

EFFECT OF PRESERVATION PROCESSES ON THE SHELF STABILITY AND ANTIOXIDANT PROPERTIES OF RADISH JUICE

GURPREET KAUR* AND POONAM AGGARWAL

Department of Food Science and Technology, Punjab Agricultural University, Ludhiana, Punjab 141 004

e-mail: gurpreet86@pau.edu

KEYWORDS

Radish
juice
Phytochemicals
Antioxidant activity
storage

Received on :

09.07.2016

Accepted on :

22.10.2016

*Corresponding
author

ABSTRACT

The present investigation was conducted with the aim to compare the effect of thermal processing with the addition of chemical additives namely Sodium benzoate, Potassium metabisulfite (KMS) and their combination, on the physicochemical and phytochemical attributes and antioxidant activity of Radish juice. The storage was done for 6 months at room temperature and the analysis was conducted at the interval of one month. The parameters like TS and TSS did not change significantly. The minimum increase in acidity during storage was found in thermally treated samples. The color was best preserved by addition of KMS where the L value changed from 54.8 to 52.22 after 6 months. The ascorbic acid and total phenols content was best preserved by KMS with final values of 33.86mg/100g and 41.47 mg/100g respectively that were far better than the thermally treated samples with values 23.72 mg/100g and 22.59mg/100g respectively. Similarly, both KMS and combination samples maintained the Antioxidant activity well with values of 28.64% and 27.92% respectively whereas thermally treated sample showed 17.45% antioxidant activity. Keeping in view all the parameters KMS was found to be the most appropriate agent for preservation.

INTRODUCTION

Radish (*Raphanus sativus*) L. (Brassicaceae) is most valued by the inhabitants of many Western and Eastern countries as a food and medicine (Mayer 1981). In Greeco-Arab or Unani medicine as well as in Indian folklore, radish is administered as a household remedy for the prevention and treatment of gall stone, jaundice, flatulence, indigestion, and in various gastric ailments (Prahoveanu and Esanu 1990). The juice of radish has a tonic, diuretic and laxative action on the intestine and indirectly stimulates the flow of bile (Aman 1969). Radishes are extremely low in calories, naturally fat-free and carry a low glycemic load. Radish was found to possess an antioxidative effect which could be related to the prevention of carcinogenesis (Ippoushi *et al.*, 2007).

Interest in the role of free radical scavenging-antioxidants in human health has prompted research in the fields of horticulture and food science to assess the antioxidant phytochemicals in fruits and vegetables. Radish is a cool season crop and during the harvesting season, large quantities get spoiled due to excess production. So a Long term preservation method is required that could be useful to prevent spoilage of Radish such that it could be consumed in off seasons as well. India is second largest producer of fruits and vegetables in the world after china, the present quantity of fruit and vegetable processing is very meager (around 2.2%) as compared to 80% in USA, 70% France, 80% Malaysia and 30% Thailand (Singh *et al.*, 2014).

For such reasons, Radish can be processed into juice in order to increase its shelf stability. Some studies have been conducted to quantify the physicochemical, phytochemical and

antioxidant activity of Radish and members of this family. Ayub *et al.* (2013) investigated the shelf life of fresh cut radishes. Aruna and Nishadh (2014) studied the effect of thermal processing on the biochemical parameters of radish using the dehydration method. But so far, there has not been a systematic study on the effect of chemical and thermal preservation techniques on the shelf life of Radish juice. Keeping in view, the present study was conducted to process and preserve the Radish juice and to find out the better technique to preserve radish.

MATERIALS AND METHODS

Raw materials

The study was conducted in the Department of Food Science and Technology, Punjab Agricultural University, Ludhiana. Radish was procured from the local market. Fresh Radish were washed thoroughly and cut off from the top and were not peeled. The Radish juice was extracted in a juicer extractor (Kalsi: 9001-2008). The juice was pasteurized at 83°C for 3 min and citric acid @ 0.15% was added to maintain the required acidity, followed by chemical preservatives.

