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Research Article

Effect of head space, processed water and vitamin C on pasteurized hot filled orange juice during storage

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Abstract

This study is revealed on pasteurized Hot-fill orange juice the sequel of ascorbic acid, processed water and head space for 90 days during storage at room temperature. Outcomes were exhibited the significant reduction of the *Alicyclobacillus* growth with the addition of an amount of 150mg/lit of vitamin C and head space finishing of thermosetting container. While, initial bacterial count was controlled by ozonated and ultraviolet light processed water. Savour and color impermeability in the pasteurized Hot-fill orange juice were significantly ($p < 0.05$) pompous by both addition of vitamin C and leaving no head space. While in contrast the non- significant effect on acidity, pH, ^{0}Brix, reducing and non-reducing sugar were observed during the storage. Hence, concluded that during processing as well as in storage the microbial growth especially *Alicyclobacillus* could be inhibited by processed water, leaving no head space and ascorbic acid fortification.

Keywords: Pasteurized orange juice; Processed water; Sensory analysis; Vitamin C

Introduction

Orange (*Citrus sinensis*) is the fruit of the citrus species belongs to the the family rutaceae. Orange is a hybrid ofpomelo (*Citrus maxima*) and mandariin (*Citrus reticulata*) genus, its being cultivated since ancient times **[1]**.Orange bears seasonal fruits that measure about 3 inches in diameter and weigh about 100-150 g, evergreen small flowering tree growing to about 5 to 8 m tall tropical to semitropical.In Pakistan, total area under orange cultivation is 193977 hectares, with annual production of 2147340 tones. Punjab province contributes more than 94 % towards total production **[2]**. Fresh fruits are a source of rational income as well as healthy supplement to the diet. But due to the high moisture content the fruits are short lived. Hence many recent developments were made to overcome these losses but these efforts proved to be non-result oriented **[3]**.The contamination of *Alicyclobacillus* may occur from soil and infected water **[4]**. Acido-thermophilic bacteria which could obtain oxygen from oxidizing the iron were isolated from acidic (pH 2.7–3.7) and geothermal (30–83 °C) site **[5]**. Because of high viscosity, reduced water activity, high

sugar content, low oxygen contents and low pH the concentrated fruit products are supposed to be resistant to microbes [6]. In contrast, *Alicyclobacillus* development in a medium also depends on the available oxygen which can be can be rendered by adding vitamin C [7].

This is accomplished that the pasteurized juice is mainly spoiled by the *Alicyclobacillus* spores forming bacterium which can survive pasteurization temperature. The bacterium is soil inhabited and can only grow when the oxygen is available. This suggests that if the contamination from the soil is controlled and render the oxygen availability in orange juice filled in any air tight container the chances of spoilage might be reduce to a safe level. Pasteurization, using processed water and then do hot filling without leaving the head space in the bottle and closed the bottle air tight to create anaerobic condition will further preserve/enhance shelf life of the orange juice.

Materials and methods

This experimentation was thoroughly conducted in the laboratory of Department of Food Science and Technology, The University of Agriculture Peshawar. Fresh Oranges were bought from the local market of Peshawar in tardy spring 2013. Oranges were washed, peeled, and the juice was extracted by utilizing a juice extractor.

Formulation of orange juice

The juice was formulated as narrated by Glevitzky [8].

Juice= (50)%, Water= (50)% , Citric acid= 2.5 g/L , Sugar= 45g /L

Research study plan

Juice was yare according to the research plan below (Table 1) [14, 27]

Table 1. Treatments wise samples study plan

Treatments	Pasteurized	Head space	Water	Vitamin C
PO0	Done	Present	Unprocessed	Omit
POU	Done	Omit	Unprocessed	Omit
POT	Done	Omit	Processed	Omit
POTC	Done	Omit	Processed	Present(150mg/l)
POUC	Done	Omit	Processed	Present(150mg/l)

