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*Full Length Research Paper*

# Role of sodium benzoate as a chemical preservative in extending the shelf life of orange juice

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**A study was carried out to examine the effect of sodium-benzoate ($NaC_6H_5CO_2$) with different concentrations on orange juice packed in various popular packing materials for various time intervals of storage. The effect of sodium-benzoate on chemical compositions of orange juice like total sugars, reducing sugars, TSS and pH was highly significant ($P<0.01$). In case of non-reducing sugars, acidity and vitamin-C, the effect of sodium-benzoate was non-significant ($P>0.05$). On the basis of higher total sugars, reducing sugars, total soluble solids and lower acidity, the quality of preserved orange juice was better when sodium benzoate was added at a level of 1.0g/1000ml. Total sugars, reducing sugars and total soluble solids increased during storage. The quality of preserved orange juice packed in Amber bottles was relatively better than rest of the packing materials. Results revealed that fresh orange juice with sodium-benzoate without the additions of sugar could be useable up to 30 days. Sensory analysis showed that colour, flavour, taste and appearance of preserved orange juice remained excellent up to 30 days. The study concludes that Amber bottles may be preferred for orange juice and sodium benzoate may be applied at the rate of 1.0g/1000ml for prolonging shelf life of orange juice.**

**Keywords:** Orange juice, sodium benzoate and packaging materials

## INTRODUCTION

Orange (*Citrus sinensis)* is a hybrid of ancient cultivated origin. Oranges originated in Southeast Asia. The fruit of *Citrus sinensis* is called sweet orange to distinguish it from *Citrus aurantium*, the bitter orange (Muratore *et al.* 2005). Oranges are widely grown in warm climates worldwide and the flavors of oranges vary from sweet to sour. The fruit is commonly peeled and eaten fresh or squeezed for its juice (Crowell, 1999). In Pakistan, the production of citrus fruits during 2004-2005, 2005-2006, 2006-2007, 2007-2008 and 2008-2009 was 1843, 2458, 1472, 2294 and 2299 thousand tons respectively (GOP, 2010).

Fruit juices are becoming an important part of the modern diet in many communities. They are nutritious beverages and can play a significant part in a healthy diet because they offer good taste and a variety of nutrients found naturally in fruits. Juices are available in their natural concentrations or in processed forms. Juices are fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health (Franke *et al.* 2005). Orange juice is rich in vitamin C, an excellent source of bio-

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**Table 1.** Total sugars (%) in orange juice as influenced by various concentration of sodium benzoate and packing materials for various storage periods.

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh Juice | **3.7378a** | **3.7378a** | **3.7378a** | 3.7378 C |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 4.3700 | 4.3800 | 4.4633 | d 4.4044 |
| 0.6 g | 4.4500 | 4.3767 | 4.4533 | a 4.4267 |
| 0.8 g | 4.4167 | 4.2833 | 4.5800 | c 4.4267 |
| 1.0 g | 4.4600 | 4.4500 | 4.4733 | b 4.4611 |
| Mean | a 4.4242 | a 4.3725 | a 4.4925 | 4.4297 B |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 4.3767 | 4.4667 | 5.3433 | a 4.7522 |
| 0.6 g | 4.5200 | 4.2733 | 5.0867 | a 4.6267 |
| 0.8 g | 4.1733 | 4.1767 | 5.1167 | b 4.4889 |
| 1.0 g | 5.0467 | 4.2567 | 4.4367 | a 4.5800 |
| Mean | b 4.5217 | c 4.3183 | a 4.9958 | **4.6120 A** |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 2.4233 | 2.6900 | 3.4867 | a 2.8667 |
| 0.6 g | 2.4533 | 2.3867 | 3.3600 | a 2.7333 |
| 0.8 g | 2.2467 | 1.9230 | 2.4667 | b 2.2121 |
| 1.0 g | 2.6633 | 3.4367 | 2.3033 | a 2.8011 |
| Mean | c 2.4467 | b 2.6091 | a 2.9042 | **2.6533 D** |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage period** | |
| **S.E.** | 0.0825 | 0.0952 | 0.0952 | |
| **LSD 0.05** | 0.1634 | 0.1887 | 0.1887 | |
| **LSD 0.01** | 0.2161 | 0.2496 | 0.2496 | |

**Table 2.** Reducing sugars (%) in orange juice as influenced by various concentration of sodium benzoate and packing materials for various storage periods.

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | 2.34a | 2.34a | 2.34a | 2.3400 **C** |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 2.8067 | 2.8667 | 2.9033 | a 2.8589 |
| 0.6 g | 2.9133 | 2.9100 | 2.9100 | a 2.9111 |
| 0.8 g | 2.9100 | 2.8733 | 2.9067 | a 2.8967 |
| 1.0 g | 2.9000 | 2.8967 | 2.9167 | a 2.9044 |
| Mean | a 2.8825 | a 2.8867 | a 2.9092 | 2.8928 **B** |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 3.1433 | 3.3400 | 3.6067 | a 3.3633 |
| 0.6 g | 3.2467 | 3.1433 | 3.5267 | a 3.3056 |
| 0.8 g | 3.1233 | 2.7533 | 3.4200 | b 3.0989 |
| 1.0 g | 3.4267 | 3.1267 | 3.2967 | a 3.2833 |
| Mean | b 3.2350 | c 3.0908 | a 3.4625 | 3.2628 **A** |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 1.2167 | 1.4533 | 2.2167 | a 1.6289 |
| 0.6 g | 1.2433 | 1.2367 | 2.1867 | a 1.5556 |
| 0.8 g | 1.2033 | 1.2867 | 1.2200 | b 1.2367 |
| 1.0 g | 1.4367 | 2.2433 | 1.2400 | a 1.6400 |
| Mean | c 1.2750 | b 1.5550 | a 1.7158 | 1.5153 **D** |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage period** | |
| **S.E.** | 0.0735 | 0.0849 | 0.0849 | |
| **LSD 0.05** | 1.1456 | 0.1682 | 0.1682 | |
| **LSD 0.01** | 0.1926 | 0.2224 | 0.2224 | |