Dose distribution of chemical additives

T₁ sample was given the pasteurization treatment followed by processing at 100°C for 20 min in boiling water bath and gradually cooled to a low temperature under running tap water. The prepared juice samples were bottled in pre-sterilized glass bottles (200 ml capacity) by leaving 2 cm head space. These bottles were stored at ambient temperature for further studies. Observations were taken just after preparation of the product and therefore, one month till 6 months of storage.

Sample	Chemical additives	Dose(ppm)
T ₂	Na-benzoate	3000
T ₃	KMS	3000
T ₄	Na-benzoate + KMS	1500 + 1500

Analytical evaluation

Physico-chemical analysis

Carrot juices were analysed at regular interval of one month for the parameters like Total solids, Titratable acidity using AOAC methods (AOAC 2000). TSS was taken using hand refractometer (ERMA, Japan), Color (Lab) using Minolta Hunter colorimeter.

Determination of Vitamin C

Ascorbic acid was extracted from the sample with 0.4 per cent oxalic acid and determined by titrimetric method using 2, 6-dichlorophenol indophenol dye solution (0.04 per cent) which was standardized against standard L-ascorbic acid (0.1 mg/ml of 0.4 per cent oxalic acid). 5g sample was taken for estimation and volume was made to 100 ml with 0.4 per cent oxalic acid solution. It was filtered and 10 ml aliquot was titrated with standardized dye. The end point was recorded as pink color, which persisted for atleast 15sec. The results were expressed as ascorbic acid mg percent of sample (Ranganna 1997).

Determination of Total Phenolic Content

The total phenolic content of blended juice was determined with the Folin–Ciocalteu method (Singleton *et al* 1999). Five gram of RTS juice was taken and refluxed with 80% methanol for two hours in a round bottom flask and residue was then further refluxed for an hour. After filtration of the extract volume was made to 100 mL with 80% methanol. Filtrate (0.5 ml) was taken into a test tube containing 0.5 ml water. The Folin-Ciocalteu reagent (5 ml) then kept for 5 min, and saturated solution of sodium carbonate (1 ml) was mixed. A standard curve was plotted by taking known amount of Gallic acid as reference standard and concentration was calculated from the standard curve

Determination of % Anti oxidant activity

Free radical scavenging activity was determined by DPPH (2,2-di phenyl picryl-1- hydrazyl) method. A method according to Brand- Williams *et al.* (1995) was followed with some modifications. Five gram of blended RTS juice was taken and refluxed with 80% methanol for two hours in a round bottom flask and residue was then further refluxed for one hour. After filtration of the extract volume was made to 100 ml with 80% methanol. To 1ml of methanolic extract of sample, 2ml of 1mM freshly prepared DPPH and 1ml of 50 mM tris buffer was added and absorbance was determined at 517 nm (blank as 80 per cent methanol and tris buffer) after 30 minutes. The free radical scavenging activity was evaluated by comparing the absorbance of the sample solution with control solution to which distilled water was added instead of sample. BHT was taken as a standard at a fixed concentration of 5mg/ml.

$$\% \text{ AA} = \frac{\text{Control OD}(0 \text{ min}) - \text{Sample OD}(30 \text{ min})}{\text{Control OD}(0 \text{ min})} \times 100$$

Statistical analysis

The results were evaluated by Analysis of Variance (ANOVA) and Tukey's post hoc tests using Systat statistical program version 16 (SPSS Inc., USA).

RESULTS AND DISCUSSION

The samples were studied for the effect of different chemical additives on Physicochemical [TS, TSS, Acidity, Color (L, a, b)], Phytochemical (Ascorbic acid, Total phenols) and % antioxidant activity for the storage period of 6 months.