Orange juice preparation

Juice was heated without leaving the head space to 88± 2°C for 2 min and was filled in the bottles at that temperature. Thermosetting plastic bottles of 500ml containing Samples were stored at ambient temperature. The interval for the analyzing of product was 15 days with the 90 days storage period

Chemical analysis

The standard method of [9] of pH as communicated with the avail of pH meter for the culled samples pH was quantified. The standard method of AOAC [9] was utilized for the resoluteness of total soluble solids.Titration of titratable acidity was resolute against the prototype alkali solution by prototype procedure of AOAC [9]. The ascorbic acid was tenacious by the volumetric analysis pattern as described in AOAC [9]. The reducing sugar and non-reducing sugar was resolute by lane Eynon method as communicated in AOAC [9]. A morsel of orange sap was evaluated organoleptically for overall acceptability, savor and color by the method as described by [10].

Microbial tests

The microbial test for yeast and bacteria was performed by the prototype procedures as communicated by Javed [11].

Statistical analysis

All the data was analyzed statistically by utilizing two factorial Complete Randomized

Design (CRD), and denotes was disunited by LSD test as described by [12].

Results and discussion

Chemical analysis

The pH of pasteurized orange juice during storage by the sequel of treatments like head space, processed water and Vitamin C is conceal in (Table 2) at room temperature. Ascribed to treatments and storage non-significant differences were perceived in the pH. Vitamin C fortification might be the cause of samples POUC and POTC initial low pH [13]. Same pH (7.4) of processed and unprocessed water at beginning did not exploit samples pH of POU and POT. Owing to the destruction of spoilage microorganisms by pasteurization the slightest disturbance in pH with storage was noted [14]. Processed water, head space and Vitamin C merely checks the *Alicyclobacillus* which did not changed the pH of juice the treatments [15]. Similarly [16] also noted a non-significant variation in pasteurized orange carrot blend juice pH. Consequently, concluded that the pH in pasteurized orange juice doesn't pompous the treatments and storage.

Titratable acidity of pasteurized orange juice during room temperature storage caused due to processed water, Head space, and Vitamin C is shown in (Table 3). In the acidity of pasteurized orange juice the non-significant changes statistically originated by the treatments and storage. The pH of the juice was non-significant may be the reason the fact that titratable acidity and pH are vice versa [17]. Likewise, during storage of pasteurized apple juice [18] noted no significant changes in the acidity. Pasteurization and pulsating electric field did not produced significant effects on orange carrot blend juice acidity. It was noted that Titratable acidity did not vitiate with storage and treatments.

The Vitamin C content of pasteurized orange juice at room temperature storage is pronounced in (Table 4) by the aftermath of Head space, Processed water and Vitamin C. The treatments and storage was pretentious significantly ($p<0.05$) on the concentration of Vitamin C of the juice. In fruit juices the storage causes the Vitamin C to decomposed [19]. Similarly, higher Vitamin C content was retained in Vitamin C fortified orange juice compared to un-fortified pasteurized orange juice [20].

Total soluble solid was significantly increased with storage perceived from the results. However, on the TSS of juice samples treatments of no headspace, addition of vitamin C and processed water has no significantly effects. Storage causes the inversion of polysaccharides in the presence of acids and high temperature might be due to this significant increase in TSS [21]. Non-significant increase in the TSS of orange carrot pasteurized blend juice was found by [22].

The reducing sugars of pasteurized Hot-fill orange juice demonstrated in (Table 5). The treatments sequel on reducing sugars were non-significantly ($p>0.05$) whereas the storage ramifications were significant ($p<0.05$) on reducing sugar. [21] reported the conversion of non-reducing sugar to reducing sugar was impede by high temperature& Acid. Changes in acidity because of treatments were not effective (Table 6). No significant changes in reducing sugars were noted. Non-reducing sugars changes to reducing sugars in storage [23]. Reducing sugars of orange juice increases in storage [17]. Study found that storage affects the reducing sugars.

