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Research Note

Development of Freeze Dried Functional Carrot Muskmelon Juice Powder

M.C. Pandey, * P.T. Harilal , K.A. Srihari, K. Jayathilakan and K. Radhakrishna

Department of Freeze Drying & Animal Products Technology, Defence Food Research Laboratory, Siddarthanagar, Mysore, Karnataka, India

Email: pandeymc@yahoo.com

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Abstract

Freeze dried (FD) functional carrot muskmelon juice powder meeting the recommended daily allowance requirement of ascorbic acid and β-carotene was developed. Carrot and muskmelon juice proportion was standardized using factorial design and sensory evaluation as response. The standardized product was freeze dried and subjected for storage at ambient temperature conditions (30±2 ^{0}C). Physico-chemical and microbiological quality attributes have been evaluated initially and during storage. The degradation patterns of ascorbic acid and β-carotene, microbial quality, sensory and color values were used for establishing the shelf life stability of the product. Effect of storage on retention of ascorbic acid and β-carotene clearly established the stability of the product till 6 months of storage. Significant variation ($p<0.05$) in ascorbic acid, β-carotene and sensory attributes were observed beyond 6 months of storage. Water activity (a_w) of the product was found to be 0.190 and adsorption and desorption studies revealed a Brunauer-Emmett-Teller (BET) monolayer value of 2.414g/100g.

Keywords: Carrot, Muskmelon, freeze drying, water activity, β-carotene, Ascorbic acid

Introduction

Carrot (*Daucus carota*) and muskmelon or cantaloupes (*Cucumis melo*) are widely grown in India because of their health importance and popularity. They are well known for their richness in phenolic and various other active functional compounds. Foods rich in antioxidant, vitamins such as vitamins C (ascorbic acid), vitamin E (α-tocopherol), phenolic compounds, and carotenoids (leutin, α and β-carotene) have proven effect on preventing cancer and cardio vascular diseases (Burns *et al.,* 2003; Block *et al.,* 2001; Gardner *et al.,* 2000). Supplementing dietary intake with carrot is a major remedy for night blindness ignited by the deficiency of provitamin-A (El-Arab *et al.,* 2002). With its high carotenoid content, the carrot is regarded as a good source of vitamin A. However, in recent years high intakes of dietary carotene have been investigated as a means to inhibit lipid oxidation (Lavy *et al.,* 1993) and to prevent certain types of cancer in humans (Muto *et al.,* 1995; Van Poppel and Goldbohm 1995). Muskmelon is one of the most consumed fruit crops worldwide due to its pleasant aroma and high nutritional value (Ismail *et al.,* 2010). High antioxidant potential and anti inflammatory properties of muskmelon pulp extracts are being studied and proved (Vouldoukis *et al.,* 2004). Muskmelon is a premium source of ascorbic acid and contains comparatively low carotenoid. Ascorbic acid is an effective anti oxidant and an important component of our nutrition. It is used as an additive in many foods because of its anti-oxidant capacity. Thus, it enhances quality and properties of food as well as nutritional value (Larisch *et al.,* 1998; Solomon *et al.,* 1995). Literature is very scanty on the processing and quality parameters on muskmelon beverages and dehydrated products. Torregrosa *et al.*

(2006) studied the ascorbic acid stability in refrigerated orange–carrot juice mix subjected for pulsed electric field processing and pasteurization.

Optimization and standardization of a balanced drink from carrot and muskmelon provides a good amount of vitamin A, carotenoids and ascorbic acid to the diet. Raw carrot is very rich in vitamin A and total carotenoids where as muskmelon is very rich in ascorbic acid. The combination of carrot and muskmelon juice mutually balances the amount of total carotenoids and ascorbic acid in the final product and it deliver the required daily allowance (RDA). The RDA of ascorbic acid and β-carotene are 60mg and 3-6 mg respectively (Levine *et al.,* 1996; Nambiar and Parnami 2008). Freeze drying is the best method of water removal with final products of highest quality compared to other conventional drying methods (Genin and Rene 1995; Irzyniec *et al.,* 1995). Freeze drying is the removal of moisture by the sublimation of frozen water from the product at low temperature and pressure conditions. Due to the absence of liquid water and low temperatures required for the process, most of the deterioration and microbiological reactions are stopped which gives a final product of excellent quality (Ratti 2001). Freeze drying of foods decrease the water activity (a_w) and increase the shelf stability, however, it has got a negative impact on the carotenoid retention as carotenoids undergo auto catalytic oxidation in dehydrated systems (Goldman *et al.,* 1983) and the low water activity plays a complex role in the oxidation. Knowledge on the water sorption properties of different food matrices would be helpful in order to predict the relative rate of oxidative degradations. Yen *et al.* (2008), studied on the effect of freeze drying (FD)and hot air drying (HAD) of carrot on the stability of ascorbic acid and reported low and insignificant degradation in freeze drying (FD) sample compared to HAD sample. Considering the above facts freeze dried carrot musk melon juice powder was developed and retention of functional ingredients like â-carotene and ascorbic acid was evaluated at ambient temperature conditions (30±2 ^{0}C). Development of ready-to-reconstitute (RTR) FD functional fruit beverages is having importance in service and civilian sectors to meet the nutritional requirements and instant energy.

