Changes in the Radical-Scavenging Activity of Bitter Gourd (*Momordica charantia* L.) during Freezing and Frozen Storage with or without Blanching

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ABSTRACT: The effects of blanching, freezing, and frozen storage on the retention of radical-scavenging activity (RSA), total phenolics, and ascorbic acid in bitter gourd were investigated. Blanching of sliced bitter gourd resulted in considerable losses of RSA and total phenolics, and most extensively, of ascorbic acid. In the subsequent frozen storage at −18 °C, RSA and total phenolic content of unblanched and blanched bitter gourd underwent little change for 90 d then gradually declined, but at −40 °C, they practically remained unchanged throughout the entire storage period. On the contrary, ascorbic acid content of both unblanched and blanched bitter gourd decreased abruptly at the early stage in frozen storage. The results show that blanching of bitter gourd improves the retention of RSA and total phenolics during subsequent frozen storage but markedly aggravated loss of ascorbic acid. Finally, it is to be noted that RSA, total phenolics, and ascorbic acid originally contained in the raw bitter gourd were overall best retained by quick freezing followed by frozen storage at −40 °C without preceding blanching.

Keywords: bitter gourd, blanching, freezing, frozen storage, radical-scavenging activity

Introduction

The presence of phytochemicals, in addition to vitamins and provitamins, in fruits and vegetables has been recently considered to be of crucial importance in the prevention of chronic diseases such as cancer, cardiovascular disease, and diabetes (Willet 1994). Many of these phytochemicals have been found to possess much stronger antioxidant activities than vitamins C and E and β-carotene contained in the same food (Cao and others 1996; Eberhardt and others 2000). Consumption of fruits and vegetables has been associated with the prevention of chronic diseases such as cancer and cardiovascular disease (Ames and others 1993; Amin and Lee 2005). Today, consumers are aware of the need to consume a variety of fresh vegetables and fruits every day and frequently use frozen vegetables in place of fresh vegetables. Blanching is one of the most important steps in food processing for various frozen vegetables. The primary objective of blanching is to inactivate undesirable enzymes that cause unfavorable effects on the quality of frozen vegetables (Barrett and Theerakulkait 1995). However, the severity of the process should be limited in order to maintain color, texture, flavor, and nutritional quality (Barrett and others 2000).

Bitter gourd (*Momordica charantia* L.), also known as bitter melon, is one of the most popular vegetables in Asia. In Okinawa prefecture of Japan, it is used for the preparation of several dishes. It can be fried, deep-fried, boiled, pickled, juiced, and dried to drink as tea. In recent years it became a popular vegetable in Japan. Bitter gourd has many medicinal applications and is used as a hypoglycemic agent for diabetic patients. And in addition, it is also beneficial against piles, blood and respiratory disorders, and cholera (Khattak and others 2005). Fruitpulp, seed, and whole plant of bitter gourd have been investigated for their potent hypoglycemic effects (Ali and others 1993; Srivastava and others 1993; Jayasooriya and others 2000). Season of bitter gourd is summer and nowadays in Japan it is eaten as a seasonal vegetable. Postprocessing temperature conditions and temperature fluctuations determine the rate of quality deterioration and the shelf life of frozen vegetables. Improper frozen storage causes evident changes in sensory characteristics that can influence consumer acceptability and also leads to products of reduced nutritive value, mainly in vitamin C. A considerable body of study on the different modes of quality degradation of different frozen vegetables has been published and reviewed in the earlier and recent literature (Kramer 1974; Labuza 1982; Martens 1986; Hung and Thompson 1989; Martins and Silva 1998). Vegetables are a major source of ascorbic acid, a nutrient that besides its vitamin action is valuable for its antioxidant effect. The level of vitamin C, besides being an indicator of nutrient value, can be used, in the case of frozen vegetables, as a reliable and representative index for estimation of the quality deterioration at any point of the marketing route of a product to its final destination, the consumer. Recent studies report vitamin C contents of several frozen green vegetables (Hagg and others 1995; Lisiewska and Kmiecik 1996, 1997; Favell 1998; Howard and others 1999; Kmiecik and Lisiewska 1999) and the effect of pretreatments, as well as storage temperatures, on the preservation of ascorbic acid in fruits and vegetables, but there is no report on the changes in the antioxidant activity and total phenol content of vegetables during frozen storage. Furthermore, the applicability of shelf-life models, under possible temperature fluctuations, has not been fully verified to