The status of non-reducing sugar after the application of treatments such as head space, processed water and Vitamin C fortification to pasteurized hot fill orange juice samples was allude to (Table 7). The treatments were left un-pretentious while the mean values of non-reducing sugars were significantly ($p<0.05$) decreased with storage. [21] Reported that due to acidity and high storage

temperature the non-reducing sugars are converted into reducing sugars. Non-reducing sugars was reduced in the juices [17]. It is found that during storage the reducing sugars are reduced in pasteurised Hot-fill orange juice.

The (Table 8) shows the effect of processed water, Head space and Vitamin C on flavor retention in pasteurized orange juice during storage. Retention of flavor is affected by treatments and storage significantly (P <0.05) in pasteurized orange juice. These assumptions are in close agreement with the results by [24], who reported reduction in organoleptic attributes of pasteurized orange juices, kept in transparent glass bottles.In pasteurized hot fill orange juice the fortification of Vitamin C and leaving no head space could be allude to positive correlated of flavor.

In (Table 9) signifies the stability of color of pasteurized orange juice due to treatments. Juice color was highly (p<0.05) effected by treatments and storage.

Color and viscosity of water melon juice which was processed with higher intensity pulse electric field and heat, major reduction in the color of water melon was detected [25]. This shows that no head space and Vitamin C fortification could be retained in the pasteurized orange juice.

Overall acceptability regularly reduced during storage for pasteurized orange juice samples as shown in (Table 10). During storage the overall acceptability mean scorewassignificantly (p<0.05) decreased direct effect on quality of juice might be due to oxygen caused the fairly less acceptability of the unprocessed sample PO0 compared to POU and POT [17]. The results of comparative effect of heat pasteurization to pulse electric field on the quality of orange juice, are in agreement with those of [14]. Addition of Vitamin C and no head space caused product microbiolly and physio-chemically accepted.

Microbial tests

In (Table 11) separation of *Alicyclobacillus* colonies with storage indicated an increase. During storage the colony forming unit was take note of a Significant (P < 0.05) increase. Parallel the bacterial development pompous significantly by treatments on the other hand. Due to sternly aerobic nature of *Alicyclobacillus* microbes the presence of oxygen in the head space of control sample PO0 might be perceive relatively more bacterial growth [26]. During storage the development of *Alicyclobacillus* in the pasteurized apple juice significantly pretentious by head space was divulge [27].

Table 2. The Effect of processed water, Vitamin C & head space on the pH of pasteurized orange sap at ambient temperature during storage

Treatments	Storage Period (Days)						% Inc	Means
	Initial	15	30	45	60	90		
POo	3.62	3.62	3.63	3.65	3.67	3.71	2.43	3.66a±0.03
POU	3.62	3.63	3.63	3.66	3.68	3.70	2.41	3.65a±0.03
POT	3.62	3.63	3.64	3.64	3.67	3.70	2.41	3.65a±0.02
POUC	3.60	3.62	3.65	3.67	3.68	3.69	2.39	3.65a±0.03
POTC	3.60	3.63	3.65	3.66	3.68	3.70	2.42	3.65a±0.03
Means	3.61a ±0.01	3.63a ±5.4	3.64a 0.01	3.66a 0.01	3.68a ±5.4	3.70a ±7.07		

Table 3. Effect of Head space, processed water and Vitamin C on the acidity of pasteurized orange juice at room temperature storage

Treatments	Storage Period (Days)						% Dec	Means
	Initial	15	30	45	60	90		
PO0	0.726	0.726	0.724	0.720	0.716	0.708	2.63	0.720a±7.04
POU	0.726	0.724	0.724	0.718	0.714	0.710	2.58	0.719a±6.40
POT	0.726	0.724	0.722	0.722	0.716	0.710	2.60	0.720a±5.93
POUC	0.730	0.726	0.720	0.716	0.714	0.712	2.65	0.719a±6.03
POTC	0.730	0.724	0.720	0.718	0.714	0.710	2.67	0.718a±6.02
Means	0.728a ±1.41	0.725a ±1.09	0.721a ±2	0.718a ±2.28	0.715a ±1.09	0.711a ±1.41		