Materials and methods

Preparation of sample

Carrot and muskmelon were procured from the local market, thoroughly washed in running water; skin peeled and cut in to medium pieces. High performance blender (Waring, Model31BL67, New Harvard, USA) was used to extract the juice and filtered using a standard sieve (Mesh No 60, 170-190µm) to retain fiber. Juice yield from carrot and muskmelon was 52% and 65 % respectively. Ratio of carrot and musk melon juice was optimized using statistical design. Selected combination was taken for freeze drying after adjusting the brix with cane sugar to sweetness (17 ^{0}Bx). Juice was pasteurized prior to freeze drying at 90 ^{0}C for 30 sec. Freeze drying of the juice was done in a freeze dryer(Martin and Christ, Germany) keeping drying parameters as condenser temperature -40 ^{0}C, heater at 55 ^{0}C and a vacuum of 100ì (Pandey *et al.,* 2009). The whole cycle of freeze drying took 16 h and the FD powder was packed in paper, aluminium foil, low density polyethylene (LDPE) (45gsm paper/20ì aluminium/37.5 ì LDPE) packets of size 10x12 cm to contain 60g. The samples were kept for storage studies under ambient temperature conditions (30±2 ^{0}C).

Microbiological analysis

Studies at different stages of processing and storage of carrot muskmelon juice powder were carried out to establish the microbial quality as per APHA (1992) procedure for standard plate count (SPC), coliforms, *Escherichia coli* (*E. coli*), *Salmonella*, *Staphylococcus aureus*, yeast and molds.

Proximate and physical characteristics of Freeze Drying carrot muskmelon juice powder

Proximate composition with respect to moisture, protein, total fat, total carbohydrate and ash were determined as per AOAC method(1997). Color was measured in terms of color coordinates (‘*L’*-lightness/darkness, ‘*a’*-redness/green, ‘*b’*-yellowness/blue) using Hunter colorflex (Hunter Lab, VA, USA) equipped with a D_{65} illuminant and standardized using a standard white and black tile to establish the pigment retention in the juice powder during storage at ambient temperature conditions (30±2 ^{0}C). A_w of the sample was determined using a pre-equilibrium conditioned water activity meter (Labmaster.aw, Novasina, Switzerland) at 25 ^{0}C. Adsorption and desorption isotherms were determined using Hydrosorb (Quantachrome Instruments, FL, USA). 0.25g of sample was taken in a 9mm dia and 150mm long pellet tube and attached to the sample port in the isotherm flask at 298 kelvin (K). BET equilibrium pressure points were distributed between a p/p_0 value of 0.0500 to 0.3000 (5

points). Adsorption (15 pressure points) and desorption (10pressure points) equilibrium pressure points between 0.0500 to 0.9975 p/p_0 Pressure tolerance of 0.05mm Hg, time tolerance of 60 s and time to achieve equilibrium was kept at 600 for the whole isothermal analysis.

Titrable acidity was determined as described by Antonio *et al.,* (2009). The prepared sample was diluted using double distilled water and titrated against standard NaOH using ethanolic phenolphthalein as an indicator. Soluble solids expressed in ^{0}Bx were measured using a hand refractometer. A meter of model cyber scan 510 (Eutech Instruments, Singapore) was used for pH measurements. Triplicate determinations were made in each analysis depicted in the work.

Ascorbic acid

Quantification of total ascorbic acid was done using modified 2, 6-dichlorophenol indophenol (DIP) method (Yen *et al.,* 2008; Klein and Perry 1982). 1g of sample was extracted using 50ml 1% meta-phosphoric acid (v/v) for 1h. The extract was then centrifuged and to 9ml of 0.05mM DIP, 1ml of the above supernatant was added and mixed thoroughly and absorbance was measured at 515 nm against a blank using Lamda-25 UV-Visible spectrophotometer (Perkin Elmer, Singapore). The optical density was correlated against a standard curve.

