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EFFECT OF REFRIGERATION STORAGE PERIOD ON ASCORBIC ACID, TOTAL PHENOLIC CONTENT AND NARINGIN CONTENT OF WHEY BASED KIWI-POMEGRANATE JUICE BLENDS

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ABSTRACT

The experiments were conducted to study the effect of 15 day refrigeration storage period on Ascorbic acid, total phenolic content and Naringin content were determined. In this study, two blends were prepared i.e. Blend-I and Blend-II in which the ratio of kiwi: pomegranate juice and whey were 70:20:10 and 60:30:10. Ascorbic acid content was determined by volumetric method, Total Phenolic content was determined by Folin-Ciocalteu reagent and naringin content were determined by colorimetric method. The results indicated that ascorbic acid and total phenolic content were decreased significantly, whereas naringin content were increased significantly in both Blends.

Keywords: Kiwi, Ascorbic Acid, Naringin, Total Phenolic Content

INTRODUCTION

The antioxidant property of phenolic compounds is mainly due to their redox properties. They act as reducing agents, hydrogen donors and metal chelators (Kaviarasan *et al.*, 2007 and Yen *et al.*, 2005). Citrus fruit juices contain most important antioxidant i.e. ascorbic acid, flavonoids and phenolic compounds which protects the organism from oxidative stress (Zvaigzne *et al.*, 2009), and are important to human nutrition (Fernandez-Lopez *et al.*, 2005; Jayaprakasha *et al.*, 2007; Ebrahimzadeh *et al.*, 2004). Kiwifruit, (*Actinidiadeliciosa*), belong to the family *Actinidiaceae*. Kiwifruit are rich in bioactive compound (Park *et al.*, 2006), such as lutein, phenolics, flavonoids, carotenoids and chlorophyll (Cassano *et al.*, 2006). It has low calories and high ascorbic acid content (Singh *et al.*, 2008) and helps to reduce the risk of cardiovascular disease (Chang and Liu, 2009).

Pomegranate (*Punicagranatum L.*) belongs to the *Punicaceae* family. Pomegranate juice contains ascorbic acid, Vitamin B₅, and polyphenols such as tannins, flavonoids (Heyn, 1990). Flavonoids inhibited low density lipoprotein oxidation and cardiovascular diseases in humans (Aviram and Domfeld, 2001).

Due to high polyphenolic content pomegranate juice shows a high antioxidant activity in compare to common fruit juices in vitro (Lansky and Newman, 2007).

Whey is the watery liquid remaining after milk has been curdled and strained. It is good source of precious nutrients like whey proteins, lactose, milk salts and also contains most of water-soluble vitamins (Naik *et al.*, 2009).

Whey proteins exhibit antibacterial, immune associated protection and anti-cancer properties (McIntosh *et al.*, 1998). It acts as good appetizer; acceptable to consumers and at the same time makes the product more delicious (Sirohi *et al.*, 2005).

Despite the enormous health benefits of kiwi, pomegranate and whey the present study were focused on the preparation and analysis of the effect of storage period on ascorbic acid, total phenolic content and Naringin content of Whey based Kiwi-Pomegranate Juice blends.

MATERIALS AND METHODS

Preparation of Kiwi Juice

Mature, ripe kiwi fruit were washed and peeled by hand and juice was prepared by blending and squeezing. The prepared juice is stored at refrigerator at 4±1°C for further analysis.

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Preparation of Pomegranate juice

The pomegranates were washed in running tap water peeled and the juice was prepared mechanically. The prepared juice is stored at refrigerator at $4\pm 1^{\circ}\text{C}$ for further analysis.

Preparation of Whey

Whey is prepared according to a previously reported method (Dhamsaniya and Varshney, 2013). The milk was heated in a stainless steel vessel at 80°C for 10 minutes and boiled milk was coagulated at 72°C with the addition of 1 percent citric acid followed by continuous stirring until the coagulation of milk proteins (casein) takes place. The milk whey and casein were allowed to cool at room temperature and then filtered using the muslin cloth. The milk whey which was obtained filled in a pre-cleaned and pre-sterilized glass bottles and kept in the refrigerator at $4\pm 1^{\circ}\text{C}$ temperature for further use.

Experimental Design

Two blends of different ratio of kiwi juice, pomegranate juice and whey were prepared as Blend-I (Kiwi juice: Pomegranate juice: whey) (70:20:10) and Blend-II (Kiwi juice: Pomegranate juice: whey) (60:30:10).

Statistical Analysis

Results were expressed as mean \pm SD. One-way analysis of variance (ANOVA) was used to find the significant differences among the means followed by Tukey's HSD test ($p<0.05$) using SPSS.

