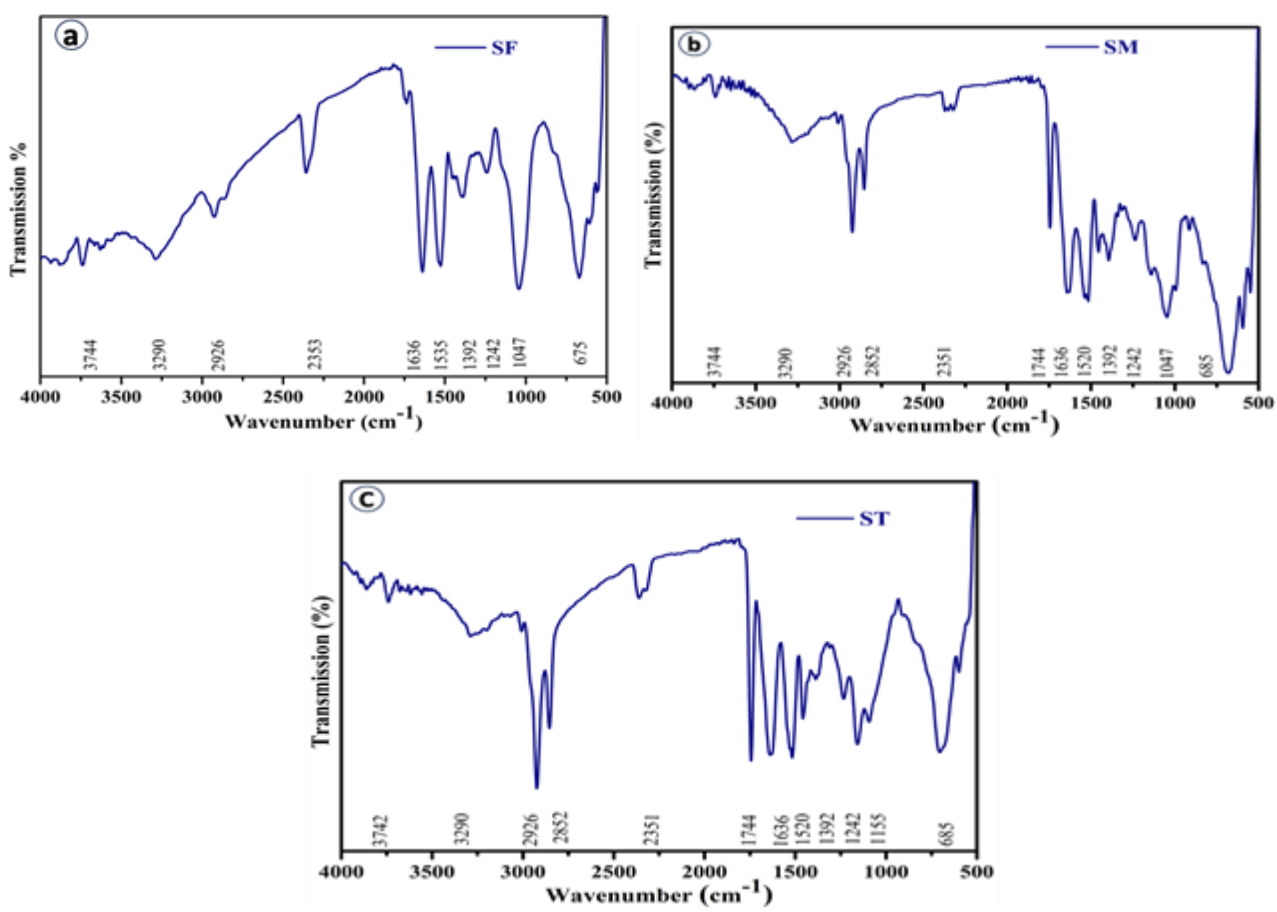

Figure 3: FT-IR spectra (a) Soy flour -SF, (b) Soy milk- SM & (c) Soy tofu -ST

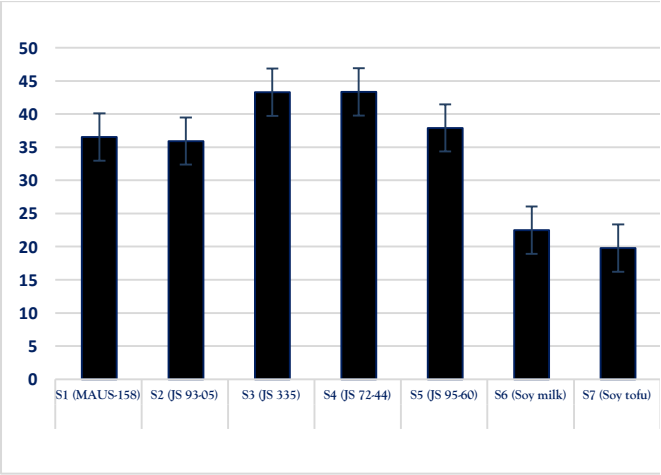

Figure 4: Total trypsin inhibitor activity (% TIA) of different soy flour and product

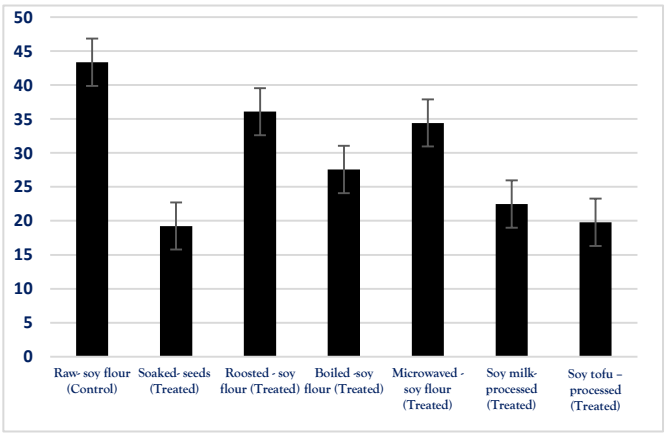

Figure 5: Total trypsin inhibitor activity (% TIA) of different soy flour and product after processing for inactivation of trypsin inhibitor.

http://doi.org/10.29369/ijrbat.2024.010.3.00016

CONCLUSION

Soybean trypsin inhibitors where activity were successfully studied in soy-based commercial products and some soybean varieties that are cultivated in the Vidarbha region of Maharashtra. The study focused on the protein profile by SDS-PAGE, FTIR and estimation of trypsin inhibitor activity (TIA) of soybean varieties and commercial products available in the market. We also investigate changes in the levels of KTI due to soaking, roosting, boiling, microwave irradiation, and processed products like soy milk and soy tofu by SDS-PAGE. The applied method of inactivation shows the partial inactivation of STI in the feasible time, the complete inactivation of STI would be achieved by increasing the time of processing which may not be feasible nutritionally and economically as increased time and energy consumption as well as prolonged heating at high temperatures can degrade heat-sensitive nutrients. Trypsin inhibitor activity and band density appeared in the processed products procured from the market when analyzed on the same methodology. A more nutritionally and economically feasible method of inactivation was needed to adopt soy and soy-based products as nutrient-rich food to overcome the nutritional security.

ACKNOWLEDGMENTS:

The authors are grateful to the Head Department of Botany R. T. M., Nagpur University, Nagpur and the Department of Molecular Biology and Genetic Engineering, R. T. M., Nagpur University, Nagpur for providing the research facilities. The authors are also thankful to Principal D. D. Bhoyar College of Arts and Science, Mouda, Nagpur for providing the research facilities and support during our work.

REFERENCES

Agarwal, D. K., Billore, S. D., Sharma, A. N., Dupare, B. U., & Srivastava, S. K. (2013). Soybean: introduction, improvement, and utilization in India—problems and prospects. *Agricultural Research*, *2*(4), 293-300.

Agyei, D. (2015). Bioactive proteins and peptides from soybeans. *Recent patents on food, nutrition & agriculture*, *7*(2), 100-107.

