Hydrothermal effects on physicochemical, sensory attributes, vitamin C, and antioxidant activity of frozen immature Dolichos lablab

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ABSTRACT

Immature Dolichos bean is popular in the Asian region, which is a potent source of vitamin C, antioxidants as well as other macro and micronutrients. Extending shelf life with retaining maximum quality parameters through hydrothermal treatments (HTT) followed by freezing of immature Dolichos bean were carried out. In particular, samples were undergone HTT (n = 9), cooled at room temperature, packed in HDPE packs and stored in a freezer (-18 °C±2), whereas samples without HTT considered as control. The samples were analyzed for physicochemical, sensory attributes, vitamin C and antioxidant activity after three months of freeze storage. In the case of vitamin C, antioxidant activity and color retention, significant differences (p < 0.05) were observed among HTT samples. HTT resulted in improving the cooking quality, sensory characteristics and retaining fresh like traits especially antioxidant activity and vitamin C in Dolichos bean. The study revealed that higher temperature (85 °C-98 °C) HTT for a shorter period of time (3 min) could be suitable for immature Dolichos bean to retain quality parameters without great loss during storage.

1. Introduction

Dolichos bean is known as ‘poor man’s protein’ as it is rich in protein and a cheap source. It also contains high amounts of fiber, complex carbohydrates and minerals (Akpapunam, 1996; Karachi, 1997; Elamin et al., 2013; Shaahu et al., 2015; Longvah et al., 2017) and is low in fat (Hardallo et al., 1980). Immature Dolichos bean possesses considerable amounts of vitamin C and antioxidants, which reduce towards maturity of the beans due to biotransformation (Bhattacharya and Malleshi, 2012).

Dolichos bean is used in multiple ways, both as vegetable and pulse. Tender pods are used as green beans or mixed with other vegetables, immature seeds as shelled beans, young leaf either raw or cooked with other leafy vegetables, flowers as raw or steamed, and mature dried seeds as pulses. Even it is used in fermented foods like Tofu and Tempe as well as sprouted bean (Byregowda, 2007). Legumes harvested at an immature stage undergo quick spoilage as it contains active enzymes and high moisture, and hence are not usually harvested at an early stage (Bhattacharya and Malleshi, 2012). These are often harvested at the immature stage for consumption as green vegetables and are common in some Asian countries including India (Maheshu et al., 2013).

Freezing refers to foods maintained in a frozen condition usually -18 °C or bellow, which is the established and convenient method for longer preservation. Other than preservation it also improves processing properties of foods and controls the rate of certain chemicals and enzymatic reactions as well as the rates of growth and metabolism of microorganisms (Porter and Hotchkiss, 2007). Fresh and frozen vegetable products differ only in texture (Canet, 1989). It gives the taste of fresh products in all aspects of its characteristics over a long period without greater losses. Freezing at the immature stage can retain most of the quality parameters over a long period. Consumers seem to prefer frozen foods because of the nutrient content more than its taste (Ridler and Ridler, 2015). That the texture of final products (green beans) can be improved by applying stepwise blanching such as low-temperature-long-time, high-temperature-short-time followed by cooling (Steinbuch, 1976, 1977) is established. Hydrothermal treatment or blanching is a thermal method applied to a variety of food crops in order to preserve their quality. It is the application of heat and moisture together, where the product is heated by immersion into hot water (50–100 °C) or by steaming (100 °C). In general, mostly used blanching temperature ranging from 70-100 °C which destroys the catalytic activity of enzymes present in fruits and vegetables. But the inactivation of these enzymes depends on the products exposure to thermal duration (Queiroz et al., 2008). Its application...
as a pre-treatment in preparing immature legumes (peas) prior to freezing is well documented (Canet, 1989; Lin and Brewer, 2005). HTT also help in improving and sterilizing microorganisms, other insecticides and improves green vegetable colour, removes off-flavours which are produced during harvesting and processing (Manay and Shadaksharswamy, 2017).