β-carotene determination

Total carotenoid and β-carotene were determined using solvent extraction method (Yen *et al.,* 2008; Prakash *et al.,* 2004). 2g juice powder was taken in a 250ml volumetric flask and 40ml acetone was added. Flask was placed on a stirrer and stirring was continued till the residue became colorless to extract maximum carotene. The acetone extract was filtered and petroleum ether (100 ml) was added along with a squeeze of sodium sulphate (to absorb any moisture) were placed in a separating funnel and shaken for 1 min. The yellow upper layer was carefully transferred into a 250 ml standard flask and the lower layer was drained to another separating funnel. It was again extracted using petroleum ether and the yellow upper layer was collected. Same procedure was repeated twice and the collected yellow layer was made up to 250 ml volume mark and an aliquot from the solution was taken for optical density measurement. Optical density was measured in terms of absorbance in Lamda-25 UV-Visible spectrophotometer (Perkin Elmer, Singapore) at 452nm and the result was compared with a standard curve for the determination of β-carotene.

Organoleptic evaluation

Sensory evaluation of the samples were carried out on monthly basis on a 9 point hedonic scale (9- like extremely, 1- dislike extremely) (Murray *et al.,* 2001) to establish the overall acceptability (OAA) by 14 trained panelists.

Statistical analysis

The experimental design was set up using factorial design with two factors i.e. carrot and musk melon juice each having four levels (ratio, 1to 4), three replications. Response data were collected as overall acceptability (Table 1). Analysis of variance was carried out taking the effect of factors and interaction into model. Based on the design final ratio of the carrot and musk melon juices was optimized for further processing and storage of functional FD product. State-Ease (Design Expert version 6.0.10) was used for statistical and graphical analysis of the data. Standard deviation and significance of variation ($p<0.05$) of the data wherever required were also done using MS Excel and Costat (2003).

Results and discussion

Optimization of ratio for carrot and musk melon juices

Analysis of variance of the experimental data indicates that model is significant with carrot, muskmelon juices and with their interaction. Numerical optimization of the design was obtained keeping carrot muskmelon juices as in the range and overall acceptability (OAA)as maximum. Total sixteen experimental designs were obtained with 5 repetitions (Table 1). Optimized ratio of carrot and musk melon juices was found as 1: 3 with overall acceptability of 8.33 and 0.97 desirability.

Table 1. Two factors and four level experimental design set up for the standardization of carrot muskmelon juice.

Sample	Factor 1Carrot juice (parts)	Factor 2 muskmelon juice (parts)	Response OAA
1.	1	1	6.32±0.45
2.	2	1	5.84±0.21
3.	3	1	5.32±0.26
4.	4	1	4.87±0.53
5.	1	2	7.60±0.71
6.	3	2	6.05±0.33
7.	1	3	8.33±0.36
8.	2	3	7.03±0.13
9.	4	3	7.19±0.21
10.	1	4	7.96±0.32
11.	3	4	7.29±0.48

Values are mean ± standard deviation

Proximate composition:

The proximate compositional data of FD carrot-muskmelon powder showed a very good nutritional profile. Moisture was found to be 2.3% which was very nearer to the BET monolayer value obtained for the product (2.414 g/ 100g solids, where the product shows maximum stability(Fabra *et al.,* 2009). The protein content was 4.89 %, fat 1.80 % and total carbohydrate 89.5%. The product had a moderate percentage of fat, protein and considerable amount of carbohydrate and a calorific value of 396 Kcals/100g.

Initial a_w of carrot muskmelon juice before FD was 0.96. The lowest value of a_w needed for growth of natural bacteria, molds and salt tolerant bacteria is 0.90, 0.80 and 0.75 respectively (Potter and Hotchkins 1996). Therefore a_w must be reduced to below critical a_w to preserve the food and this was achieved by subjecting the product to FD as the a_w of the product was found to be 0.190.

Hunter color values on storage

Colour of the freshly processed carrot muskmelon juice mix was orange-red in color and that of FD sample was whitish orange predominantly came from the carotenoid pigments present in the carrot juice. The orange-red color of carrots is caused by the two carotenoids all trans-α and β-carotenes (Khachik *et al.,* 1991; Mangels *et al.,* 1993). These two pigments account for more than 90% of carotenoids in carrot (Simon and Wolf 1987). Evaluation of the colour values is a good quality marker of FD products during storage. Table 2 depicts the changes in *L, a, b* values of FD carrot muskmelon juice powder during storage. Results revealed a significant ($p<0.05$) decrease in Hunter *L* (lightness) *a* (redness) and *b* (yellowness) values after 6 m of storage at ambient temperature conditions (30±2 ^{0}C). Significant ($p<0.05$) changes in *L,* and *b* values may be triggered by the auto catalytic oxidation of β-carotene in the sample or the formation of *cis* isomers (Tang and Chen 2000). Hunter *a* value decreased significantly ($p<0.05$) after 6 months of storage revealing the direct relation with β-carotene concentration (Skrede *et al.,* 1997). Reports underlined a positive correlation between β-carotene degradation and changes in Hunter *L,* and *a* color values during storage. The data reflected in Table 3 for β-carotene degradation is in positive correlation with Hunter color readings.

