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Influence of blanching and frozen storage on quality characteristics of vegetable soybean (*Glycine max*)

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ABSTRACT

Effect of blanching on freezing and quality attributes during frozen storage of vegetable soybean [*Glycine max* (L.) Merrill] was investigated during 2015–16 at ICAR-RCER, RC Ranchi. Seeds were blanched for 5 and 10 min in boiling water, steam and microwave. Freezing was carried out at -20°C and stored in frozen condition (-18°C) for 12 months. Quality attributes such as drip loss, ascorbic acid, chlorophyll, hardness, colour, microbiological status and sensory trait were examined. Green seeds (58.3%) were recovered from harvested vegetable soybean pod, in which 54.8% were good seeds and 3.5% were physically damaged. Drip loss of frozen samples was between 3.68% and 5.54% during the storage period. A loss of 35.7% was found in ascorbic acid after blanching that further reduced to 71.9% after 12 months of frozen storage. Chlorophyll reduced to 21.9% due to blanching and freezing that further decreased to 42.4% at the end of storage. Blanching treatments did not show significant variation in hardness while losses of hardness were significant during storage. Colour retention was better in samples blanched in boiling water or steam. Bacterial and fungal count at the end of 12 months frozen storage suggested that samples were microbiologically safe to consume. Sensory examination revealed that 10 min blanching either in boiling water or in steam was more acceptable vis-à-vis samples blanched in microwave.

Keywords: Blanching, Freezing, Physical characteristics, Storage, Vegetable soybean.

Vegetable soybean [*Glycine max* (L.) Merrill] is a rich and better source of dietary fibres and health promoting phytochemicals. It is believed that vegetable soybean (VS) would become popular in India being a good protein source along with other essential nutrients. VS are specialty beans harvested at about 80% of physiological maturity in green-yellow pods. At this stage, seeds are still green, large and soft having no or negligible trypsin inhibitors. VS varieties are rich in protein, fat, phosphorus, calcium, iron, thiamin, riboflavin, tocopherols and isoflavones. Fresh VS pods are cooked with salt for 10–15 min until tender and consumed. Green seeds of VS can also be cooked along with rice to add taste and complementary protein.

Freezing and storing, after blanching, constitute a possible method to commercialize vegetable soybeans (Song *et al.* 2003). Kumar *et al.* (2012) assessed two boiling treatments for minimizing the losses of biochemical attributes of immature seeds of four vegetable-type soybean genotypes. Samples were subjected to boiling under pressure (5 min) and at atmospheric pressure (10 min). Both the boiling treatments equally deactivated lipoxygenases, while boiling at atmospheric pressure was more effective in reducing trypsin inhibitor. Freezing decreases the rate of most deteriorative reactions, such as senescence, enzymatic decay, chemical decay, and microbial growth (Savas *et al.* 2005). Freezing preserves taste, texture, and nutritional value of foods better than any other preservation method; as a result, ever-increasing quantities of food are being frozen throughout the world. Majority of vegetables require a high temperature short time heat treatment or blanching before freezing. Blanching is a thermal process to inactivate enzymes responsible for generating flavours and odours.

To promote consumption of VS, its large-scale production and processing is essential. Grading may be one of the important criteria for export of good quality fresh VS. With a view to devise a low-cost process protocol, the present experiment was undertaken to determine the effect of blanching on freezing characteristics of VS green seeds and to evaluate changes in quality during frozen storage.

MATERIALS AND METHODS

Samples and physical characteristics: Vegetable soybean (Cv. Swarna Vasundhara) was harvested at maturity

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level between R6 and R7. Defective and immature grains were sorted out from the seed lot after shelling. Bold sized green seeds free from all defects were kept in refrigerated condition (8^{o}C) till the completion of experiments. Average weight of pod and seed (n=50) was determined. Size of seed was evaluated by measuring linear dimensions using digital vernier caliper. Geometrical mean diameter (Dp) and sphericity (ǿ) was determined using Eq 1 and Eq 2 respectively (Koocheki *et al.* 2007).

$$Dp = (L.W.T)^{1/3} \quad (1)$$

$$ǿ = [(L.W.T)^{1/3}]/L \quad (2)$$

where L, W and T are length, width and thickness in mm

Bulk density was measured by taking the weight of sample occupying 1/4th cubic meter volume. Moisture content was determined by oven drying method at a constant temperature of 110^{o}C for 48 h.