Effect on Total solids and TSS

TS increased non-significantly ($p \leq 0.05$) in all the juices during the storage. The TSS values of samples T₁ to T₄ on day first was 7.3 for each sample which gradually increased to 7.6, 7.5, 7.4 and 7.4 respectively after 6 months of storage. Although TSS increased for all the samples but the changes were non-significant ($p \leq 0.05$). An increase in soluble content of apple pulp was reported during storage when preserved with chemical preservatives (Kinh *et al.*, 2001). Also when jamun RTS beverages were stored on thermal treatment, the increase in TSS content was found (Kesharwani *et al.*, 2015). The treatments

Table 1: Effect of storage period and treatments on Titratable acidity (%) of Radish juice*

Treatments	0	1	2	3	4	5	6
T1	0.34 ^{eb}	0.378 ^{db}	0.401 ^{cdB}	0.425 ^{cA}	0.467 ^{bA}	0.504 ^{aB}	0.524 ^{aBC}
T2	0.383 ^{gA}	0.415 ^{fA}	0.449 ^{eA}	0.493 ^{dA}	0.529 ^{cA}	0.561 ^{bA}	0.598 ^{aA}
T3	0.383 ^{eA}	0.403 ^{eAB}	0.433 ^{dA}	0.467 ^{cAB}	0.492 ^{bcB}	0.515 ^{bB}	0.546 ^{aB}
T4	0.383 ^{eA}	0.403 ^{deAB}	0.425 ^{cdAB}	0.452 ^{cB}	0.485 ^{bb}	0.506 ^{abB}	0.517 ^{aC}

Table 2: Effect of storage period and treatments on the color values (L a b) of Radish juice*

Treatments	0	1	2	3	4	5	6	
L	T1	46.24 ^{aD}	46.07 ^{aD}	45.8 ^{abD}	45.12 ^{abD}	43.43 ^{bcD}	42.24 ^{cD}	41.27 ^{cD}
	T2	49.06 ^{aC}	49.01 ^{aC}	48.86 ^{aC}	48.01 ^{abC}	47.39 ^{abC}	46.2 ^{bc}	46.02 ^{bc}
	T3	54.8 ^{aA}	54.64 ^{abA}	54.36 ^{abA}	53.89 ^{abA}	53.34 ^{abA}	53.17 ^{abA}	52.22 ^{ba}
	T4	52.44 ^{aB}	52.32 ^{aB}	51.53 ^{abB}	51.12 ^{abB}	50.29 ^{abB}	50.07 ^{abB}	49.17 ^{bb}
a	T1	0.38 ^{cA}	0.42 ^{bcA}	0.49 ^{abcA}	0.56 ^{abcA}	0.61 ^{abcA}	0.67 ^{abA}	0.73 ^{aA}
	T2	0.43 ^{aA}	0.45 ^{aA}	0.48 ^{aAB}	0.52 ^{aA}	0.57 ^{aA}	0.61 ^{aAB}	0.68 ^{aA}
	T3	0.33 ^{ba}	0.36 ^{ba}	0.4 ^{abAB}	0.45 ^{abAB}	0.52 ^{abAB}	0.58 ^{abA}	0.65 ^{aA}
	T4	0.22 ^{aA}	0.23 ^{aB}	0.25 ^{aB}	0.28 ^{aB}	0.31 ^{aB}	0.35 ^{aA}	0.39 ^{aB}
b	T1	-0.01 ^{aA}	-0.03 ^{aA}	-0.06 ^{aA}	-0.09 ^{aA}	-0.12 ^{aA}	-0.16 ^{aA}	-0.21 ^{aA}
	T2	0.15 ^{aA}	0.13 ^{aA}	0.11 ^{aA}	0.08 ^{aA}	0.05 ^{aA}	0.01 ^{aA}	-0.03 ^{aA}
	T3	0.01 ^{aA}	-0.02 ^{aA}	-0.05 ^{aA}	-0.08 ^{aA}	-0.11 ^{aA}	-0.15 ^{aA}	-0.19 ^{aA}
	T4	0.09 ^{aA}	0.07 ^{aA}	0.05 ^{aA}	0.02 ^{aA}	-0.01 ^{aA}	-0.05 ^{aA}	-0.09 ^{aA}

Table 3: Effect of storage period and treatments on Ascorbic acid content (mg/100g) of Radish juice*