Determination of Ascorbic Acid

Ascorbic acid was measured by previously reported method (Sawhney and Singh, 2015). Briefly, 2 ml juice was diluted up to 10 folds with 6% Meta phosphoric acid. Transfer 20ml standard Vitamin C solution in Erlenmeyer flask and titrate against 2, 6 Dichlorophenol indophenols solution till the appearance of a light pink colour. Note the volume of the dye used, say x ml. Now transfer the sample in Erlenmeyer flask and titrate against 2, 6 Dichlorophenol indophenols solution till the appearance of a light pink colour. Note the volume of the dye used, say y ml.

Amount of ascorbic acid in 100ml of undiluted juice was calculated by using the formula.

Ascorbic Acid (Vitamin C) (mg/100ml) = $(y/x) \times 10\text{mg}$

Determination of Total Phenolic Content

Total phenolic content was measured by previously reported method (Makkar *et al.*, 1993). Briefly, in 1ml juice sample 0.5ml Folin-Ciocalteu and 2.5 ml Na_2CO_3 (20%) was added in test tubes. The mixture was allowed to stand for 40-45 minutes in dark at room temperature, and then the absorbance was taken at 725nm with UV-VIS spectrophotometer. Total phenolic content were calculated against standard curve of Gallic acid and result were reported as mg GAE/L.

Determination of Naringin Content

The naringin content in the fruit was estimated by previously reported method (Davis, 1947). Briefly, 0.2 ml of juice sample was taken in the test tube, add 10 ml of 90% diethylene glycol in it and then add 0.2 ml of 4M NaOH and incubate at room temperature ($30\pm 2^{\circ}\text{C}$) for 5 minutes for yellow colour development. After development of yellow colour absorbance was at 420nm. Naringin content were calculated against standard curve of naringin prepared in the range of 5-30 $\mu\text{g/ml}$ and result were reported as $\mu\text{g/ml}$.

RESULTS AND DISCUSSION

Effect of Storage Period on Ascorbic Acid

Ascorbic acid content was decreased continuously during the storage period of 7 to 15 days at refrigeration temperature. Table 1 shows that in case of Blend-I ascorbic acid content decreases from 68.34 mg/100ml to 45.57 mg/100ml and in case of Blend-II ascorbic acid content decreases from 57.56 mg/100ml to 34.77 mg/100ml during the storage period. Figure 1 show graphical presentation of result.

The oxidation of ascorbic acid into dehydro ascorbic acid may be the reason in decreasing trend in Ascorbic acid content. Our finding favors the findings of Kumar *et al.*, (2017) who reported that Ascorbic acid content is reduced during the refrigeration storage period in case of Pear- Amla Based Ready-To-Serve (RTS) Beverages.

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Table 1: Effect of Storage Period on Ascorbic Acid, Total Phenolic Content and Naringin Content of Whey Based Kiwi-Pomegranate Juice Blends

Juice Blends (Kiwi Juice: Pomegranate Juice: Whey)	Storage Period (Days)	Ascorbic Acid (mg/100ml)	Total Phenolic Content (mg GAE/L)	Naringin Content (µg/ml)
Blend-I (Kiwi juice: Pomegranate juice: whey) (70:20:10)	0	68.34±0.67 ^a	2561.66±0.57 ^a	157.9±0.36 ^b
	7	49.16±1.02 ^b	2411.03±0.83 ^b	153.3±0.65 ^c
	15	45.57±0.70 ^c	2259.53±0.50 ^c	242.43±0.70 ^a
Blend-II (Kiwi juice: Pomegranate juice: whey) (60:30:10)	0	57.56±0.63 ^a	2531.30±1.09 ^a	175.64±0.41 ^c
	7	50.26±0.70 ^b	2358.7±0.34 ^b	162.26±0.45 ^b
	15	34.77±0.98 ^c	2436.33±0.50 ^c	242.67±0.59 ^a

Values are mean ± standard deviation of triplicate determinations

Means within columns with different letters are significantly different ($p < 0.05$)

Effect of Storage Period on Total Phenolic Compounds (TPC)

Total Phenolic Compounds (TPC) was decreased continuously during the storage period of 7 to 15 days at refrigeration temperature.

Table 1 shows that in case of Blend-I TPC decreases from 2561.66 mg GAE/L to 2259.53 mg GAE/L and in case of Blend-II TPC decreases from 2531.30 mg GAE/L to 2436.05 mg GAE/L during the storage period.

Figure 2 show graphical presentation of result. Our finding favors the findings of Dar *et al.*, (2016) who reported that total phenolic content is reduced during the storage period in case of bran enriched snacks. The decrease in polyphenols may be due to the oxidation of these compounds and polymerization with proteins (Liu *et al.*, 2014).