Table 4. The Effect of processed water, Vitamin C &head space on the ascorbic acid of pasteurized orange sap at ambient temperature during storage

Treatments	Storage Period (Days)						% Dec	Means
	Initial	15	30	45	60	90		
POo	10.03	6.44	3.95	2.13	1	1	90	4.24b±3.57
POU	10.04	7.10	4.79	2.98	1.52	1	90	4.85b±3.49
POT	10.07	7.26	4.43	2.65	1.34	1	90	4.67b±3.58
POUC	17.91	16.45	15.07	14.21	13.59	12.10	32.89	14.96a±2.07
POTC	18.11	17.04	16.21	14.92	14.06	12.86	32.39	15.19a±1.95
Means	13.21a ±4.36	10.65b ±5.38	8.17c ±6.18	7.23cd ±6.57	6.46de ±6.87	5.49e ±6.29		

Table 5. Total Soluble Solids Scenario after the application of Vitamin C, Head space and processed water to the pasteurized orange juice

Treatments	Storage Period (Days)						% Increase	Means
	Initial	**15**	**30**	**45**	**60**	**90**		
PO0	11	11.1	11.1	11.3	11.3	11.4	3.07	11.20a±0.15
POU	11	11.1	11.2	11.3	11.4	11.4	3.07	11.19a±0.16
POT	11	11.1	11.2	11.2	11.3	11.3	2.35	11.18a±0.11
POUC	11	11	11.1	11.2	11.4	11.4	3.07	11.18a±0.18
POTC	11	11	11.2	11.3	11.3	11.3	2.35	11.18a±0.14
MEANS	11.00d ±0.00	11.06d ±0.05	11.16c ±0.05	11.26b ±0.05	11.34a ±0.05	11.36a ±0.05		

Table 6. The Effect of processed water, Vitamin C &head space on the reducing sugar of pasteurized orange sap at ambient temperature during storage

Treatments	Storage Period (Days)						% Inc	Means
	Initial	15	30	45	60	90		
PM0	4.5	4.6	4.7	4.9	5.1	5.5	40.00	4.88a±0.37
POU	4.6	4.6	4.7	4.0	5.1	5.3	26.92	4.88a±0.45
POT	4.5	4.6	4.8	4.0	5.1	5.4	36.63	4.88a±0.48
POUC	4.5	4.6	4.6	4.1	5.2	5.3	30.00	4.88a±0.45
POTC	4.4	4.6	4.7	4.9	5.3	5.5	45.83	4.90a±0.42
Means	4.50d ±0.07	4.60cd ±0.00	4.76c ±0.07	4.96b ±0.47	5.16ab ±0.08	5.40a ±0.10		

Table 7. The Effect of processed water, Vitamin C &head space on the non-reducing sugar of pasteurized orange sap at ambient temperature during storage

Treatments	Storage Period (Days)						% Dec	Means
	Initial	15	30	45	60	90		
PO0	5.3	5.1	5.0	4.7	4.5	4.2	17.46	4.75a±0.40
POU	5.4	5.2	5.9	4.6	4.4	4.3	17.18	4.78a±0.63
POT	5.2	5.1	5.0	4.7	4.4	4.3	16.51	4.78a±0.37
POUC	5.1	5.0	5.9	4.8	4.5	4.2	18.75	4.75a±0.58
POTC	5.2	5.1	5.9	4.7	4.5	4.2	17.46	4.77a±0.60
Means	5.26a ±0.11	5.13b ±0.07	5.95b ±0.49	4.70bc ±0.07	4.48c ±0.05	4.24c ±0.05		

Table 8. The Effect of processed water, Vitamin C & head space on the flavor of pasteurized orange sap at ambient temperature during storage