Ali, A., Devarajan, S., Manickavasagan, A., & Ata, A. (2022). Antinutritional factors and biological constraints in the utilization of plant protein foods. In *Plant protein foods* (pp. 407-438). Cham: Springer International Publishing.

Avilés-Gaxiola, S., Chuck-Hernández, C., & Serna Saldivar, S. O. (2018). Inactivation methods of trypsin inhibitor in legumes: A review. *Journal of food science*, *83*(1), 17-29.

Baker, E. C., & Mustakas, G. C. (1973). Heat inactivation of trypsin inhibitor, lipoxygenase and urease in soybeans: effect of acid and base additives. *Journal of the American Oil Chemists' Society*, *50*(5), 137-141.

Benedetti, S., Prudencio, E. S., Müller, C. M. O., Verruck, S., Mandarino, J. M. G., Leite, R. S., & Petrus, J. C. C. (2016). Utilization of tofu whey concentrate by nanofiltration process aimed at obtaining a functional fermented lactic beverage. *Journal of Food Engineering*, *171*, 222-229.

Bilawal, A., Jiang, Z., Li, Y., & Qi, B. (2022). Isolation, bioactivity, identification, and commercial application of soybean bioactive peptides. In *Phytochemicals in Soybeans*(pp. 217-234). CRC Press.

http://doi.org/10.29369/ijrbat.2024.010.3.00016

Birk, Y. (1985). The Bowman-Birk inhibitor. Trypsin-and chymotrypsin-inhibitor from soybeans. *International journal of peptide and protein research*, *25*(2), 113-131.

Chauhan, G. S., & p Joshi, O. (2005). Soybean (Glycine max)-the 21st century crop. *The Indian Journal of Agricultural Sciences*, *75*(8).

Chen, X., Ru, Y., Chen, F., Wang, X., Zhao, X., & Ao, Q. (2013). FTIR spectroscopic characterization of soy proteins obtained through AOT reverse micelles. *Food Hydrocolloids*, *31*(2), 435-437.

Chen, Y., Xu, Z., Zhang, C., Kong, X., & Hua, Y. (2014). Heat-induced inactivation mechanisms of Kunitz trypsin inhibitor and Bowman-Birk inhibitor in soymilk processing. *Food Chemistry*, *154*, 108-116.

de Oliveira, C. F. R., Vasconcelos, I. M., Aparicio, R., Freire, M. D. G. M., Baldasso, P. A., Marangoni, S., & Macedo, M. L. R. (2012). Purification and biochemical properties of a Kunitz-type trypsin inhibitor from Entada acaciifolia (Benth.) seeds. *Process Biochemistry*, *47*(6), 929-935.

Dixit, A. K., Antony, J., Sharma, N. K., & Tiwari, R. K. (2011). 12. Soybean constituents and their functional benefits. *Research signpost*, *37*(661), 2.

Egounlety, M., & Aworh, O. C. (2003). Effect of soaking, dehulling, cooking and fermentation with Rhizopus oligosporus on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. Walp) and groundbean (Macrotyloma geocarpa Harms). *Journal of food engineering*, *56*(2-3), 249-254.

Gao, Y. L., Wang, C. S., Zhu, Q. H., & Qian, G. Y. (2013). Optimization of solid-state fermentation with Lactobacillus brevis and Aspergillus oryzae for trypsin inhibitor degradation in soybean meal. *Journal of Integrative Agriculture*, *12*(5), 869-876.

Ghahri, S., Mohebby, B., Pizzi, A., Mirshokraie, A., & Mansouri, H. R. (2018). Improving water resistance of soy-based adhesive by vegetable tannin. *Journal of Polymers and the Environment*, *26*, 1881-1890.

Gilani, G. S., Xiao, C. W., & Cockell, K. A. (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *British Journal of Nutrition*, *108*(S2), S315-S332.

Isanga, J., & Zhang, G. N. (2008). Soybean bioactive components and their implications to health—a review. *Food reviews international*, *24*(2), 252-276.