MS 20080137 Submitted 2/22/2008, Accepted 5/27/2008. *Author Myojin is with Graduate School of Humanities and Sciences, authors Enami, Nagata, Yamaguchi, Takamura, and Matoba are with Dept. of Food Science and Nutrition, and author Takamura is with KYOUSEI Science Center for Life and Nature, Nara Women's Univ. Kitauoya-Nishimachi, Nara, 630-8506, Japan. Direct inquiries to author Takamura (E-mail: takamura@cc.nara-wu.ac.jp).*

doi: 10.1111/j.1750-3841.2008.00886.x

make it possible to predict, in a reliable way, the nutritional level of a product based on its temperature history.

The objective of this study was to evaluate the effect of blanching, freezing, and long-term frozen storage on radical-scavenging activity (RSA), total phenolic content, and ascorbic acid content of bitter gourd with special reference to the effect of storage temperature and frozen storage time. The ultimate aim of this study was to establish the optimum condition of freezing and long-term frozen storage to best preserve the antiradical scavenging capacity, total phenol content, as well as ascorbic acid content, of bitter gourd without appreciable damages to its sensorial and nutritional qualities.

Materials and Methods

Reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), tris(hydroxymethyl) aminomethane (Tris), L-ascorbic acid, 2,4-dinitrophenylhydrazine, 2,6-dichloroindophenol, Folin–Ciocalteu reagent, sodium carbonate, triethylamine, and gallic acid were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Metaphosphoric acid, stannous chloride, ethyl acetate, sodium chloride, and ethanol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Acetonitrile, n-hexane, 2-propanol, dichloromethane, and methanol (HPLC grade) were also from Wako Pure Chemical Industries. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.). Water used in this study was purified with a Milli-Q-Labo equipment (Millipore Japan, Tokyo, Japan).

Preparation of samples

Bitter gourd was purchased from a vegetable market in Nara, Japan, on the day of the experiment. Bitter gourds were washed, dried by pressing paper towel gently, and cut lengthwise into halves. The seeds and inner tissue were scraped out of the halved bitter gourds-using a plastic spoon. Then, bitter gourds were sliced into 1-cm thickness with a ceramic knife.

Blanching

The bitter gourd (1 kg) was put into boiling tap water (4000 mL) in a stainless pot (30 cm i.d. × 50 cm deep) and blanched uncovered for 4 min. The blanching time was predetermined by negative peroxidase tests (Masure and Campbell 1944; Yemenicioğlu and others 1998). Blanched bitter gourd was drained and cooled in a low-temperature room (4 °C).

Packaging

Blanched and unblanched bitter gourds (40 g) were rapidly frozen in liquid nitrogen and packaged in Ziploc freezer bag (polyethylene bags, 0.068 × 196 × 177 mm, Asahi Kasei Home Products Corp., Tokyo, Japan) and stored in a temperature-controlled freezer at −18 or −40 °C for 6 mo. The content of 35 g per bag was taken out, rapidly frozen in liquid nitrogen, and freeze-dried for 24 h using a freeze-dryer (VD-400F, Taitec, Saitama, Japan). The dried samples were ground into fine powder using a food grinder (IFM-300DG, Iwatani, Tokyo, Japan), packaged in polyethylene boxes with drying agent (Sheet drying agent 1.5 × 2 cm, Taisei Co. Ltd., Oita, Japan) and deoxidizer (Well pack B-30 Taisei, Taisei Co. Ltd.), and kept at −80 °C until analyses.

Preparation of vegetable extracts

Fifty milligrams of the powdered samples were mixed with 6 mL of 90% methanol containing 0.5% acetic acid (Myojin and others 2008). The mixture was vortexed for 1 min followed by ultrasonication for 10 min. The procedure of vortexing and ultrasonication was repeated thrice at a few minutes interval, and the mixture was then centrifuged at 1500 × *g* for 10 min. The resulting supernatant was filtered through a 0.45-μm filter (Ekicrodisc 25 mm syringe filter, Nacalai Tesque) and, if necessary, diluted appropriately before subjected to analyses for RSA, total phenolic content, and ascorbic acid content.

