IJFAAS International Journal of Food and Allied Sciences
ISSN: 2415-0290 (Print)
ISSN: 2413-2543 (Online)
DOI:10.21620/ijfaas.2015256-62

Research Article

Effect of water extracts of apple and pomegranate peel on the quality of orange squash

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History

Submitted: Sep 20, 2015
Revised: Nov 30, 2015
Accepted: Dec 01, 2015
Online: Dec 15, 2015

Keywords
Aqueous extract
Pomegranate
Apple
Quality characteristics

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Abstract

Punica granatum (pomegranate) and Malus domestica (apple) are rich source of bioactive compounds i.e. phenolics, anthocyanins and tannins that can be potential preservative agents. The aim of the present research plan was to evaluate the quality characteristics of orange squash after addition of aqueous extracts of apple and pomegranate peels. For this purpose aqueous extracts of pomegranate and apple peels were added in orange squash and then the final product was examined for physiochemical and sensory attributes during storage. Results of current study demonstrated that although physico-chemical parameters of orange squash were changed with the storage period in all the treatments yet the changes in the squash prepared by standard preservative and 100% extracts of pomegranate and apple peel were least. The total soluble solids (°Brix) were gradually decreased but the acidity was increased with storage period. Sensory examination of the squash elaborated the suitability of apple peel extract as natural preservative in the squash whereas the addition of pomegranate peel extract was rejected due to dark color contributed by the extract.

1. Introduction

Food is the basic requirement of life and is a mean for the provision of different nutrients. A food is termed as functional if it provides positive functions to the body other than enough nourishment, increases the fitness level and minimizes risks of diseases (Shafi et al., 2014). It may be used as a medium for the growth of different kinds of microbes like bacteria, fungi and viruses which are major threats to the public health (Mojab et al., 2008). The microorganisms are responsible to lower the quality of foods and food products. In order to control the microbial threats, the specific synthetic antimicrobial components are being used (Raghuveer & Tendon, 2009). Due to resistance in the microorganisms, the lower efficiency of synthetic antimicrobial components has been reported. In addition, some other side-effects of synthetic antimicrobial agents have also been noticed. For example Pongsavee (2015) reported that the use of sodium benzoate in food products as preservative caused mutagenic and cytotoxic toxicity in lymphocytes through the formation of micronucleus and also by breaking of chromosome. For this purpose the new researches are focused to investigate the antimicrobial potentials of different fruits and their wastes to provide natural preservation through bioactive compounds for the increment in shelf life and improved food safety (Singh, 2002).

Among fruits, apple (*Malus domestica*) is recognized for a number of health benefits because of phytochemicals (Chan, Graves, & Shea, 2006). The apple peel or skin has phenolic compounds and also contains flavonoids which are not present in pulp, such as cyanidin glycosides and quercetin glycosides (Van-Der-Sluis et al., 2001). Similarly, pomegranate (*Punica granatum*) is one of the historical fruit used for edible purpose. This fruit possess powerful antioxidant (Lansky & Newman, 2007) and antimicrobial properties (Sadeghian et al., 2011). Pomegranate peel contribute near about 50% of the whole weight of fruit, which contains bioactive compounds such as phenolic components, tannins, proanthocyanidins components, flavonoids (Li et al., 2006) and complex polysaccharides (Jahfar, Vijayan, & Azadi, 2003). It

also consists of minerals i.e. K, N, Ca, P, Mg, and Na (Mirdehghan & Rahemi, 2007). Flavonoids present in pomegranate peel contain anthocyanins, catechins, epicatechin, epigallocatechin and complex flavonoids. Tannins such as pedunculagin, punicalin, punicalagin, gallic acid and ellagic acid are also found in apple peel. These tannins are hydrolysable (Afaq et al., 2005; Negi, Jayaprakasha, & Jena, 2003).

Similarly, the citrus fruit has become the world's foremost fruit in all fruits. Orange (*Citrus sinensis*) amongst all fruits is the most widely consumed fruit all over the world (USDA, 2004). People had found out several ways to protect the citrus fruit for consumption during off seasons, so several kinds of useful products are made from citrus fruits such as jellies, marmalade, jam, fruit squashes, juices, and many other drinks. For the preservation of these products antimicrobial agents are necessary (Mubeen et al., 2008).

Keeping in view the antimicrobial activities of apple and pomegranate peel extracts and the importance of preservation of citrus products for their utilization in off season, the present research work was planned to prepare orange squash by the addition of natural preservative (apple and pomegranate peel extracts) and also to assess the suitability of pomegranate and apple peel aqueous extracts as preservative in the preparation of orange squash.