Blanching treatment: Two batches of 0.5 kg seeds each were blanched in boiling water for 5 min (B5) and 10 min (B10). Similar batch of green seeds was steam blanched for 5 min (S5) and 10 min (S10) separately. Another batch was blanched by heating in a microwave oven for 5 min (M5) and 10 min (M10) at power level 800 W. Blanching time was decided based on several pre-experimental trials. The blanched samples were packed in polypropylene envelops in 100 g and envelops was sealed using heat sealing device. A total of 60 envelops were made out of total six treatments.

Freezing: Packaged samples were placed inside the freezer at -20^{o}C. Freezing was accomplished by cold air blast in refrigerator. After 24 h, one packet of each from six treatments were taken out from compartment for initial (termed as 0 duration of frozen storage) quality analysis. Frozen samples were thawed before quality analysis. Thawing was carried out in normal tap water (30±1^{o}C). Thawing was considered complete when temperature of water stabilized at 10±1^{o}C.

Estimation of drip loss: Drip loss of thawed samples was examined by taking the weights of samples before and after thawing (Eq 3).

$$\textit{Drip loss}\ (\%) = \frac{W1}{W2} \times 100 \quad (3)$$

where, $W_1$and $W_2$are weight in g before and after thawing.

Determination of ascorbic acid and chlorophyll: Ascorbic acid of fresh, freezed and frozen stored samples was determined by standard 2, 6-dichlorophenol indophenols visual titration method (Rangana 2000). Chlorophyll content was determined as described by Li *et al.* (2008). The concentration of chlorophyll was evaluated using spectrophotometer at 652 nm wavelength. Calculations were made using the Eq 4.

$$\text{Chlorophyll content (mg/100 g)} = \frac{A652 \times V}{34.5 \times W} \times 100 \quad (4)$$

where, A is absorbance of chlorophyll extract, V is volume of chlorophyll extract in ml and W is weight of sample in g.

Estimation of textural properties and colour: The hardness of fresh, freezed and frozen stored samples was evaluated using a texture analyzer (TA-HDPlus, Stable Micro System) with cylindrical stainless-steel probe of diameter 2 mm. The pre and post-test speed of probe was set at 3.0 mm/s while speed during the test was set at 2.0 mm/s. Height of the peak point in the curve was recorded as hardness. The colour attributes in L*, a*, b* values of CIE system for samples were measured using a pre-calibrated handy colourimeter. L* indicates lightness, a* is the chromaticity on green (-) to red (+) axis and b* is the chromaticity on blue (-) to yellow (+) axis. Total colour difference (ΔE) between fresh, blanched and frozen stored sample was calculated using Eq 5.

$$\Delta E = \sqrt{(L^* - Lo^*)^2 + (a^* - ao^*)^2 + (b^* - bo^*)^2} \quad (5)$$

Microbiological evaluation: Population of microorganism in frozen stored samples was evaluated by standard plate count method. Samples were crushed in a mortar to a fine paste, diluted appropriately and placed on a standard plate count nutrient agar medium for bacterial count. Similarly, medium of potato dextrose agar was used for fungal growth count. Colonies were enumerated after incubation for 48±2 h at around 36°C.

Frozen storage: Packaged freeze samples were stored in frozen condition at a temperature of -18^{o}C for a period of 12 months. Quality parameters were evaluated at every three months interval. One packet from each treatment was taken out from freezer, thawed appropriately and evaluated for drip loss, ascorbic acid, chlorophyll, hardness, colour, microbiological tests and sensory evaluation. Observations were taken in triplicate and average value was reported.