Immature Dolichos beans which resemble green peas somewhat are a candidate for freezing. Frozen green Dolichos beans thus, may add to the consumer's food basket. Dolichos beans are popular legume among Asians because of unique aroma when used as a green vegetable. Due to changing lifestyles, dual-income, technological advances and long working hours, in India and elsewhere in Asia people are ready to use convenience food products and frozen foods are gaining popularity (Pendse and Patil, 2016).

At ambient temperature, untreated Dolichos beans retains its quality a maximum of one day, whereas pre-treated beans show 3 days and at refrigerated conditions shows 12 days (John et al., 2018). Therefore, a maximum of one day, whereas pre-treated beans show 3 days and at

2.1. Procurement of sample

Hebbal Avare 4 (HA 4), a special derivative from HA 3 and Magadi local, which is photo-insensitive, highly produce (40–50 q/ha of green pods) and a characteristic odour (Sogadu) of the green pods. Consumer has a great attraction to Dolichos beans because of its unique odour (Byregowda, 2007). Freshly harvested Dolichos beans (20 kg) variety HA 4 was collected from the farmer’s fields under the direction from the University plant breeder. After collection the samples were cleaned to remove dust and other foreign matter at the time of harvesting. Moreover, the fresh seeds of the Dolichos beans were shelled as soon as possible (within 24 h) and subjected to specific hydrothermal pretreatments before freezing.

2.2. Hydrothermal treatments (HTT)

The immature beans were divided into nine equal portions, of 50 g each, and different HTT were applied to these nine portions. These equal portions were coded and were weighed into perforated high-density polyethylene (HDPE) packs and immersed in a temperature-controlled water bath. Different time and temperature variations (n = 9) were tried based on the inactivation of enzymes described by Queiroz et al., (2008); these were 70 °C for 3, 6 and 9 min; 85 °C for 3, 6 and 9 min; and 98 °C for 3, 6 and 9 min and sample without HTT in HDPE packs served as control. After HTT seeds were patted dry with clean tissue paper. Samples were worked in triplicates for each HTT. Both hydrothermal treated and untreated (control) samples were ready for freezing.

2.3. Frozen storage

Samples subjected to different HTT and control as described in the previous paragraph were immediately frozen in a deep freezer at -18 °C (Remimake, Model: RQF-170). Samples were withdrawn from frozen storage after three months, brought to room temperatures, patted dry with tissue paper and made ready to carry out observations.

2.4. Weight loss

The freeze storage samples which were ready for observations were assessed for any loss in weight during processing (HTT followed by freezing) by recording their weights after three months of freezing storage and just before the samples were hydrothermally treated. For determining the physiological loss in weight, samples were weighed before imposing into HTT which was as considered the initial weight. The same sample was weighed after freezing storage on the observation day, this was the final weight. Final weight was subtracted from the initial weight and divided by the initial weight and the results were expressed as % weight loss.

2.5. Weight gain

The ready-made samples for observations were weighed and this was considered as initial weight. Weighed samples were pressure cooked for 5 min followed by patted dry and final weight was recorded. Weight gained by the sample was determined by subtracting initial weight from final weight after cooking and divided by the initial weight. The value was expressed as % weight gain of the beans after cooking.

2.6. Volume increase

Increase in volume was determined after estimation of the weight gain of the cooked samples. The volume of the sample in a 100 ml measuring cylinder before and after the sample was cooked was measured. Recorded volume was subtracted and divided by the volume recorded before cooking and expressed as % volume increase of the beans after cooking.

2.7. Cooking time

Among two domestic cooking methods, we used pressure cooking instead of open pan boiling, which might retain nutrients mostly than the other one (Gayathri et al., 2004). Accurately 25 g of sample was weighed into stainless containers in 100 ml distilled water and lids were shut. These were immersed in a pressure cooker and cooked initially 4 min at 15 p.s.i. Beans were considered as cooked when the beans were soft enough to mash easily. To find out optimum cooking time few beans were pressed between two fingers. If they were not cooked the cooking was resumed with samples from the same batch. In all observations, readings were taken at 30 s intervals. Time to cook the beans was recorded.