2. Material and methods

The research work was conducted in Food Microbiology Laboratory at Institute of Food Science and Nutrition, University of Sargodha, Sargodha Pakistan to check the effect of aqueous extracts of pomegranate and apple peels on the quality of orange squash.

2.1. Preparation of aqueous extracts of pomegranate and apple peel

Aqueous extracts of pomegranate and apple peel were obtained by the method as suggested by Tanveer (2013). For this purpose initially the aqueous extract was obtained from 10g sample (in 100 mL water) at 60°C for 2 hours in shaking incubator at 120 rpm. Then the extract was filtered through Wattman filter paper (No. 1) and was concentrated by evaporation in rotary evaporator at 50°C and finally the extract was dried by heating at 45°C in water bath. This dried powder of the extracts was later on used as natural preservative for the preparation of orange squash.

Table 1. Treatment Plan for the preparation of orange squash

Treatments	Apple Peel Extract (g)	Pomegranate Peel Extract (g)	Sodium benzoate(g)
T_0	-	-	-
T_1	-	-	0.4 (100%)
T_2	0.4 (100%)	-	-
T_3	0.2 (50%)	-	0.2 (50%)
T_4	-	0.2 (50%)	0.2 (50%)
T_5	-	0.4 (100%)	-

2.2. Preparation of orange squash

Fresh oranges were purchased from local market of Sargodha, and juice was extracted after washing using rose head machine. Then juice was filtered by muslin cloth and orange squash was prepared by following the recipe recommended by Awan and Rehman, (2004) as given in Table 1. During preparation of the squash, the standard preservative (Sodium Benzoate) was replaced with the aqueous extracts as obtained earlier. The standard preservative was replaced by the extracts with different combinations and levels as shown in Table 1.

The prepared squash was stored at room temperature and was evaluated for its physiochemical and microbiological quality after every 15 days during storage at ambient temperature for a period of 60 days. The following quality tests were performed to observe the preservative potential of the extracts.

2.3. Physico-chemical analysis

The physico-chemical attributes like pH, acidity, total soluble solids and solid to acid ratio were analyzed to assess the quality of the squash. The Total Soluble Solids (TSS), titratable acidity and pH was determined by the standard method of AOAC (2000). However, the ratio between total soluble solids to acid (TSS/TA) was calculated simply by dividing the total soluble solids (in percentage) by its acidity percentage.

2.4. Microbiological evaluation

Microbiological analysis of squashes was performed by following the method of Harrigan (1998). The samples were prepared by 10 fold serial dilution and the total viable count was estimated by using plate count agar as

medium. The colonies were manually counted and viable count was determined by the following formula (standard acceptable colony range was 25-250).

$$N = \frac{\sum C}{[(1\times n_1)+(0.1\times n_2)\times(d)}$$

Where:

N = Number of colonies per ml or g of product
∑C = Sum of all colonies on all plates counted
n_1 = Number of plates in first dilution counted
n_2 = Number of plates in second dilution counted
d = Dilution from which the first counts were obtained

2.5. Sensory Evaluation

Quality of orange squash was evaluated for sensory characteristics (color, taste, flavor and overall acceptability) during storage on Hedonic Rating Scale by a panel of assessors to judge the quality of orange squash as done by Nelson & Trout (1964).

2.6. Statistical analysis

The data obtained during entire research work was statistically analyzed by using factorial design through statistics software (Statistix version 8.1) according to the method recommended by Steel, Torrie, & Dickey (1997).

3. Results

The research was conducted to study the quality of orange squash by physicochemical and sensory parameters during storage after addition of aqueous extracts of apple and pomegranate peel in different concentrations.

Table 2. Effect of Treatment and Storage on the Chemical and Microbial quality of Orange Squash