Determination of DPPH-RSA

An aliquot of sample solution (200 μL) was mixed with 100 mM Tris-HCl buffer (pH 7.4, 800 μL) and 1 mL of 500 μM DPPH solution in ethanol was added. The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. Blanks were without the sample solution. The absorbance at 517 nm due to DPPH was measured by a UV-2100PC UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). DPPH-RSA was calculated from the difference in DPPH radical absorbance detected at 517 nm between a blank and a sample. The DPPH-RSA was evaluated from the difference in decrease of the absorbance of the DPPH radical detected at 517 nm. The activity was expressed as micromoles of Trolox equivalent in 100 g of each fresh vegetable.

Determination of total phenol content

Total phenol content was measured according to the method of Singleton and Rossi (1965). The sample solution (200 mL) was added to 800 mL of 7.5% sodium carbonate solution, 1 mL of Folin–Ciocalteu reagent was added, and the mixture was left to stand for 30 min. The absorbance was measured at 765 nm using a Shimadzu UV-2100PC UV-VIS spectrophotometer. The total phenol content was expressed as micromoles of gallic acid equivalent in 100 g of each fresh vegetable.

Determination of ascorbic acid content

Since dehydroascorbic acid has no RSA toward DPPH and hydroxyl radicals (Yamaguchi and others 2001), we measured only ascorbic acid content. It was determined by high-performance liquid chromatography (HPLC) according to the method of Kishida and others (1992) as follows: the sample solution (100 μL), with and without 50 μL of 0.2% 2,6-dichloroindophenol, was mixed with 50 μL of 1% stannous chloride in 5% metaphosphoric acid (50 μL), and 120 μL of 2% 2,4-dinitrophenylhydrazine in 4.5 M sulfuric acid were added. The mixture was incubated in a water bath for 3 h at 37 °C, and then ethyl acetate (1 mL) and water (1 mL) were added. After shaking and centrifuging (1500 × *g*, 4 °C) for 5 min, 300 μL of the ethyl acetate layer were pipetted and dried under flashing nitrogen gas. The residue was dissolved in 200 μL of acetonitrile and applied to HPLC analysis. The HPLC analysis was carried out on a Cosmosil 5C18-AR-II column (4.6 × 250 mm, Nacalai Tesque) using a detector set at 505 nm. The mobile phase was acetonitrile-water (50:50, v/v) adjusted to pH 3.5 with 0.1% triethylamine and phosphoric acid. The flow rate was 1 mL/min. The ascorbic acid content was calculated by subtracting the value of sample without 2,6-dichloroindophenol from that containing 2,6-dichloroindophenol. The data were expressed in milligrams per 100 g of each fresh vegetable.

Statistical analysis

All data represent the means of 4 replicates. Student's *t*-test was accomplished using Microsoft Excel. Differences at $P < 0.05$ were considered to be significantly different.