2.8. Sensory evaluation

With the ethical approval of “UAS Ethical Committee” a panel of semi-trained judges (n = 21) were selected from the Department of Food Science and Nutrition, UAS, GKVK, Bangalore, and preliminary assessment procedures were explained. Cooked frozen samples of Dolichos beans were coded and served to the panel inside a sensory evaluation chamber. The panel members were requested to rinse their mouth in between tasting the samples. The beans were evaluated for appearance, texture, colour, aroma, taste and overall acceptability on a nine-point hedonic scale (Meilgaard et al., 2016). Before each sensory evaluation a written consent was taken from all judges.

2.9. Colour measurement

Colour measurements of hydrothermal treated and untreated frozen Dolichos beans were carried out by using Konica Minolta CM-5 spectrophotometer (Konica Minolta Sensing Americas, Inc.) with a 30 mm diameter window. Frozen samples were brought to normal temperature and crushed. Three consecutive measurements with replacing of samples were carried out to avoid the heterogeneity of the surface part of the seed. The measurements of the colour coordinate CIE-L° (lightness), a°
(greenness/redness), and \( b^* \) (yellowness/blueness) were obtained from the reflection spectrum of the samples using D65 illuminant/10° observer.

### 2.10. Vitamin C

Vitamin C content was estimated by using 2, 6-dichlorophenol indophenol (DCPIP) method (AOAC, 2006). Sample (5 g) was mixed thoroughly with 4% oxalic acid solution, squeezed by using a muslin cloth and volume made up to 50 mL. Standard ascorbic acid solution using oxalic acid was titrated against dye solution to get dye factor. Vitamin C content present in the solution was estimated by titrating a known volume of the extract against DCPIP (Sisco Research Laboratory Pvt. India). Results were expressed as mg of vitamin C per 100 g of the fresh sample by using a standard curve of L-ascorbic acid (Sigma-Aldrich Ltd, St Louis, MO, USA).

### 2.11. Antioxidant activity

Antioxidant activity was quantified by DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) radical scavenging activity method described by Kavitha et al. (2014). A concentration of 0.02 mM DPPH was used by dissolving in absolute methanol. A standard curve was prepared using ascorbic acid standards. A 0.2 mL aliquot of methanol extract of the sample was mixed with 0.8 mL of 80% methanol, 0.3 mL of 10 mmol/L-1 acetate buffer (pH 5.5) and 2.5 mL of DPPH followed by vigorous shaking and stored in dark place for 30 min and absorbance was measured at 517 nm. Simultaneously, the blank sample was prepared using 0.3 mL of acetate buffer, 1 mL of 80% methanol and 2.5 mL of DPPH. However, results were expressed as mg of ascorbic acid equivalents antioxidant capacity (AEAC) per 100g of the fresh sample.

### 2.12. Statistical analysis

All experiments were carried out in triplicates. Three-factor experimental analysis of variance (ANOVA) as well as Games-Howell Post Hoc test was used to determine the effects of hydrothermal treatments temperature and time on studied parameters using SPSS statistics software (version 24.0, SPSS Inc., Chicago, IL, USA). Results were reported in this study as mean with standard deviation. Statistical significance was accepted at 5% probability level (\( p < 0.05 \)).

### 3. Results and discussion

In general blanching temperature used in industries ranges from 50-100 °C. But too high or too low blanching temperature will give poor quality of products in terms of physical, sensory, shelf life as well as in nutrients. Therefore, a particular blanching temperature and even more specific time duration is crucial to retain quality parameters and the most important nutrients as well during storage.

#### 3.1. Weight loss during processing

Table 1 depicts the changes in weight due to processing treatments of immature Dolichos beans. Small but significant (\( p < 0.001 \)) decrements in weight were observed. The highest percentage (2.5%) of weight loss was seen for the treatment high-temperature-short time, longer duration of hydrothermal treatment resulted in lower weight loss. It may be due to the swelling of cell walls, disruption of membranes and shrinkages of intracellular spaces etc. (Nieto et al., 1998). Decrements in weight due to hydrothermal treatments of food products after freezing were observed by Aberoumand (2013), Jany et al. (2008), Montdragon-Portocarrero et al. (2006), Briley (2002) and Vedrina-Dragojevic et al. (1997). From these reports it may be inferred that the extent of HTT did not influence the extent of weight loss in food products.