		Storage Period (Days)					
Parameter	**Treatments**	**0**	**15**	**30**	**45**	**60**	**Mean**
°Brix	T_0	44.67a	44.33a	43.83b	43.67b	43.67b	44.03 A
	T_1	44.00a	44.33a	44.00a	43.83b	43.33b	43.90 B
	T_2	44.00a	44.00a	43.83b	43.67b	43.17b	43.73 B
	T_3	43.67b	43.83b	43.67b	43.67b	43.00b	43.57 B
	T_4	43.50b	43.17b	43.00b	43.00b	42.83c	43.10 B
	T_5	43.50b	42.83c	43.00b	43.00b	42.83c	43.03 B
	Mean	**43.89 A**	**43.75 A**	**43.56 A**	**43.47 A**	**43.14 A**	
Acidity (% Citric Acid)	T_0	0.43c	0.47c	0.50b	0.53b	0.59a	0.50 A
	T_1	0.43c	0.48c	0.51b	0.56ab	0.60a	0.52 A
	T_2	0.43c	0.47c	0.50b	0.54b	0.58a	0.50 A
	T_3	0.42c	0.46c	0.49c	0.53b	0.55b	0.49 B
	T_4	0.42c	0.45c	0.47c	0.52b	0.54b	0.48 B
	T_5	0.42c	0.45c	0.47c	0.52b	0.53b	0.48 B
	Mean	**0.43 D**	**0.47CD**	**0.49 C**	**0.53 B**	**0.57 A**	
Brix/Acid Ratio	T_0	7.33b	5.00d	4.00e	3.33f	2.00g	4.33 B
	T_1	8.00a	7.33b	6.67c	6.00c	4.67e	6.53 A
	T_2	7.67b	7.33b	6.67cc	6.00c	4.33e	6.40 A
	T_3	7.67b	7.00b	6.67c	6.00c	4.33e	6.33 A
	T_4	5.00d	4.67e	4.00e	3.33f	2.33g	3.87 C
	T_5	4.33e	3.67f	3.33f	3.00f	2.33g	3.33 C
	Mean	**6.67 A**	**5.83 B**	**5.22 B**	**4.61 C**	**3.33 D**	
Microbial Load	T_0	0.00f	0.00f	159.33b	146.00c	247.67a	110.60A
	T_1	0.00f	0.00f	0.00f	0.00f	0.00f	0.00D
	T_2	0.00f	0.00f	0.00f	0.00f	13.00e	2.60C
	T_3	0.00f	0.00f	0.00f	0.00f	12.00e	2.40C
	T_4	0.00f	0.00f	0.00f	0.00f	19.00d	3.80B
	T_5	0.00f	0.00f	0.00f	0.00f	18.33d	3.67B
	Mean	**0.00C**	**0.00C**	**26.56B**	**24.33B**	**51.67A**	

The values with same letters in a column or row are similar while the values with different letter are significantly different from each other

3.1. Chemical analysis

3.1.1. Brix

The statistical results revealed that the effect of treatments and treatments x storage period was found to be highly significant on the °Brix of orange squash. The effect of treatment on the °Brix of the orange squash showed that the value of °Brix was gradually decreased from 44.03 to 43.03 in all the treatments. Similarly, due to storage period the °Brix was also reduced with the passage of time however the results for storage period were found to be non-significant. The mean reduction in °Brix was 43.89-43.14 during a period of 60 days at room temperature. The combined effect of treatments and storage period on the Brix of the orange squash indicated that the value of Brix was gradually decreased with increment in storage period in all the treatments (Table 2). However, the decline in the value of °Brix without preservative (T_0) was from 44.67 to 43.67 whereas the value of °Brix in case of squash prepared by preservative (T_1), 100% apple extract (T_2), combination of preservative and apple extract (T_3), combination of preservative and pomegranate extract (T_4) and100% pomegranate extract (T_5) were decreased from 44.00-43.33, 44.00-43.17, 43.67-43.00, 43.50-42.83 and 43.50-42.83, respectively.

3.1.2. Titratable acidity

The statistical results revealed that the effect of treatments, storage period and treatments x storage period was found to be highly significant on the acidity of orange squash. The effect of treatment on the acidity of the orange squash showed that value of acidity was gradually decreased (0.50-0.48) in all the treatments. But the effect of storage period showed that the value of acidity of orange squash was increased (0.43-0.57) with increment of storage duration of 60 days. The mutual effect of treatments and storage period on acidity of the orange squash described that acidity was gradually increased with increment in storage period in all treatments (Table 2). However, the maximum value of acidity during storage was observed in case of squash prepared by preservative (T_1), followed by without preservative (T_0), 100% apple extract (T_2), combination of preservative and apple extract (T_3), combination of preservative and pomegranate extract (T_4) and 100% pomegranate extract (T_5), respectively as shown in Table 2.