Treatments	Storage Period (Days)						% Dec	Means
	Initial	15	30	45	60	90		
POo	8	7.1	6.0	5.6	4.3	2.7	62.19	5.73b±1.91
POU	8	7.4	7.0	6.2	5.4	5.0	37.50	6.46b±1.17
POT	8	7.5	6.8	6.3	5.7	5.1	35.80	6.61b±1.09
POUC	8	8	7.9	7.8	7.7	7.7	6.20	7.78a±0.13
POTC	8	8	8	7.9	7.9	7.8	2.43	7.93a±0.08
Means	8.00a ±0.00	7.66ab ±0.39	7.14abc ±0.82	6.74bcd ±1.03	6.26cd ±1.55	5.82d ±2.43		

Table 9. The Effect of processed water, Vitamin C &head space on the color stability of pasteurized orange sap at ambient temperature during storage

Treatment	Storage intervals (Days)						% Dec	Means
	Initial	15	30	45	60	90		
PM0	8.2	7.5	6.5	5.6	3.1	2	62.19	5.73b±2.46
POU	8.2	7.6	7.2	6.5	5.8	5.3	37.50	6.61b±1.10
POT	8.2	7.8	7.3	6.4	5.9	5.1	35.00	6.46b±1.18
POUC	8.2	8.2	8	7.9	7.9	7.9	5.00	7.82a±0.14
POTC	8.2	8.2	8.1	8.1	8.0	8.0	3.75	7.88a±0.08
Means	8.00a ±0.00	7.66ab ±0.32	7.24abc ±0.65	6.74bcd ±1.06	6.26cd ±1.99	5.82d ±2.46		

Table 10. The results for overall acceptability of pasteurized orange juice samples

Treatment	Storage Intervals						% Dec	Means
	Initial	15	30	45	60	90		
POo	8.1	7.2	6.1	5.0	3.8	2.2	60.24	5.72d±2.19
POU	8.1	7.8	7.3	6.5	6.2	5.4	35.52	6.12c±1.03
POT	8.1	7.7	7.1	6.4	6	5.5	34.71	6.48b±1
POUC	8.1	8	7.9	7.7	7.7	7.6	5.22	7.85a±0.19
POTC	8.1	8.1	8	7.8	7.7	7.7	4.76	7.90a±0.18
Means	8.10a ±0.00	7.62b ±0.35	7.00c ±0.76	6.12cd ±1.14	5.90d ±1.60	5.63de ±2.23		

Table 11. The isolation of *Alicyclobacillus* colonies with storage per 10^{-2} ml

Treatment	Storage intervals (Days)						% Inc	Means
	Initial	15	30	45	60	90		
PM0	17	46	78	92	122	304	95.13	94.33a±109.8
POU	15	21	26	34	47	83	86.36	39.83b±24.83
POT	2	5	14	21	37	53	94.60	35.00b±19.69
POUC	19	23	31	35	41	48	64.44	34.66b±10.88
POTC	3	5	7	10	16	19	61.34	8.50b±6.32
Means	11.00b ±8.07	21.20b ±16.85	32.40b ±27.83	41.54b ±31.67	55.78ab ±40.51	101.18a ±115.51		

Conclusion

Careful observations revealed that best keeping qualities during room temperature storage maintained by samples POUC and POTC. While keeping in mind the initial count and the microbial development the sample POTC may have prolonged shelf life. The positive influence of head space and Vitamin C on the color, flavor and Vitamin C retention perceive in the samples while the control of *Alicyclobacillus* initial count only be done by processed water.

Authors' contributions

Conceived and designed the experiments: QA Sultan & S Wahab, Performed the experiments: QA Sultan, Analyzed the data: QA Sultan, Contributed reagents/ materials/ analysis tools: S Wahab, MN uddin, Y Durrani, A Muhammad, H Bilal & MU Din, Wrote the paper: QA Sultan.

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