#### 3.2. Cooking characteristics

Increments in weight and volume after cooking of frozen immature Dolichos beans due to different HTT are shown in Table 2. Very few significant differences (\( p < 0.05 \)) were observed between treatments, but no significant differences were found when compared with control in case of weight gain. Increase in volume did not differ significantly between treatments but differed when compared with control. The highest percentage of increase in weight and volume was observed in HTT for higher-temperature-short-time (98° for 3 min). Overall, longer time duration (9 min) of HTT increased weight and volume greatly. HTT has an impact on expanding the volume of legume seeds due to its high protein and complex carbohydrate content, which is desirable in cooking. Blanching solubilizes the pectic substances and alters cell structures and increase the permeability of cytoplasmic membranes to enter water inside (Canet and Alvarez-Torres, 2005) which may affect to increase in weight and volume as well as the eating quality. Therefore, it is important to make this observation. In other studies, Acevedo et al. (2017), Kajihua et al. (2014), Tiwari et al. (2008) and Mwangwela et al. (2006) reported that the hydrothermal treatment significantly increased the weight and volume of different beans after cooking. In this study, longer the treatment times more increments in weight and volume were observed.

Significant differences (\( p < 0.05 \)) in cooking time were found between different HTT when compared with control but no differences were observed among different HTT (Table 2). Higher temperatures of HTT of frozen immature beans resulted in a short duration of cooking time. Kinyanjui et al. (2015), Mustafa et al. (2015), Obasi et al. (2014) and Tiwari et al. (2008) investigated the effects of HTT on cooking quality. They too, observed that HTT resulted in decreasing cooking time.

| Table 1. Effect of processing treatments on weight loss (%) of frozen immature Dolichos beans. |
| Duration of HTT | Control (No HTT) | Temperature of HTT |
|-----------------|------------------|-------------------|
|                 |                  | 70° C | 85° C | 98° C |
| 3 min | 1.61 ± 0.91 | 2.25 ± 0.99° | 1.45 ± 3.11° | 2.48 ± 1.23° |
| 6 min | 1.14 ± 0.59° | 1.08 ± 1.13° | 0.71 ± 0.22° |
| 9 min | 1.02 ± 0.86° | 0.80 ± 0.93° | 0.44 ± 0.62° |

Values are represented as Mean ± SD; Different superscripts (a, b and c) within a column indicate significant differences due to HTT duration according to ANOVA test \(( p < 0.05 )\). \( n = 27. \) Significantly differed from control. There were no significant differences among HTT temperatures i.e. within row.

| Table 2. Effect of hydrothermal treatments on cooking quality of frozen immature Dolichos beans. |
|-----------------|------------------|-------------------|
| Duration Control of HTT | Temperature of HTT |
|-----------------|------------------|
|                 | 70° C | 85° C | 98° C |
| % weight gain |                  |                  |      |
| 3 min | 9.40 ± 1.19 | 10.82 ± 0.75° | 10.99 ± 1.01° |
| 6 min | 8.63 ± 1.98° | 9.87 ± 1.20° | 10.82 ± 1.19° |
| 9 min | 10.94 ± 2.04° | 10.49 ± 0.96° | 10.68 ± 0.94° |
| % volume increase |                  |                  |      |
| 3 min | 5.24 ± 0.78 | 5.43 ± 1.09° | 5.25 ± 1.22° | 6.09 ± 1.35° |
| 6 min | 5.40 ± 0.91° | 5.15 ± 1.34° | 5.62 ± 0.76° |
| 9 min | 5.51 ± 1.0° | 5.01 ± 0.92° | 6.07 ± 1.26° |
| Cooking time (sec) |                  |                  |      |
| 3 min | 317.67 ± 12.25 | 268.33 ± 15.69° | 262.11 ± 17.77° |
| 6 min | 273.56 ± 15.42° | 268.0 ± 15.34° | 261.22 ± 17.0° |
| 9 min | 271.0 ± 13.76° | 265.44 ± 14.66° | 258.89 ± 17.05° |

Values are represented as Mean ± SD; Different superscripts (a, b) within a column indicate significant differences due to HTT duration according to ANOVA test \(( p < 0.05 )\). \( n = 27. \) Significantly differed from control. There were no significant differences among HTT temperatures i.e. within row.
Freezing immature Dolichos beans adds to the convenience of the consumers due to this effect.