Treatments	0	1	2	3	4	5	6
T1	45.26 ^{ab}	41.11 ^{bc}	38.74 ^{cC}	34.69 ^{dC}	31.46 ^{eC}	27.33 ^{fC}	23.72 ^{gC}
T2	47.37 ^{aA}	44.18 ^{bB}	41.02 ^{cB}	38.77 ^{dB}	35.66 ^{eB}	32.83 ^{fB}	29.12 ^{gB}
T3	48.29 ^{aA}	45.87 ^{bA}	43.33 ^{cA}	41.83 ^{cA}	38.19 ^{dA}	36.51 ^{eA}	33.86 ^{fA}
T4	48.76 ^{aA}	46.64 ^{bA}	43.92 ^{cA}	41.32 ^{dA}	38.15 ^{eA}	36.09 ^{fA}	32.21 ^{gA}

Table 4: Effect of storage period and treatments on Total Phenols (mg/100g) of Radish juice*

Treatments	0	1	2	3	4	5	6
T1	48.66 ^{ad}	45.55 ^{bd}	42.81 ^{cd}	38.63 ^{dD}	34.08 ^{eD}	28.16 ^{fD}	22.59 ^{gD}
T2	52.79 ^{aC}	51.46 ^{bC}	50.17 ^{cC}	47.05 ^{dC}	44.49 ^{eC}	40.87 ^{fC}	35.37 ^{gC}
T3	54.32 ^{ab}	53.73 ^{ab}	52.59 ^{bb}	50.41 ^{cB}	48.21 ^{dB}	45.32 ^{eB}	41.47 ^{fB}
T4	57.22 ^{aA}	56.24 ^{bA}	54.87 ^{cA}	52.86 ^{dA}	50.19 ^{eA}	47.12 ^{fA}	43.62 ^{gA}

Table 5: Effect of storage period and treatments on % Antioxidant activity of Radish juice*

Treatments	0	1	2	3	4	5	6
T1	35.68 ^{ab}	33.67 ^{abB}	31.12 ^{bcB}	28.43 ^{cdB}	25.66 ^{dB}	21.28 ^{eB}	17.45 ^{eC}
T2	40.08 ^{aA}	38.83 ^{abA}	36.67 ^{abA}	34.83 ^{bcA}	31.43 ^{cdA}	27.51 ^{deA}	23.87 ^{eB}
T3	41.33 ^{aA}	39.77 ^{aA}	37.19 ^{abA}	35.07 ^{bcA}	33.15 ^{bcA}	30.78 ^{cdA}	28.64 ^{dAB}
T4	41.67 ^{aA}	39.54 ^{abA}	37.81 ^{abA}	35.52 ^{bcA}	33.23 ^{cdA}	31.55 ^{cdA}	27.92 ^{eA}

* Data is expressed as means of three readings and values followed by different upper case or lower case letters are significantly different ($p \leq 0.05$) within columns and rows respectively

had no significant effect ($p \leq 0.05$) on Total solids as well as TSS. The increase in TSS content can be attributed to the breakdown of polysaccharides into oligosaccharides and monosachharides.

Effect on acidity

According to the results, chemical additives as well as storage showed an increasing trend on acidity of the Radish juice. The titratable acidity of sample T₁ on day first was found to be 0.341 and 0.383 for the three chemically treated samples (T₁ to T₃) that gradually increased to 0.524, 0.598, 0.546 and 0.517 respectively, being the minimum increase in T₄ (Table 1). The present findings are also in conformity with the reported works of Byanna and Gowda (2012) in sweet orange beverages, Yadav *et al.* (2013) in Banana RTS. The acidity of the thermally treated sample (T₁) increased more as compared to other chemically treated samples and the change was least in T₄ sample. The increase in acidity in RTS during 90 days of storage may be due to formation of organic acids by ascorbic acid degradation

Effect on Color (L a b values)

Color is one of the most important visual attributes for juices. The L value varied significantly ($p \leq 0.05$), both for storage as well as chemical treatments. On the day of preparation, the lightest sample was T₃ followed by T₄, T₂ and heat treated sample T₁ was found to be the darkest. This trend remained the same till the end of 6 months. Aguilo-Aguayo *et al.* (2009) found that strawberry juice subjected to heat treatment exhibited decreased L values during storage at 40 C. Heating caused the accumulation of dark colour compounds in the juice and consequently decreased the L value (Klim and Nagy 1988). According to Rivas *et al.* (2006), the L values of thermally pasteurized blended orange and carrot juice decreased significantly during storage at 120 C. The lightness of sample T₃ containing KMS is attributed to the bleaching action of KMS that helped to maintain the whitish color of the juice. The 'a' value changed significantly ($p \leq 0.05$). T₄ was found to have the lowest 'a' value and retained the minimum value than the other 3 samples at the end of 6 months also (Table 2). But there was non-significant ($p \leq 0.05$) change in the b values.