Sensory estimation: Sensory assessment of frozen seeds was carried using a 5-point balanced Hedonic scale ranging from extreme approval (excellent) to extreme disapproval (poor) (Thakur and Jain 2006, Thakur *et al.* 2019). Sensory assessment carried out in terms of colour and appearance, texture and consistency, taste and flavour and overall acceptability at the beginning of experiment and at an interval of 3 months up to a period of 12 months. Sensory scores were analyzed by 2-factor analysis of variance at 5% significance (Levine 2012).

RESULTS AND DISCUSSION

Physical characteristics: The physical characteristics of VS pod and seed are presented in Table 1. VS can be classified into categories, viz. perfect pods (3-seed pod and 2-big seed pod) that contained 86.6% seeds, rest were imperfect pods including 2-small seed pod, 1-seed pod, shrunken pod, twisted pod, and defected pod. In any harvested lot, 3-seeded pod were only 6.4%, 2-seeded pod were 80.2% which is highest and 1-seeded pod was 13.4%. After shelling of pods, 58.3% of green seeds were recovered, out of which 54.8% were good seeds and 3.5% were physically damaged. Observed dimensions of seeds suggested that it was oval shaped because of sphericity around 0.66. Weight of single seed varied from 0.36 to

Table 1 Physical characteristic of vegetable soybean pod and seed

Parameter	Value (mean ± SD)			
	3-Seed Pod	2-Seed Pod	1-Seed Pod	Only seed
Length (L), mm (n=50)	50.05 ± 5.02	52.33 ± 4.60	37.74 ± 7.97	13.71 ± 1.33
Width (W), mm (n=50)	13.26 ± 1.21	13.10 ± 0.93	13.06 ± 1.06	8.56 ± 0.63
Thickness (T), mm (n=50)	8.72 ± 0.93	8.87 ± 0.60	8.81 ± 0.69	6.34 ± 0.80
Pod wt, g (n=50)	3.38 ± 0.78	2.37 ± 0.27	1.27 ± 0.24	-
Geometrical mean dia. (D_p), mm	-	-	-	9.10 ± 0.84
Sphericity (ø)	-	-	-	0.662 ± 0.02
1000-seed weight, g	-	-	-	667.00 ± 0.13
Bulk density, kg/m^3	343.34 ± 2.16	347.34 ± 2.56	354.12 ± 1.66	569.50 ± 5.56
Moisture content, % w.b	70.11 ± 1.35	71.34 ± 1.12	71.89 ± 1.65	66.89 ± 1.02

0.88 g. Average length of seed was 1.6 and 1.35 times, respectively, of its width and thickness. Seeds of VS were considered bold enough as its geometrical mean diameter varied from 7.1 to 10.0 mm. Bulk density of seed was 64% higher than bulk density of pod. Average moisture content of pod and seed was 71.11% and 66.89%, respectively.

Drip loss: Drip Loss (DL) is an indicator of reversibility effect of the frozen process on tissue, cells and fibres of vegetables (Pukszta and Palich 2007). DL varied from 3.68 to 5.54 among treatments and frozen duration (Table 2). DL gradually increased at a significant level during frozen storage in all treatments ($P<0.05$), whereas it was non-significant among blanching treatments. Therefore, blanching method does not have any significant effect on DL. Increasing trend of DL during the period of frozen storage suggests that the cells may become disrupted due to loss of turgor created by ice-crystals of inter cellular spaces. At beginning of frozen storage, the minimum DL was observed in blanched samples of either 5 or 10 min under microwave followed by steam and hot water blanching, however the difference was non-significant ($P>0.05$). DL of 3.96% in vegetable soybean just after freezing at -20°C in household refrigerator, where soybean seeds were blanched under hot water immersion at 95°C (Chang *et al.* 2014).