Results and Discussion

Effect of blanching on RSA, total phenolic content, and ascorbic acid content

The effect of blanching on RSA, total phenolic content, and ascorbic acid content of bitter gourd is shown in Table 1. RSA of blanched bitter gourd was 536.6 μmol Trolox eq. per 100 g fresh weight but markedly decreased to 431.2 μmol by blanching ($P < 0.05$), corresponding to 80% retention. It is known that RSA in most vegetables decreases after blanching, and Yamaguchi and others (2007) showed that RSA lost in the cooking water during blanching and that remained in the blanched bitter gourd nearly add up to the initial RSA before blanching. This indicates that no substantial chemical changes of the antioxidant compounds occurred during blanching. They also reported that the loss of the antioxidant activity in cooking water was generally aggravated by prolonged heating (Yamaguchi and others 2003, 2007). A similar result was reported by Amin and Lee (2005), who carried out a detailed study on the effect of the blanching time for red cabbage, Chinese cabbage, cabbage, mustard cabbage, and Chinese white cabbage. The 4-min blanching adopted in the present study is the minimum required to nullity the peroxidase activity in bitter gourd and seems to be a reasonable compromise between the retention of RSA and preservation of sensorial and nutritional qualities of bitter gourd that can severely be deteriorated by under blanching. The total phenolic content in unblanched bitter gourd was 263.3 μmol gallic acid eq. per 100 g fresh weight but significantly decreased to 226.9 μmol after blanching ($P < 0.05$), that is, 86% retention. Amin and Lee (2005) reported that the retention of total phenol content of 5 varieties of cabbage after 5-min blanching ranged from 43% to 95%. According to Yamaguchi and others (2007), the average retention of the total phenolics in most vegetables after 5-min blanching was 69%, and 17% was in the cooking water. It may be notable that the retention of the total phonolics of blanched bitter gourd in the present study is comparatively high, although it may partly be due to a tissue peculiarity of bitter gourd. The ascorbic acid content in unblanched bitter gourd was 79.9 mg/100 g fresh weight but significantly decreased to 54.6 mg after blanching ($P < 0.05$), corresponding to 68% retention. Lisiewska and Kmiecik (1996) reported that in the whole processing, the step of blanching caused the most loss of ascorbic acid, for example, 41% to 42% for broccoli and 28% to 32% for cauliflower. Yamaguchi and others (2007) reported that the loss of vitamin C in green vegetables could amount to 75% depending on the length of blanching. Yamaguchi and others (2007) also showed that ascorbic acid retained in green vegetables after 5-min blanching is 47% and that 25% was in the cooking water. It is conceivable that oxidation of ascorbic acid during blanching occurred in the cooking water to an extent to account for the missing 28%. In agreement with the results previously reported by many researchers, ascorbic acid of bitter gourd was also found highly liable to loss during blanching. The loss of ascorbic acid (32%) much exceeds that of total phenolics (14%), which is rather close to the loss of RSA (20%). This seems to indicates that the loss of RSA is in large part attributed to the decrease of total phenolics; that is, the contribution of ascorbic acid to the overall RSA in bitter gourd is minor. Since the effect of blanching is markedly affected by the surface area of vegetables in contact with the blanching water (Howard and others 1999), the retention of RSA, total phenolics, as well as ascorbic acid, after blanching may be improved by decreasing surface area of bitter gourd, that is, by increasing appropriately the thickness of the slices.

Effect of blanching, freezing, and long-term frozen storage on RSA, total phenolic content, and ascorbic acid content

Table 2 shows RSA of unblanched and blanched frozen bitter gourd stored at -18 and -40 °C for up to 180 d. Since the initial difference in RSA between unblanched and blanched bitter gourd before freezing is solely due to the blanching effect, it is reasonable to base discussion relevant to the frozen storage on the percentage basis of remained RSA relative to that of bitter gourd of 0-d storage. Table 2 shows that RSA in both unblanched and blanched bitter gourd stored at -18 °C practically remained unchanged for up to 90 d but significantly decreased after 120 d of storage period. It is important to note that the retention of RSA is significantly improved by blanching, indicating possible involvement of enzymatic reactions persisting at -18 °C significantly in unblanched bitter gourd and in a less extent still in the blanched samples. In contrast, RSA in both unchanged and blanched bitter gourd stored at -40 °C remained unchanged for up to 180 d of the storage period. Before concluding this section, it may need to be added that the observed numerical differences of RSA before and after freezing (Table 2) are statistically insignificant. In Table 3 are shown the changes of total phenolic content and in Table 4, the changes of ascorbic acid content in unblanched and blanched frozen bitter gourd stored at -18 and -40 °C for up to 180 d. As ascorbic acid is known to react stoichiometrically with Folin–Ciocalteu reagent (Wu and Prior 2005; Yamanaka and others 2007), the measured values of total phenolic content must include a contribution from ascorbic acid.

It is remarkable that the trend of the RSA retention (percent) against frozen storage period at -18 °C is in close parallel with that of total phenolic content for both unblanched and blanched bitter gourd, indicating that RSA of bitter gourd originates for the most part from total phenolics. On the contrary, the abrupt and drastic diminution of ascorbic acid at an early stage in storage period, that is, within 30 d, is not correspondingly reflected in RSA retention. However, the poor retention of ascorbic acid brings forward a serious nutritional problem: such a large loss of ascorbic acid during frozen storage severely devaluates the nutritional qualities of stored bitter gourd. The loss of ascorbic acid during frozen storage at both -18 and -40 °C is apparently aggravated by the preceded blanching, and even at -40 °C the loss in blanched sample is appreciable.

