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Effect of Different Thermal and Non-Thermal Treatments on Total Phenols and Antioxidant Content of Soybean

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Abstract: Soybean is the most explored commodity for protein and fat as well as for obtaining products like soymilk and tofu. However, the presence ofanti-nutritional factors in soybean is not favorableand these are needed to be inactivated. Similarly, soybean is known for potential source of isoflavones, total phenolics and antioxidants.To inactivate anti-nutritional factorsdifferent treatments had been applied and consequently their corresponding effect on total phenolics (TP) and antioxidant capacity (AOC) were studied. High Pressure Processing (HPP), autoclave and microwave boiling treatments wereemployed to the soybean samples in soaked condition, and correspondingly their TP and AOC were estimated. HPP was not observed to affect the TP and AOC content significantly. The microwave treatment was observed to be affecting the TP and AOC but did not exhibit any severe effect while autoclave boiling lead to degradation of the bioactive by more than 50% and it was found to be severe. Conclusively, HPP was found to be suitable treatment to inactivate anti-nutritional factor and retain the bioactives like TP and AOC. The most elevated concentration of phenolic was acquired for HPP treatment at 400 Mpa for 10 min (1.88 mg/100g of the sample) and was almost similar to 400 Mpa for 5 min(1.87 mg /100g of the sample) and was observed that at higher pressure treatment time was not significant. The antioxidant capacity of the grains was similarly affected by the other processing techniques, but maximum was observed for raw sample (230.56 mg/100g of the sample). The autoclaveboilingimpaired severely total phenolics, and antioxidant capacity and it was observed to be 1.07mg/100 g of sample and 94.77 mg/100 g of the sample, respectively.

Keywords: Soybean, Total Phenolics, Antioxidant, Microwave, High Pressure

I. INTRODUCTION:

One of the real crops on the planet, this seed is enriched with protein and a great source of oils, proteins and for production of animal feed. (Yin et al., 2011). Soybean seeds, besides oil and proteins, contain sugars as well. In addition, soybean is also a rich source of bioactive like phenolics and antioxidants. These antioxidants, isoflavones and phenolics are considered as strong free radical scavenging ailments. Since free radicals are created as a piece of ordinary metabolic physiological procedures.

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These are amazingly receptive, flimsy and conceivably debasing transient asubstance species.

Under the physiological express, the cell redox state is immovably constrained by cancer prevention agent enzymatic frameworks and substance foragers, for example, endogenous chemicals, dietary cell reinforcements just as certain hormones.The degree of phenolic and alternative auxiliary metabolites in soybean are influenced by various variables, as well as soil, water system, and climate. Soil development ofharvests could likewise induce year-to-year inconstancy within the constituents of phytochemicals and in complete yield (Bourgaud et. al, 2001).Total phenolics and antioxidants are essential source of protecting from many diseases. Like skin maturing, numerous malignant growth atherosclerosis, cirrhosis, and waterfalls (Halliwell and Gutteridge, 2000). A cell reinforcement is a concoction substance that hinders the oxidation of different synthetic compounds. They ensure the basic cell segments by killing the harming impacts of free radicals, which are inborn results of cell digestion. However, destruction of these bioactive compounds by various treatment and processing activities are very prominent. It was found that around 30-40% of phenolswould be lost within fundamental seeds (P. Vulgaris) by submerged warmth treatment and draining the cooking media (Molina, Fuente and Bressani, 1975). In another investigation, right around 43-62% of absolute phenolic, 68-78% of all flavonoid, 28-36% of concentrated tannins, and 88-95% of monomeric anthocyanin was diminished in treated dark seeds. Altogether,pressure boiling and ordinary boiling caused major total phenols and total flavonoid losses than heat treatments by steaming in black soybeans (Hang, et. al, 2008). In this investigation, the soybean samples are treated with various techniques, which are ordinarily utilized for the preparing of the soybean, during inactivation of the counter dietary factors, and results were broke down and contrasted with the sample without treatment, the sample with broken hull and High-Pressure. The high-pressure method is a novel system of preserving the greater part of the constituents of sustenance under tests.

2. Materials and Methodology:

2.1 Procurement, cleaning and storage of raw material

Soybean glycine max. (cv. JS-9305) was procured from NRCS, Indore (Madhya Pradesh,India). The obtained raw material was cleaned manually to remove immature, discoloredand mechanically damaged beans, and other impurities like stalks, leaves, stones and dust.The cleaned soybeans were stored at 4 °C for further studies (Tripathi et. al., 2015).