3.1.3. Brix /Acid ratio

The statistical results showed that the effect of treatments, storage period and treatments x storage period was observed to be highly significant on the Brix/Acid ratio of orange squash. The effect of treatment on the Brix/Acid ratio of the orange squash showed that the Brix/Acid value had gradually decreased (6.53-3.33) from T_1-T_5 except T_0. But the storage period gradually decreased the value of Brix/Acid ratio (6.67-3.33) of orange squash in all the treatments. The combined effect of treatments and storage period on the Brix/Acid ratio of the orange squash indicated that the Brix/Acid ratio was gradually decreased with increment in storage period in all the treatments (Table 2).

3.1.4. Microbiological evaluation

The statistical results (ANOVA) revealed that the effect of treatments, storage period and treatments x storage period was found to be highly significant on the microbial count of orange squash. The effect of treatment on the microbial status of the orange squash revealed that T_4 and T_5 showed value of viable count 3.80CFU/mL and 3.67 CFU/mL, respectively whereas T_0, T_1, T_2 and T_3 showed value of viable count 110.60 CFU/mL, 0.00 CFU/mL, 2.60 CFU/mL and 2.40 CFU/mL respectively. The storage period increased the no. of viable count (0.00 CFU/mL -51.67 CFU/mL) in all the treatments. The combined effect of treatments and storage period on the microbial count of the orange squash revealed that the microbial count gradually increased with increment in storage period in all the treatments. However, the maximum increase in the value of microbial count during storage was observed in case of squash prepared without preservative (T_0). However, the lower microbial load was observed in case of standard and natural preservatives (aqueous extracts of apple and pomegranate peel) as shown in Table 2. The results clearly indicated the presence of antimicrobial potential (preservative potential) in the extracts as compared to the control and standard treatment.

Table 3. Effect of Treatment and Storage on the Sensory parameters of Orange Squash

Parameter	Treatments	Storage Period (Days)					
		0	**15**	**30**	**45**	**60**	**Mean**
Taste	T_0	7.33b	5.33d	3.67f	3.00f	2.00g	4.27 B
	T_1	8.00a	7.67b	7.00b	6.33c	4.00e	6.60 A
	T_2	8.00a	7.33b	6.67c	6.00c	4.00e	6.40 A
	T_3	8.00a	7.00b	6.33c	5.67d	3.67f	6.13 A
	T_4	5.33d	4.33e	3.33f	2.67g	1.33h	3.40 C
	T_5	4.67e	4.00e	3.00f	2.33g	1.33h	3.07 C
	Mean	**6.89 A**	**5.94 B**	**5.00 C**	**4.33 D**	**2.72 E**	
Flavor	T_0	6.67b	5.33c	4.67d	3.33e	1.33g	4.27 B
	T_1	7.67a	7.33a	7.00a	6.67b	5.67c	6.87 A
	T_2	7.67a	7.33a	7.00a	6.33	5.67c	6.80 A
	T_3	7.67a	7.33a	6.67b	6.00b	5.33c	6.60 A
	T_4	5.00c	4.33d	3.33e	2.67f	1.33g	3.33 C
	T_5	5.33c	4.67d	3.67e	3.00e	1.67g	3.67 C
	Mean	**6.67 A**	**6.06 A**	**5.39 B**	**4.67 C**	**3.50 D**	
Color	T_0	7.33b	5.00d	4.00e	3.33f	2.00g	4.33 B
	T_1	8.00a	7.33b	6.67c	6.00c	4.67e	6.53 A
	T_2	7.67b	7.33b	6.67c	6.00c	4.33e	6.40 A
	T_3	7.67b	7.00b	6.67c	6.00c	4.33e	6.33 A
	T_4	5.00d	4.67e	4.00e	3.33f	2.33g	3.87C
	T_5	4.33e	3.67f	3.33f	3.00f	2.33g	3.33C
	Mean	**6.67 A**	**5.83 B**	**5.22 B**	**4.61 C**	**3.33 D**	

The values with same letters in a column or row are similar while the values with different letter are significantly different from each other

3.1.5. Sensory evaluation

3.1.5.1. Color

Highly significant statistical results (treatments, storage period and treatments x storage period) about color of orange squash were observed. The effect of treatment on the color of the orange squash showed that color scores given to the orange squash decreased from T_1-T_5 (6.53-3.33) except T_0. Similarly, the storage period also gradually decreased (6.67-3.33) the color scores of orange squash in all the treatments. The mutual effect of treatments and storage period on the color of the orange squash showed that the quality of color was gradually decreased with increment in storage period in all the treatments (Table 3). However, the minimum decline in the quality of the color during storage was observed in case of squash prepared without preservative (T_0), followed by preservative (T_1), 100% apple extract (T_2) and combination of preservative and apple extract (T_3) as shown in Table 3.