### 3.3. Colour retention

Retention in colour of frozen immature Dolichos beans variety subjected to different HTT are shown in Table 3. Higher values on a scale of 0–100 of \( L^* \) values indicate lightness in colour. Significant differences (\( p < 0.01 \)) in the lightness of beans were observed among the hydrothermally treated beans when compared with control. Negative \( a^* \) values indicate greenness (desirable) of samples and did not differ significantly among hydrothermally treated beans. Control samples had higher \( a^* \) values (\( p < 0.01 \)) compared to hydrothermally treated samples. Positive \( b^* \) values indicate yellowness rather than blueness in samples. Significant changes in yellowness of beans (\( p < 0.01 \)) were observed among hydrothermally treated beans as well as when compared with control. As for Dolichos bean light, yellowish-green is preferable, which is found better at high-temperature-short-time (98 °C for 3 min) hydrothermally treated beans. Colour in green vegetables and legumes starts degrading immediately after harvesting due to peroxidase activity. Early processing can reduce colour degradation to a great extent. HTT prior to freezing of immature Dolichos beans. Colour in green vegetables and legumes starts degrading immediately after harvesting due to peroxidase activity. Early processing can reduce colour degradation to a great extent. HTT prior to freezing of immature Dolichos beans.

### 3.4. Sensory attributes

Characteristic “appearance, texture, colour, aroma, taste and overall-acceptability” did not differ among different HTT, although small variations were observed. However, values were higher for samples which were subjected to HTT at high temperatures (85 °C and 98 °C) (Figure 1). HTT showed significantly (\( p < 0.05 \)) better sensory acceptance for attributes appearance, texture, colour, taste and overall-acceptability compared to control. Sensory attributes like appearance, texture, colour and aroma are more important for any food products. But when it comes to vegetables and legumes, sensory parameters are an important factor for purchasing as well as consumption of immature legumes. The initial forms of fresh produce are always preferable to consumers (Canet and Alvarez-Torres, 2005). But due to enzymatic activity and environmental factors alteration in sensory parameters of vegetables and legumes is the very common scenario. Peroxidase and lipoxygenase are mainly responsible for the deterioration of vital sensory attributes-colour and aroma (Chen and Hwang, 1988; Chen et al., 1987). HTT (high-temperature for short-time) prior to freezing offers excellent advantages on texture, colour and aroma (Mwangwela et al., 2006; Brewer et al., 1995b; Sheu and Chen, 1991). The current study also supports that, high temperature and short time HTT could be effective in retention of sensory attributes of frozen legumes.

### 3.5. Vitamin C

Table 4 shows the changes in vitamin C contents of frozen immature Dolichos beans due to different HTT. Significant changes (\( p < 0.05 \)) in vitamin C contents among different hydrothermal treated and control samples were observed. Vitamin C values were higher in hydrothermally treated samples compared to control. Exposure to the HTT for longer time resulted in a decrease in vitamin C. HTT of immature Dolichos beans at 85 °C resulted in a better retention of vitamin C. Enzymatic activity, tissue structure along with other contributing factors leads to alteration of vitamin C in vegetables and legumes (Rickman et al., 2007; Canet and Alvarez-Torres, 2005). Studies confirmed lipoxygenase inactive at lower blanching temperature where peroxidase requires more but longer duration causes more losses of vitamin C (Kaack, 1994; Hartzler and Guerrant, 1952). As HTT inactive enzyme activity therefor prior to freezing the process has effects on retaining vitamin C significantly (Bahceci et al., 2005; Lee and Kader, 2000; Inyang and Ike, 1998). Obtaining an optimum point of HTT to specific vegetables and legumes is, therefore, necessary to retain nutrients as possible. According to current findings HTT at 85 °C for the duration of 3–6 min could be suitable in the retention of vitamin C for immature Dolichos beans.