Table 2. Hunter *L, a, b* color values for FD carrot muskmelon juice powder during storage at ambient temperature conditions (30±2 ^{0}C)

Storage time (Months)	*L*	*a**	*b**
0	80.08^a ±0.16	17.91^a ±0.18	24.80^a ±0.59
1.	79.93^a ±0.12	17.89^a ±0.09	24.69^a ±0.22
2.	79.51^a ±0.50	17.75^a ±0.17	24.47^a ±0.50
3.	79.39^a ±0.53	17.55^a ±0.10	24.47^a ±0.44
4.	79.28^a ±0.62	17.27^a ±0.44	24.44^a ±0.49
5.	79.27^a ±0.26	17.24^a ±0.84	24.42^a ±0.37
6.	79.24^a ±1.15	17.13^a ±0.37	24.11^a ±0.29
7.	77.33^b ±0.57	14.90^b ±0.79	21.71^b ±0.55
8.	74.93^c ±0.38	12.97^c ±0.43	20.01^c ±0.59

Values are mean ± standard deviation

N=3, means with different superscript differ significantly ($p<0.05$)

Evaluation of β-carotene stability:

The stability of carotene was monitored after FD and storage at ambient in FD carrot muskmelon and the data generated is reflected in Table 4. Evaluation of the stability of β-carotene is important in food materials as it has got lot of functional characteristics. Apart from being precursors of pro vitamin A, carotenoid pigments exhibit functional properties as they got anti-carcinogenic properties (Krinsky, 1989).

From the data presented in Table 3, it is evident that the β-carotene values did not show any significant difference ($p<0.05$) up to 6 months of storage at ambient temperature conditions (30±2 ^{0}C). The stability of carotenoid pigments at normal temperature in FD samples of carrot pulp was reported by Tang and Chen (2000). Effect of drying treatments like FD and hot air drying on the stability of carotenoids in Taiwanese mango was studied by Chen *et al.,* (2007) and reported better retentions of pigments in FD samples during normal temperature of storage. The loss of β-carotene that is occurring after 6 months of storage may be due to auto catalytic oxidation in dehydrated systems (Goldman *et al.,* 1983) and the low water activity plays a complex role in the oxidation. The highly unsaturated

chemical structure makes them very susceptible to thermal degradation and oxidation during storage (Stefanovich and Karel 1982).

Table 3. Effect of storage on the ascorbic acid and β-carotene stability of FD carrot muskmelon juice powder stored at ambient temperature conditions (30±2 ^{0}C).

Storage (in months)	Ascorbic acid (mg/100g)	β-carotene (mg/100g)
0	280.29 ^{a} ±0.97	23.59 ^{a} ±0.53
1.	280.19 ^{a} ±0.49	23.39 ^{a} ±0.45
2.	280.00 ^{a} ±0.16	23.37 ^{a} ±0.38
3.	279.81 ^{a} ±0.56	23.31 ^{a} ±0.47
4.	279.39 ^{a} ±0.34	23.26 ^{a} ±0.38
5.	279.39 ^{a} ±0.33	23.16 ^{a} ±0.27
6.	279.33 ^{a} ±0.29	22.97 ^{a} ±0.17
7.	277.11 ^{b} ±0.62	21.00 ^{b} ±0.02
8.	275.46 ^{c} ±0.44	20.11 ^{c} ±0.19

Values are mean ± standard deviation
N=3, means with different superscript differ significantly ($p<0.05$)

Stability of ascorbic acid in FD carrot muskmelon powder:

From the values shown in Table 3, it could be ascertained that FD and storage up to 6 months at ambient temperature conditions (30±2 ^{0}C) did not show any significant difference ($p>0.05$) in the ascorbic acid values. But after 6 months of storage, the values did differ significantly ($p<0.05$). Marfil *et al.* (2008) studied the effect of different drying treatments and storage on ascorbic acid degradation kinetics in tomatoes and reported the increase in vitamin C degradation with respect to storage period. The degradation of vitamin C during storage may be attributed to the presence of trace elements and iron in moisture content (Dennison and Kirk 1982).