3.1.5.2. Taste

Taste of orange squash on statistical analysis results (treatments, storage period and treatments x storage period) showed to be highly significant. The effect of treatment on the taste of the orange squash showed that taste scores given to the orange squash decreased (6.60-3.07) from T_1-T_5 except T_0 (4.27). The storage period gradually decreased the taste scores of orange squash (6.89-2.72) in all the treatments. The combined effect of treatments and storage period on the taste of the orange squash showed that the quality of taste was gradually decreased with increment in storage period in all the treatments (Table 3). However, the minimum decline in the quality of the taste during storage was observed in case of squash prepared by preservative (T_1) after without preservative (T_0), 100% apple extract (T_2) and combination of preservative and apple extract (T_3). Moreover, the taste of the squash prepared by pomegranate extract was not accepted by the judges whereas, the quality of the taste in case of squash prepared without addition of preservative was significantly decreased during storage.

3.1.5.3. Flavor

The results (statistical results) showed that the effect of treatments, storage period and treatments x storage period was found to be highly significant on the flavor of orange squash. The effect of treatment on the flavor of the orange squash showed that flavor scores given to the orange squash decreased (6.87-3.67) from T_1-T_5 except T_0 (4.27). The storage period gradually decreased (6.67-3.50) the flavor scores of orange squash in all the treatments. The flavor quality was gradually decreased with increment in storage period in all the treatments as shown in Table 3. However, the minimum decline in the quality of the flavor during storage was observed in case of squash prepared by preservative (T_1), 100% apple extract (T_2) and combination of preservative and apple extract (T_3). Moreover, the flavor of the squash prepared by pomegranate extract was not accepted by the assessors.

4. Discussion

The total soluble solids or sugar contents of fruits and fruit products fluctuates with the passage of time because of acid hydrolysis and might be due to the formation of pectic substances from protopectin and mono-saccharides from disaccharides i.e. degradation of sucrose into glucose and fructose. The current findings of the study showed gradual decrease in total soluble solids of orange squash. Acidity increases due to the degradation of sugars into carboxyl acids. In all treatments there was a gradual increase in acidity during storage up to 60 days. Comparable acidity increasing trend was also observed by the Saito et al. (2002) during studies on fruit juices. Brix/Acid ratio is a most excellent indicator for determining relative sweetness or tartness of the product. Higher the Brix as compared to acid gives better taste (Mubeen et al., 2008).

Moreover, the results for sensory evaluation showed that color of the squash prepared by pomegranate extract was not accepted by the judges due to the presence of granatonine (main coloring agent in the pomegranate peel) in the alkaloid form N-methyl granatonine. Similar results were also narrated by Kulkarni et al. (2011) while working on pomegranate peel color. The decline in acceptability of color with the passage of time was observed which is due to chemical reactions of organic acids present in the fruit drinks. A slight decline in flavor score was observed, as the storage period increased. The gradual loss in flavor scores over the entire storage period is due to alterations in volatile compounds of the drink. Flavour deterioration in beverage products was also reported by Jain et al. (2003) and Bezman (2001). Taste is very significant parameter in organoleptic evaluation after color and flavor. The highest scores for taste was observed when it was freshly prepared. A slight decrease in taste score was experienced, as the storage time increased. The taste difference and loss is because of the duration of storage time and temperature; and similar results also reported by Jain et al. (2003).

So from the Quality evaluation of orange squash it was observed that aqueous extracts of apple and pomegranate peels preserve the quality and shelf life of orange squash for the storage period of 60 days. The maximum physico-chemical quality attributes were deteriorated in the squash prepared by without the addition of preservative whereas apple and pomegranate peel extracts preserved the quality of squash comparable to standard preservative (Sodium benzoate). These extracts provide this preservative effect might be due to their antimicrobial properties as described by Al-Zoreky (2009) and Fratianni et al. (2007). Apple peel extract also preserved the organoleptic quality of orange squash comparable to the standard preservative whereas pomegranate peel extract not preserved this quality of orange squash because of high quantity of coloring compounds as described by Kulkarni et al. (2011) while working on coloring compounds present in pomegranate peel

5. Conclusions

It was concluded from the results of the current study that aqueous extracts of apple and pomegranate peels could be used as natural preservative to preserve the organoleptic and physico-chemical quality of orange squash. Among both of the extracts apple peel extract preserve the quality of orange squash upto an acceptable level as compared to pomegranate peel extract. So apple peel extract could be used as natural preservative to extend the shelf life of orange squash upto 2 months.

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