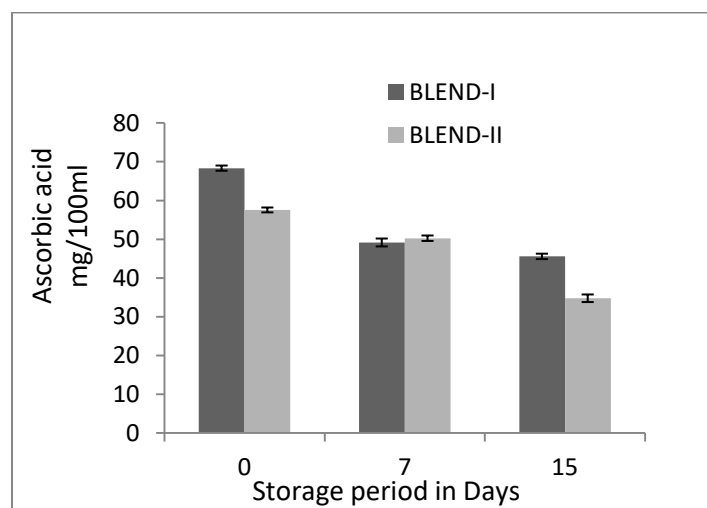


Figure 1: Effect of Storage Period on Ascorbic Acid

Effect of Storage Period on Naringin Content

Naringin content was increased during the storage period of 7 to 15 days at refrigeration temperature as shown in table 1. Naringin content of Blend-I increased from 157.9 µg/ml to 242.43 µg/ml and in case of Blend-II increased from 175.64 µg/ml to 242.67 µg/ml. These results are in agreement with those of Chaudhary *et al.*, (2016) who reported that naringin content increase during his 12 week study on Rio Red grapefruit. Figure 3 show graphical presentation of result.

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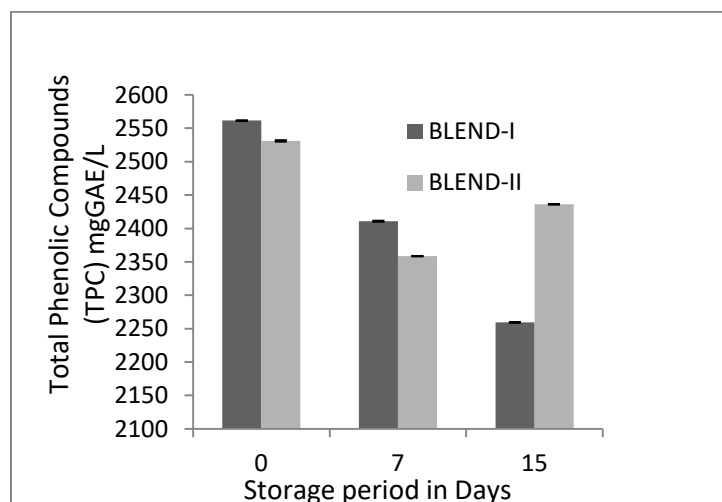


Figure 2: Effect of Storage Period on Total Phenolic Compounds (TPC)

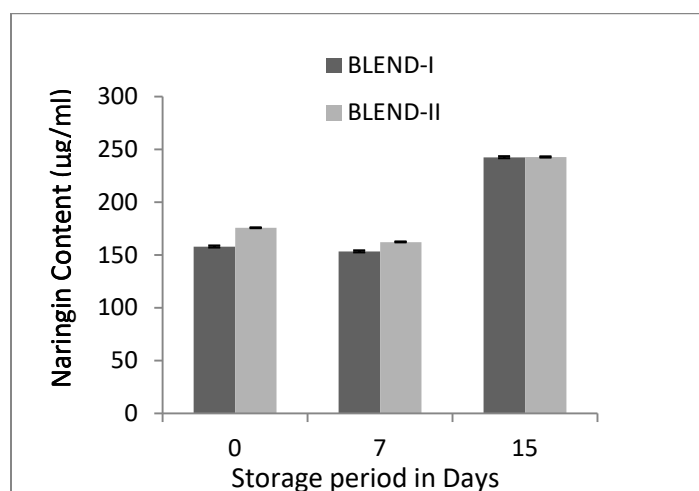


Figure 3: Effect of Storage Period on Naringin Content

Conclusion

Refrigeration storage period shows some significant effect on Ascorbic acid, total phenolic content and Naringin content of Whey based Kiwi-Pomegranate Juice Blends. The results indicated that ascorbic acid and total phenolic content were decreased significantly, whereas naringin content were increased significantly in both Blends. We conclude that instead of discarding whey it can be used to prepare value added products like beverages for use at household and commercial level.

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