2.2 Microwave, Autoclave as thermal treatment and High pressure as Novel treatment

2.2.1 Autoclaving

Hard and fast soybean was measured and took contained by beaker withdistilled food grade water which is incorporated in the extent of 1:10 (w/v). The fixed of beaker by using cotton plug nearby darker paper was done and autoclave was used for treatment (Deg C bioline vertical autoclave, bioline advancements, Mumbai). The samples were kept for 30 mins, later the rest of the water was disposed of (Yadav, et. at., 2018).

2.2.2 Microwave

Soybean seeds were warm during a microwave treatment (Intellocook 900 W, LG Technologies, South Korea), initially set at 800W. H2O was enclosed during a proportion of 1:15 (w/v) for 2 treatments as 15-and 30-min time span. Water left within the holder was later discarded (Yadav, et. at., 2018).

2.2.3 High pressure treatment

High Pressure treatment was given using the high-pressure vessels. The treatmentcondition was obtained on the basis of previous research articles, high pressure was practiced in a range of pressure to obtain a maximum amount of total phenols and antioxidants. High pressure was practiced for 200, 300, and 400 Mpa, respectively, for 5 min and 10 min each. After the treatment, sample was dry very fast, using vacuum oven dryer to remove the excess moisture uptake during pressurization to inhibit any changes or damage to the grain from moisture uptake.Afterwards dried soybean grains were ground using fine grinder, later extraction and analysis for bioactives was performed(Patras et. al., 2009).

2.2.4 Sample preparation and analysis

For sample preparation first drying was performed at 50 °C, afterwards sample was grounded (Philips Mixer grinder 3128 750 W, Amsterdam) and experienced 0.5 mm work sifter. Every one of these examinations were completed in triplicates.

2.2.5 Extraction of Total Phenolsand Antioxidants from fresh and differently treatedSoybean

For estimation, the freshsample,hot air oven-dried were ground to powder with ahigh-speed mixer grinder and sifted through a 0.5mm sieve opening. The extraction procedures of thesoybean. Summarily, soybean powders (0.5 g thrice) were extracted continuously twice with sumten ml of acetone/water (50:50, v/v). Afterwards, series of extract werekept at 4 °C for use (Yadav, et. al, 2018).

2.2.6 Estimation of phenoliccompounds:

TPC of soybean extract wasestimated according to the method described with minor modifications (Chakraborty, Rao and Mishra, 2015).

2.2.7 Radical-scavenging activity by using DPPH:

Five separate concentrations were used for each soybean extract for estimating the IC_{50}-value against 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH mixture (0.9 mL, 0.04 mg/mL in EtOH) was added into 0.1 mL of soybean extract. The absorption at 517 nm was measured since 30 min of initiation of the chemical reaction. The absorbance of a blank with 1 mL of EtOH and a control with the solution of 0.9 mL of DPPH and 0.1 mL of EtOH mixture were also measured. The percent of DPPH radical lasts after 30 min was investigated (Chung et al.,). IC_{50}indexdenote the proportion of extractneeded to neutralize50% of radicals which was free (Pourmorad, Hosseinimehr,& Shahabimajd, 2006).

Activity affectedby different kind of thermal and non-thermal treatments.

1. Results and Discussion:

This discussion deals with the course of results found during the experimentation on Total Phenolics and Antioxidant

Table 1: Effect of different treatment condition on TPC, GAEAC and IC_{50}-value

Sl no	Treatment	TPC (mg)	GAEAC (mg/100g)	IC_{50}-value (mg)
1	Raw	1.7	230.56	16.39
2	Raw(hulled)	1.83	234.71	16.03
3	200 MPa/5 min	1.68	176.11	21.85
4	200 MPa/10 min	1.74	181.86	22.15
4	300 Mpa/5 min	1.76	191.41	20.06
6	300 MPa/10 min	1.80	207.56	20.68
7	400 MPa/5 min	1.87	187.31	20.21
8	400 MPa/10 min	1.88	181.43	20.65
9	µwave/15 min	1.61	169.21	22.32
10	µwave/30 min	1.50	164.17	25.36
11	15psi, 121°C for 30 min	1.07	94.77	44.22

TPC for this soybean cultivars after different treatment is exhibited in above table. Similarly, IC-50 value and total antioxidant content are alsoshown. A decrease in TPC was seen in soybean cultivar which was exposed to a couple of heat treatments like autoclave and microwave, with different treatment conditions. However, not only the thermal treatment, but non-thermal treatment was also applied to the same cultivars in form of high-pressure processing.