Table 1—Radical-scavenging activity, total phenol content, and ascorbic acid content in bitter gourd before and after blanching.

Radical-scavenging activity (μmol Trolox eq. /100 g fresh weight)		Total phenol content (μmol gallic acid eq./ 100 g fresh weight)		Ascorbic acid content (mg/100 g fresh weight)	
Unblanched	Blanched	Unblanched	Blanched	Unblanched	Blanched
536.1 ± 18.2[a]	431.2 ± 50.5*(80)[b]	263.3 ± 9.5	226.9 ± 7.7**(86)	79.7 ± 14.6	54.6 ± 3.0**(69)

[a]The values are mean ± SD for 4 determinations.
[b]The values in parentheses are percentage relative to unblanched samples.
*Significantly different from unblanched samples ($P < 0.05$).
**Significantly different from unblanched samples ($P < 0.01$).

This may be suggestive of involvement of nonenzamatic reactions in the loss of ascorbic acid. It seems that the loss of ascorbic acid of bitter gourd during its frozen storage at −18 °C much exceeds the losses reported for whole green peas, broccoli, and spinach under a similar condition (Favell 1998). This may be partly due to large surface area of the sliced bitter gourd. In this context, an optimum combination of sample size (surface area) − blanching time and practice of strictly controlled procedure of blanching–cooling are essential, if blanching in boiling water is to be employed for bitter gourd.

Conclusions

Blanching of sliced bitter gourd in boiling water caused considerable losses of RSA, total phenolics, and, more extensively, ascorbic acid. However, the blanching process notably improved the retention of RSA and total phenolics during subsequent frozen storage at −18 °C. At −40 °C, RSA and total phenolics of both unblanched and blanched bitter gourd remained practically unchanged during 180-d frozen storage but ascorbic acid of blanched bitter gourd markedly decreased. The retention of RSA, total phenolics, and ascorbic acid originally contained in fresh

Table 2—Changes in the radical-scavenging activity of unblanched and blanched bitter gourd during frozen storage at −18 and −40 °C for up to 6 mo.

		Radical-scavenging activity (μmol Trolox eq./100 g fresh weight)			
Bitter gourd	Storage period (days)	−18 °C		−40 °C	
Unblanched	Before freezing	536.1 ± 18.2[a]	(104)[b]	536.1 ± 18.2	(97)
	0	515.2 ± 21.0	(100)	552.3 ± 21.8	(100)
	30	520.9 ± 13.8	(101)	557.7 ± 43.3	(101)
	60	500.1 ± 4.9	(97)	541.4 ± 26.4	(98)
	90	526.6 ± 14.9	(102)	551.3 ± 25.9	(100)
	120	423.2 ± 4.0*	(82)	534.9 ± 41.8	(97)
	150	364.0 ± 34.9*	(71)	597.0 ± 8.9	(108)
	180	355.6 ± 3.5**	(69)	559.5 ± 34.4	(101)
	Correlation coefficient[c]	−0.888		−0.362	
Blanched	Before freezing	431.2 ± 50.5	(103)	431.2 ± 50.5	(108)
	0	417.3 ± 23.0	(100)	397.7 ± 57.2	(100)
	30	427.4 ± 24.3	(102)	406.1 ± 6.2	(102)
	60	407.0 ± 67.9	(98)	424.3 ± 1.8	(107)
	90	402.3 ± 36.6	(96)	403.0 ± 55.6	(101)
	120	357.9 ± 77.3*	(86)	426.5 ± 9.3	(107)
	150	337.6 ± 19.8**	(81)	411.3 ± 77.7	(103)
	180	336.5 ± 39.7**	(81)	401.9 ± 11.5	(103)
	Correlation coefficient	−0.941		−0.173	

[a]The values are mean ± SD for 4 determinations.
[b]The values in parentheses are percentage relative to day−0 samples.
[c]Correlation coefficient between storage period and radical-scavenging activity.
*Significantly different from day−0 samples ($P < 0.05$).
**Significantly different from day−0 samples ($P < 0.01$).

Table 3—Changes in the total phenol content of unblanched and blanched bitter gourd during frozen storage at −18 and −40 °C for up to 6 mo.