Ascorbic acid: Loss of ascorbic acid (AA) is generally attributed to solubility in water and sensitivity to high temperatures under oxidative stress. AA of soybean seed was 23.5 mg/100 g which on blanching and just after freezing reduced to 15.1 mg/100 g (Fig 1). After 12 months frozen storage, average value of AA of all treatments was reduced to 6.6 mg/100 g. Variation among blanching treatment was non-significant ($P>0.05$) whereas the variation during frozen storage was significant ($P<0.05$). Loss of AA was little bit higher in samples of 10 min blanching in comparison of samples of 5 min blanching. AA retention was comparatively better in sample blanched for 5 min in boiling water followed by steam and microwave. In all samples, loss of AA was more prominent after nine months of frozen storage. There was 33.1% and 56.3% reduction in AA in treatments at the end of 6 and 12 months frozen storage, respectively.

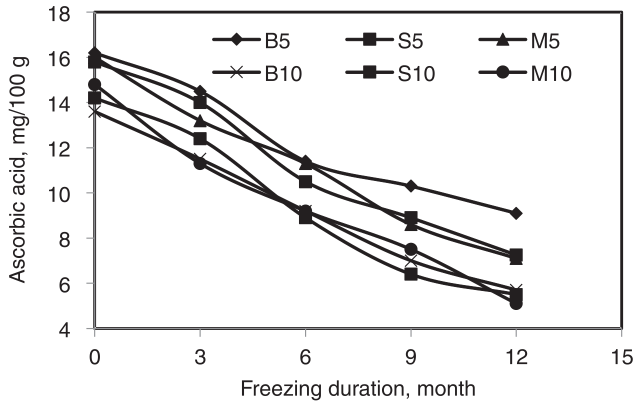

Fig 1 Effect of blanching time, method and storage duration on ascorbic acid.

Table 2 Effect of blanching time, method and storage duration on drip loss

Storage duration/Months	Drip Loss (%)					
	5 min			10 min		
	B	S	M	B	S	M
0	4.24 ± 0.12	3.95 ± 0.17	3.84 ± 0.12	4.31 ± 0.21	3.86 ± 0.09	3.68 ± 0.20
3	4.54 ± 0.18	4.25 ± 0.13	3.98 ± 0.18	4.54 ± 0.14	4.15 ± 0.14	4.22 ± 0.14
6	4.62 ± 0.14	4.35 ± 0.12	4.56 ± 0.17	4.72 ± 0.12	4.23 ± 0.12	4.68 ± 0.10
9	4.78 ± 0.17	4.76 ± 0.19	4.78 ± 0.16	4.9 ± 0.14	4.88 ± 0.10	4.97 ± 0.15
12	5.34 ± 0.20	5.14 ± 0.17	5.00 ± 0.14	5.54 ± 0.18	5.11 ± 0.18	5.20 ± 0.17

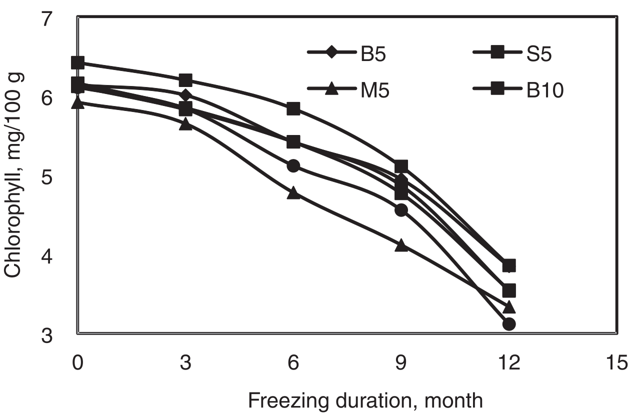

Fig 2 Effect of blanching time, method and storage duration on chlorophyll.