Microbiological profile:

The microbial quality in terms of SPC, Coliforms and yeast and molds for carrot juice were $8x10^4$, $8x10^2$ and $6x10^2$ cfu/g and that of muskmelon juice was $9x10^4$, $9x10^2$ and $3x10^2$ cfu/g respectively. After mixing both the juices in the selected ratio and after subjecting for pasteurization, SPC was reduced to $8x10^1$ cfu/g with nil coliforms count and yeast and mold count was <10 cfu/g. After FD the product, SPC was found to be $3x10^1$ cfu/g with nil coliforms, and yeast and mold count was <10 cfu/g. Pathogens viz. *E.coli*, *S.aureus* and *Salmonella* were absent in the FD sample. Though initially a few microorganisms were present, subsequently upon storage for 8 months, the microbial load was reduced to <10 cfu/g SPC, and throughout the storage period coliforms and yeast and molds and pathogens were absent indicating a good microbial profile of the product.

Sensory profile of the product:

Table 4 illustrates the sensory characteristics of the product stored at ambient temperature (30±2 ^{0}C). The chemical and microbiological evaluation to the stability characteristics of the product was earlier discussed. To support these findings at ambient temperature (30±2 ^{0}C) the sensory profile of this product was evaluated during storage on a 9-point hedonic scale and the overall acceptability score is reflected in Table 4. The product exhibited good sensory attributes upto 6 months of storage at ambient temperature (30±2 ^{0}C) producing a score of 7.9±0.2 on a 9-point hedonic scale. After 6 months of storage, the score did differ significantly ($p<0.05$).

Table 4. Sensory profile of reconstituted FD carrot muskmelon juice powder stored at ambient temperature conditions (30±2 ^{0}C).

Storage (months)	Color	Aroma	Texture	Taste	OAA
0	8.36 ^{a} ±0.53	8.57 ^{a} ±0.33	8.36 ^{a} ±0.23	8.32 ^{a} ±0.25	8.32 ^{a} ±0.32
1.	8.32 ^{a} ±0.25	8.50 ^{a} ±0.39	8.32 ^{a} ±0.50	8.21 ^{a} ±0.26	8.25 ^{a} ±0.39
2.	8.28 ^{a} ±0.26	8.46 ^{a} ±0.41	8.25 ^{ab} ±0.61	8.18 ^{a} ±0.25	8.21 ^{a} ±0.38
3.	8.25 ^{a} ±0.26	8.43 ^{a} ±0.43	8.18 ^{ab} ±0.58	8.14 ^{a} ±0.23	8.19 ^{a} ±0.31
4.	8.21 ^{ab} ±0.26	8.36 ^{a} ±0.41	8.11 ^{ab} ±0.21	8.10 ^{ab} ±0.29	8.18 ^{a} ±0.25
5.	8.19 ^{ab} ±0.24	8.32 ^{ab} ±0.42	8.11 ^{ab} ±0.24	8.07 ^{ab} ±0.27	8.04 ^{ab} ±0.13
6.	8.18 ^{ab} ±0.25	8.30 ^{ab} ±0.46	8.17 ^{ab} ±0.32	8.07 ^{ab} ±0.42	8.04 ^{ab} ±0.06
7.	8.02 ^{b} ±0.02	8.07 ^{bc} ±0.18	8.00 ^{ab} ±0.33	7.86 ^{bc} ±0.49	7.79 ^{b} ±0.54
8.	7.70 ^{c} ±0.11	7.96 ^{c} ±0.23	7.67 ^{c} ±0.37	7.61 ^{c} ±0.68	7.39 ^{c} ±0.88

Values are mean ± standard deviation
N=14, means with different superscript differ significantly ($p<0.05$)

Conclusion

Factorial design can be used to optimize the ratio of carrot and muskmelon juice to develop functional freeze dried powder. Optimized ratio of carrot and musk melon juice were obtained as 1:3. Pasteurized freeze dried carrot musk melon juice was subjected for freeze drying. The FD carrot muskmelon juice powder was found highly nutritious with its functional components. Study revealed that functional components of the product like β-carotene and Vitamin C were stable up to six month which is sufficient to provide the RDA requirements. Studies on the stability of ascorbic acid and â-carotene and their degradation patterns showed a direct correlation to other physico-chemical qualities of the juice powder considered for establishing the shelf life stability and quality. Sensory characteristics of the product were also very good during storage. The product has exhibited excellent microbial profile upto one year at ambient conditions. Finally, it is concluded that the shelf stability of the product was six months at ambient temperature conditions (30±2 ^{0}C).

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