### 3.6. Antioxidant activity

Increased exposure to HTT resulted in better retention of the antioxidant activity. The antioxidant activity was significantly higher (\( p < 0.05 \)) in hydrothermal treated frozen samples (Table 4). The duration of exposure to HTT had significant (\( p < 0.01 \)) effects on antioxidant activity. Study reported that, HTT techniques enhances the antioxidant activity of specific vegetables due to the formation of phenolic derivatives (Wang et al., 2017; Dorantes-Alvarez et al., 2011). So the different temperature and duration used in HTT could be identical to enhance or reduce antioxidant activity. Hence it also necessary to optimize the specific HTT point to retain or reach at highest antioxidant activity of desired vegetables and legumes. Although antioxidants present in fruits, vegetables and legumes are soluble in water and sensitive to heat and therefore during processing (HTT, canning, sterilizing, freezing and cooking) qualitative changes, antioxidant breakdown and leaching to surrounding water may reduce its activity (Amin et al., 2006; Hunter and Fletcher,

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**Table 3. Effect of hydrothermal treatments on colour retention of frozen immature Dolichos beans.**

| Duration of HTT | Control (No HTT) | Temperature of HTT |
|----------------|-----------------|--------------------|
|                | \( L^* \)       | 70 °C | 85 °C | 98 °C |
| 3 min          | 63.79 ± 1.01a   | 62.12 ± 2.42a   | 61.98 ± 0.64a | 65.57 ± 0.25a |
| 6 min          | 60.58 ± 0.56a   | 63.01 ± 2.14a   | 67.29 ± 1.74a |
| 9 min          | 61.67 ± 0.81a   | 62.59 ± 2.09a   | 68.09 ± 1.04a |
| \( a^* \)      | -3.40 ± 0.71a   | -5.24 ± 1.25a   | -6.61 ± 0.74a | -9.66 ± 0.47a |
| 3 min          | -5.70 ± 1.02b   | -5.28 ± 0.99b   | -4.48 ± 0.99b |
| 6 min          | -5.33 ± 0.70b   | -4.81 ± 2.19b   | -4.90 ± 0.29b |
| 9 min          | 32.48 ± 0.30a   | 31.86 ± 0.39a   | 31.93 ± 2.09a | 33.13 ± 3.06a |

Values are represented as Mean ± SD. Different superscripts (a and b) within a row indicate significant differences due to HTT temperature according to ANOVA test (\( p < 0.01 \)). n = 36. There were no significant differences due to HTT duration i.e. within column.
Figure 1. Sensory score of hydrothermal treated and control frozen immature Dolichos beans. C- control. Mean values and 95% Games Howell Post Hoc intervals according to ANOVA test (n = 36).
Table 4. Effects of hydrothermal treatments on vitamin C content (mg per 100 g) and antioxidant activity (mg of AEAC per 100 g) of frozen immature Dolichos beans.

| Duration of HTT | Control (No HTT) | Temperature of HTT | Vitamin C | Antioxidant activity |
|----------------|-----------------|-------------------|------------|---------------------|
|                | 70 °C | 85 °C | 98 °C |                 |              |
| 3 min          | 8.09 ± 0.39a | 9.31 ± 0.43b | 8.99 ± 0.23b | 31.96 ± 2.544b | 38.28 ± 0.20a |
| 6 min          | 7.96 ± 0.18a | 9.54 ± 0.28b | 8.78 ± 0.32c | 36.61 ± 0.18a | 42.37 ± 0.28a |
| 9 min          | 8.37 ± 0.34a | 9.16 ± 0.29 | 8.62 ± 0.57bc | 35.22 ± 0.34bc | 37.86 ± 0.29 | 36.16 ± 0.57bc |

Values are represented as Mean ± SD; Different superscripts (a, b, and c) within a row indicate significant differences due to HTT temperature according to ANOVA test (p < 0.05). n = 36. Different superscripts (x, y) within a column indicate significant differences due to HTT duration according to ANOVA test (p < 0.01). n = 27.

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Additional information

No additional information is available for this paper.

Declaration

Author contribution statement

Md. Mahedi Hassan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data, Wrote the paper.

Neena Joshi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.
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