Kakade, M. L. (1969). An evaluation of natural vs. synthetic substrates for measuring the antitryptic activity of soybean samples. *Cereal Chem.*, *46*, 518-526.

Kakade, M. L., Rackis, J. J., McGhee, J. E., & Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure.

Khojely, D. M., Ibrahim, S. E., Sapey, E., & Han, T. (2018). History, current status, and prospects of soybean production and research in sub-Saharan Africa. *The Crop Journal*, *6*(3), 226-235.

Kumar, V., Rani, A., Mittal, P., & Shuaib, M. (2019). Kunitz trypsin inhibitor in soybean: Contribution to total trypsin inhibitor activity as a function of genotype and fate during processing. *Journal of Food*

http://doi.org/10.29369/ijrbat.2024.010.3.00016

Measurement and Characterization, *13*(2), 1583-1590.

Laing, W., & McManus, M. T. (2002). Proteinase inhibitors. *Protein-Protein Interactions in Plant Biology. CRC Press, Boca Raton, FL*, 77-119.

Lee, B. J., Zhou, Y., Lee, J. S., Shin, B. K., Seo, J. A., Lee, D., ... & Choi, H. K. (2018). Discrimination and prediction of the origin of Chinese and Korean soybeans using Fourier transform infrared spectrometry (FT-IR) with multivariate statistical analysis. *PLoS One*, *13*(4), e0196315.

Lee, G. A., Crawford, G. W., Liu, L., Sasaki, Y., & Chen, X. (2011). Archaeological soybean (Glycine max) in East Asia: does size matter?. *PloS one*, *6*(11), e26720.

Liener, I. E. (1994). Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science & Nutrition*, *34*(1), 31-67.

Liu KeShun, L. K. (2019). Soybean trypsin inhibitor assay: further improvement of the standard method approved and reapproved by American Oil Chemists' Society and American Association of Cereal Chemists International.

Lumakso, F. A., Rohman, A., Riyanto, S., & Yusof, F. M. (2015). Detection and quantification of soybean and corn oils as adulterants in avocado oil using fourier transform mid infrared (FT-MIR) spectroscopy aided with multivariate calibration. *Jurnal Teknologi (Sciences & Engineering)*, *77*(1).

Medic, J., Atkinson, C., & Hurburgh, C. R. (2014). Current knowledge in soybean composition. *Journal of the American oil chemists' society*, *91*, 363-384.

Mumba, P. P., Chilera, F., & Alinafe, G. (2004). The effect of the length of soaking time on trypsin inhibitor, crude protein and phosphorus contents of soybeans (Glycine max). *International Journal of Consumer Studies*, *28*(1), 49-54.

Oakley, B. R., Kirsch, D. R., & Morris, N. R. (1980). A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Analytical biochemistry*, *105*(1), 361-363.

Orf, J. H., Mies, D. W., & Hymowitz, T. (1977). Qualitative changes of the Kunitz trypsin inhibitor in soybean seeds during germination as detected by electrophoresis. *Botanical Gazette*, *138*(3), 255-260.

Vagadia, B. H., Vanga, S. K., & Raghavan, V. (2017). Inactivation methods of soybean trypsin inhibitor–A review. *Trends in Food Science & Technology*, *64*, 115-125.

Vineet Kumar, V. K., Anita Rani, A. R., Priyanka Mittal, P. M., & Mohd Shuaib, M. S. (2019). Kunitz trypsin inhibitor in soybean: contribution to total trypsin inhibitor activity as a function of genotype and fate during processing.

Westfall, R. J., & Hauge, S. M. (1948). The nutritive quality and the trypsin inhibitor content of soybean flour heated at various temperatures.

Xiao, C. W. (2008). Health effects of soy protein and isoflavones in humans. *The Journal of nutrition*, *138*(6), 1244S-1249S.

Yalcin, S., & Basman, A. (2015). Effects of infrared treatment on urease, trypsin inhibitor and lipoxygenase activities of soybean samples. *Food chemistry*, *169*, 203-210.

http://doi.org/10.29369/ijrbat.2024.010.3.00016