Effect on Vitamin C content

Vitamin C is light and heat sensitive, the concentration of Vitamin C follows first order kinetics and thus storage time affects Vitamin C content (Heldman and Singh 1981). Singh *et al.* (2015) found the similar trend in grapefruit. Radish is a good source of Vitamin C. According to the results, chemical additives have significant effect on Vitamin C content. Also the Vitamin C content decreased significantly ($p \leq 0.05$) during the storage. On the day of preparation, Vitamin C

content in samples T₁, T₂, T₃ and T₄ was 45.26, 47.37, 48.29 and 48.75mg/100g respectively. The values came out to be lower in T₁ as heat treatment destroys Vitamin C. This difference in reduction rate of ascorbic acid, a heat sensitive vitamin, may be due to longer exposure time of juice blends to high temperatures (Calskantur *et al.*, 2011). At the end of 6 months, the Vitamin C content reduced to 23.72, 28.12, 33.86 and 32.21mg/100g respectively (Table 3). Out of the chemically treated samples, potassium metabisulphite retained the maximum Vitamin C. This result is in agreement to that of Negi and Roy (2000) where they found that the application of KMS reduces the loss of ascorbic acid during the storage of leafy vegetables. Besides that Nagy (1980) reported that loss of ascorbic acid in processed products is due to aerobic and anaerobic reaction of non-enzymatic nature also. The incorporation of air into the juice during extraction, finishing and bottle filling have long been recognized by Farnsworth *et al.* (2001) as cause for ascorbic acid loss.

Effect on Total Phenols

Phenolic compounds, ever-present in fruits and vegetables are the most abundant antioxidants in the human diet, and are of substantial interest due to their antioxidant properties. The total phenolic content in samples T₁ to T₄ on the first day was 48.66, 52.79, 54.32 and 57.22mg/100g respectively. The added chemicals preserved the phenolic content more than thermally treated sample (T₁). But both the treatments and storage affected the Total phenols non-significantly ($p \leq 0.05$). At the end of 6 months, the Total phenolic content came out to be 22.59, 35.37, 41.47 and 43.62mg/100g respectively (Table 4). The decrease was found to be least in sample T₃, followed by T₄ and T₂. According to the findings, a decrease in total polyphenol content of tomato juices after 3, 6 and 9 months of storage were reported (Vallverdu-Queralt *et al.* 2011). These results were consistent with the findings of Laorko *et al.* (2013) who reported a decrease in phenolic content in clarified banana juice after 6 months at 27 and 38°C. The decrease in polyphenols could mainly be resulted from oxidation of these compounds and polymerization with proteins (Liu *et al.* 2014).

Effect on Antioxidant activity

Antioxidants delay the oxidation process, inhibiting the polymerization chain initiated by free radicals and other subsequent oxidizing reactions (Halliwell and Aruoma 1991). According to the results, on the day of preparation, percent Antioxidant activity for samples T₁ to T₄ was found to be 35.68, 40.08, 41.33 and 41.67 respectively (Table 5). Significant ($p \leq 0.05$) decrease in antioxidant activity was found during storage for 6 months. At the end of 6 months, the percent antioxidant activity decreased to 6.98, 13.26, 15.97 and 14.24 percent

respectively. Also, the percent antioxidant activity for the three chemically treated was different significantly at the end of 6 months. However, the decrease was found to be least in sample T₃. It has been reported that the decrease in antioxidant activity may be linked to a decrease in total phenolic content and vitamin C during storage (Klimczak *et al.* 2007). According to them, antioxidant activity of orange juices decreased by 45 percent after 6 months of storage at 28°C. But in case of Radish, the vitamin content is relatively high. So the reduction of antioxidant activity is mainly associated with significant decrease in vitamin content.

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