Effect on chlorophyll: Initial value of chlorophyll of VS was 7.8 mg/100 g which was degraded to 6.15 mg/100 g on an average of all treatment after blanching and freezing. Chang *et al.* (2014) found the initial value of chlorophyll of fresh VS as 7.73 mg/100 g sample. Song *et al.* (2003) reported that loss of chlorophyll occurred in the range of 17.7–32.3% under different blanching condition of VS. In this study, chlorophyll reduced to 21.9% due to effect of blanching and freezing. Variation in chlorophyll among blanching treatments was non-significant (P>0.05), however, variation of chlorophyll during frozen storage was significant (P<0.05). Trend of loss of chlorophyll in different treatment during frozen storage is presented in Fig. 2. The average loss of chlorophyll in treatment samples was 13.3% and 42.4% after 6 and 12 month storage, respectively. Maximum retention of chlorophyll was found in samples of 5 min boiling and in 10 min steam blanching at the end of 12 month storage.

Textural properties: Hardness of fresh seeds was 722.0 g_f which reduced to an average of all treatment to 508 g_f after freezing (-20ºC). Decrease in hardness/firmness after blanching may be attributed to gelatinization of starch granules and dissolution of pectic substances (Song *et al.* 2003). Most vegetables are blanched prior to freezing to inactivate undesirable enzymes in tissues. Hardness reduced more rapidly after 6 months storage. Hardness reduced to 5.4% and 17.8% after 6 and 12 months frozen storage. Blanching methods and time did not show any significant difference in the hardness (P>0.05), whereas hardness significantly reduced (P<0.05) during frozen storage. Loss of hardness was slightly low in 5 min blanched samples in comparison to samples of 10 min blanching and the reason may be the occurrence of higher loss of turgor and cell damage during 10 min of blanching.

Colour characteristics: Greenish colour of samples after blanching and subsequent freezing improved to about 20% in comparison to fresh samples. Improvement in colour after blanching and freezing was due to removal of intercellular air from seeds which consequently decreases the opaqueness. At 12 months frozen storage, minimum difference in total colour was in the sample blanched in boiling water for 10 min followed by steam blanching and blanching in boiling water for 5 min. No systematic trend of colour change was observed among the treatments and during freezing storage. However, the total colour difference among treatments and during frozen storage was statistically found to be significant (P<0.05). Overall, better colour was retained in samples either blanched in boiling water and steam in comparison to microwave.

Microbiological quality: The value of bacterial plate count for all treatments at the beginning i.e. just after freezing was negligible. Similarly, there was no colony appearance with respect to fungi. Blanching either for 5 or 10 min effectively destroyed initial load of bacteria and fungi. More or less similar result was found in respect of bacterial and fungal counts in all treatment was after 3, 6, and 9 months of frozen storage. However, after 12 months of frozen storage, number of bacterial count was 2-log cfu/g and fungal count was near to 0 in all samples. This clearly indicated that the experimental process of freezing and frozen storage has a major effect on non-proliferation of bacteria and fungi.

Sensory performance: Variation in quality attributes such as colour and appearance, texture and consistency, taste and flavour of treatment samples was found significant (P<0.05), these attributes were also significantly varied during frozen storage. However, overall acceptability of the frozen stored samples of all treatments reported as non-significant (P>0.05) whereas the difference in mean score during storage as significant (P<0.05). After 9 months of storage, the overall acceptability varied from 4.0 to 4.6 (maximum score of 5) among the treatments, whereas after 12 months it varied between 3.6 and 4.0. Inferences drawn on analyzing the sensory assessment scores that frozen stored samples of all treatment comfortably be utilized for 12 months.

The results conclude that frozen VS is an excellent product and it can be preserved and utilized for round the year. Blanching either in boiling water or in steam for 10 min is sufficient and most suitable to obtain good quality frozen product. Freezing at -20ºC and frozen storage (-18ºC) for 12 months did not affect quality. The investigations suggested that commercial as well as home scale freezing of vegetable soybean has a good prospect to start an agri-entrepreneurship.

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