In addition, to see the effect of hull (outer cover) present on antioxidant and TPC, the cultivar was hulled, and comparison was done with all those treatments including, thermal, non-thermal and raw sample. Microwave boilingof soybean seeds for 30 and 15 min has shown a significant decrease in TPC. Similarly, TPC content of the cultivar was decreased to 60% of the raw after autoclave treatment for 15 min at 121 ℃, 15 psi pressure, and it was found to be 1.07mg/100g of the sample, similar observation was reported by other authors (Yadav, et. al, 2018).Further, since microwave is an instant heating treatment and it was found to be less severe than autoclave, in comparison to raw sample, after microwave treatment only 84% of the total TPC was left in the soy cultivar and it was in accordance with some authors.The most noteworthy damage and decrease in phenols observed for thermal, trailed by boiling in microwave, and High-pressure. Moderating in the level of TPC while hydrothermal treatment could be aoutcome of leaching phenols from seed to treatment media, which was noted during the media investigation (Yadav, et. al, 2018). Microwave treatment time was not showing any severe effect on TPC. These results of decrease in TPC was in contrary to some of the authors (Yadav, et. al, 2018), and it was in cohesion with some of the findings. In addition, if we compare microwave treated sample TPC content with High pressure treated, it was only 80% of the High pressure treated. The TPC content for the high pressure treated sample was almost conserved as the raw one. It was not only conserved but the extractability of the TPC was also increased in comparison to the raw ones as reported by some previous authors (Chakraborty, Rao and Mishra, 2015), because permeability of the solvent was increased to inner matrix of sample and yield was more. For raw and hulled sample there was no such difference in TPC contentobserved, but in case of high pressure treatment as thepressure increases, the extractability was increased and was observed to be maximum at 400 Mpa, for 10 min (1.88 mg/100g), at the same time it was also noted that at higher pressure, treatment time is very insignificant parameter. The advancement in TPC could be because of the advance extractability of some phenols by application of isostatic high-pressure treatment.It could be also hypothesizingbyconsidering effect of Le-Chatelier's principle, while pressing,media was more liable to penetrate deep and extract the bioactives, as the membrane functionalityextendedusinghigh pressure processing.In this study,it is not advocating that TPC is more in HPP treated sample than others but trying to emphasize on ability to enhancethe extractability of TPC due to application of instantaneous high pressure as per reported earlier (Patras et. al., 2009). There is notably increase in 5.6% of TPC after High pressure treatment comparison to raw sample.Ethanolic extracts of raw sample was examined for radical scavenging activity or total antioxidant activity of, hulled, high pressure and thermally treated soybean cultivar is represented inabove table, which were investigated byradical scavenging activity method using DPPH. Soybean cultivars exhibited a decrease in free radical reducingability after all modes of treatment, including high-pressure also, same was reported by the other authors also (Chakraborty, Rao and Mishra, 2015). This decrease was more severe than what was reported by the earlier authorsfor IC50 value, also. The amount of antioxidant was calculated in equivalent of the gallic acid (Yadav et. al., 2009). The quantitative amount was observed to be maximum for raw sample compared to all kind of treatments, there was nosuch effect observed on antioxidant content because of hulling process or because of hull removal on soybean. High pressure treatment was observed to show effect of both parameters e.g. pressurizing time and amount of pressure.Firstly, due to increase in pressure, it was observed to be increase in antioxidant content, for 200 and 300 Mpa, but after 300 Mpa this relation was changed to inversely between pressure and antioxidant content. This indicates the degradation of antioxidant starts onwards 300 Mpa, when 400 Mpa pressure was applied indication of more degradation was observed, and this may be due to compression heatinggenerated by high amount of pressure. Further, it was noted that increase in pressurizing time leads to increase in antioxidant content for 200and 300 Mpa, respectively, but similar observation was not obtained for 400 Mpa, with increase in pressurizing time, there was decrease in antioxidant activity at higher pressure. Hence, it was observed to be 32% decrease in antioxidant content after treating for 10 min at 400 Mpa and 20% for 5 min at 400 Mpa, clearly it is indicating that time is an important factor for antioxidant content at high pressure treatment. Autoclave treatment was the most severe treatment, it reduces the antioxidant content to 40% of the raw (60% loss) sample and this was opposite to what reported by previous authors (Yadav, et. al, 2018), and it was because of the leaching to treatment water and thermal degradation, IC50 value was also the maximum for the autoclaved sample. Microwave treatment was a kind of intermediate treatment in its effect in comparison to non-thermal and autoclave, there was significant decrease in antioxidant content of soybean observed after microwave boiling, but it was more than autoclave and less than high pressure. The antioxidant content was reduced to 70% (30% loss) of the raw sample.

II. CONCLUSION

High pressure treatment was noticed to be preserving the total phenolics and Simultaneously it was equal to the amount of phenolics present in raw sample, extractability of phenolics was enhanced by application of high pressure. The most loss in total phenolics and antioxidant activity was observed for autoclaved sample. Microwave treatment was found to be an intermediate kind of treatment due to its effect on total phenolics and antioxidant activity.

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