		Total phenol content (μmol gallic acid eq./100 g fresh weight)			
Bitter gourd	Storage period (days)	−18 °C		−40 °C	
Unblanched	Before freezing	263.3 ± 9.5[a]	(106)[b]	263.3 ± 9.5	(101)
	0	247.6 ± 3.3	(100)	260.5 ± 11.6	(100)
	30	233.0 ± 3.8	(94)	258.7 ± 20.1	(99)
	60	238.6 ± 5.6	(96)	245.3 ± 16.6	(94)
	90	245.5 ± 7.2	(99)	251.6 ± 7.5	(97)
	120	223.3 ± 6.5	(90)	241.8 ± 13.2	(93)
	150	223.4 ± 8.1	(90)	252.1 ± 14.7	(97)
	180	202.8 ± 10.2*	(82)	251.6 ± 6.1	(97)
	Correlation coefficient[c]	−0.836		−0.539	
Blanched	Before freezing	226.9 ± 7.7	(102)	226.9 ± 7.7	(107)
	0	222.7 ± 6.5	(100)	212.1 ± 11.4	(100)
	30	217.4 ± 15.7	(98)	202.9 ± 16.3	(96)
	60	211.9 ± 27.2	(95)	219.2 ± 8.3	(103)
	90	210.7 ± 13.1	(95)	209.7 ± 11.1	(99)
	120	191.8 ± 11.0**	(86)	216.2 ± 7.0	(102)
	150	202.3 ± 14.7*	(91)	203.6 ± 10.5*	(96)
	180	201.8 ± 8.8*	(91)	189.1 ± 1.6**	(89)
	Correlation coefficient	−0.890		−0.431	

[a]The values are mean ± SD for 4 determinations.
[b]The values in parentheses are percentage relative to day−0 samples.
[c]Correlation coefficient between storage period and total phenol content.
*Significantly different from day−0 samples ($P < 0.05$).
**Significantly different from day−0 samples ($P < 0.01$).

Table 4—Changes in the ascorbic acid content of unblanched and blanched bitter gourd during frozen storage at −18 and −40 °C for up to 6 mo.

		Ascorbic acid content (mg/100 g fresh weight)			
Bitter gourd	Storage period (days)	−18 °C		−40 °C	
Unblanched	Before freezing	79.7 ± 14.6[a]	(112)[b]	79.7 ± 14.6	(110)
	0	71.4 ± 5.5	(100)	72.3 ± 3.3	(100)
	30	62.5 ± 2.5*	(87)	69.7 ± 6.5	(96)
	60	63.3 ± 2.3*	(89)	68.2 ± 2.5	(94)
	90	61.8 ± 0.5**	(87)	71.4 ± 13.3	(99)
	120	52.3 ± 2.5**	(73)	70.0 ± 4.5	(97)
	150	54.8 ± 1.0**	(77)	70.3 ± 6.3	(97)
	180	54.6 ± 0.9**	(76)	68.6 ± 5.8	(95)
	Correlation coefficient[c]	−0.890		−0.431	
Blanched	Before freezing	54.6 ± 3.0	(95)	54.6 ± 3.0	(96)
	0	57.2 ± 3.4	(100)	56.6 ± 10.4	(100)
	30	43.8 ± 4.8**	(77)	48.2 ± 4.5	(85)
	60	40.2 ± 1.9**	(70)	45.9 ± 4.2	(81)
	90	43.0 ± 2.1**	(75)	44.6 ± 5.3	(79)
	120	40.6 ± 6.0**	(71)	41.9 ± 2.9*	(74)
	150	41.2 ± 6.4**	(72)	46.6 ± 6.0	(82)
	180	40.9 ± 1.8**	(72)	46.2 ± 4.0	(82)
	Correlation coefficient	−0.686		−0.643	

[a]The values are mean ± SD for 4 determinations.
[b]The values in parentheses are percentage relative to day−0 samples.
[c]Correlation coefficient between storage period and ascorbic acid content.
*Significantly different from day−0 samples ($P < 0.05$).
**Significantly different from day−0 samples ($P < 0.01$).

bitter gourd was best achieved by quick freezing followed by frozen storage at −40 °C without blanching.

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