available antioxidant phytochemicals (Franke *et al.* 2005) and significantly improves blood lipid profiles in people affected by hyper-cholesterolemia (Kurowska *et al.* 2000). Orange juice is one of the commodities widely

**Table 3.** Non-reducing sugars (%) in orange juice as influenced by various concentration of sodium benzoate and packing materials for various storage periods.

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | 1.4156 | 1.4156 | 1.4156 | 1.4156 B |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 1.5167 | 1.5200 | 1.5300 | 1.5222 |
| 0.6 g | 1.5133 | 1.5133 | 1.5167 | 1.5144 |
| 0.8 g | 1.5233 | 1.5100 | 1.5300 | 1.5211 |
| 1.0 g | 1.5100 | 1.5033 | 1.5100 | 1.5078 |
| Mean | 1.5158 | 1.5117 | 1.5217 | 1.5164 A |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 1.2633 | 1.4367 | 1.7400 | 1.4800 |
| 0.6 g | 1.2633 | 1.1300 | 1.5767 | 1.3233 |
| 0.8 g | 1.3200 | 1.4233 | 1.6967 | 1.4800 |
| 1.0 g | 1.6300 | 1.1300 | 1.1400 | 1.3000 |
| Mean | 1.3692 | 1.2800 | 1.5983 | 1.3958 B |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 1.2067 | 1.2367 | 1.2700 | 1.2378 |
| 0.6 g | 1.1800 | 1.500 | 1.1733 | 1.1678 |
| 0.8 g | 1.1633 | 1.1700 | 1.2467 | 1.1933 |
| 1.0 g | 1.2267 | 1.1933 | 1.1833 | 1.2011 |
| Mean | 1.1942 | 1.875 | 1.2183 | 1.2000 C |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 0.0301 | 0.0347 | 0.0347 | |
| **LSD 0.05** | NS | NS | 0.0688 | |
| **LSD 0.01** | NS | NS | 0.0910 | |

**Table 4.** Total acidity (%) in orange juice as influenced by various concentration sodium benzoate concentrations and packing materials for various storage periods

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | 0.2336 | 0.2336 | 0.2336 | 0.2336 B |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 0.1768 | 0.1733 | 0.2067 | 0.1889 |
| 0.6 g | 0.3067 | 0.1833 | 0.2300 | 0.2400 |
| 0.8 g | 0.2167 | 0.1367 | 0.1900 | 0.1811 |
| 1.0 g | 0.1300 | 0.1900 | 0.1833 | 0.1678 |
| Mean | 0.2100 | 0.1708 | 0.2025 | 0.1945 C |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 0.1733 | 0.1733 | 0.1700 | 0.1722 |
| 0.6 g | 0.1733 | 0.1733 | 0.1767 | 0.1744 |
| 0.8 g | 0.1700 | 0.1767 | 0.1733 | 0.1733 |
| 1.0 g | 0.1700 | 0.1767 | 0.1733 | 0.1733 |
| Mean | 0.1717 | 0.1750 | 0.1733 | 0.1733 D |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 0.4267 | 0.3167 | 0.2767 | 0.3400 |
| 0.6 g | 0.1700 | 0.3300 | 0.2667 | 0.2556 |
| 0.8 g | 0.2400 | 0.2200 | 0.3267 | 0.2622 |
| 1.0 g | 0.2133 | 0.2767 | 0.2533 | 0.2478 |
| Mean | 0.2625 | 0.2858 | 0.2808 | 0.2764 A |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 0.0138 | 0.0160 | 0.0160 | |
| **LSD 0.05** | NS | NS | 0.0317 | |
| **LSD 0.01** | NS | NS | 0.0419 | |

used in almost all countries of the world. This is mainly done in the home and in industry on much larger scale.

Frozen orange juice concentrate is made from freshly squeezed and filtered orange juice (Wilson, 2008). Fruit

**Table 5.** Vitamin-C (mg/kg) in orange juice as influenced by various concentration of sodium benzoate concentrations and packing materials for various storage periods

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | 67.758a | 67.758a | 67.758a | **67.758 A** |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 61.150 | 43.467 | 52.627 | 52.081 |
| 0.6 g | 45.123 | 48.147 | 41.020 | 44.763 |
| 0.8 g | 41.870 | 54.367 | 56.470 | 50.902 |
| 1.0 g | 56.310 | 44.350 | 57.610 | 52.757 |
| Mean | a 50.863 | b 47.582 | a 51.932 | **50.126 B** |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 18.833 | 36.033 | 51.353 | 35.407 |
| 0.6 g | 18.833 | 33.623 | 29.873 | 27.443 |
| 0.8 g | 19.784 | 29.750 | 28.567 | 26.134 |
| 1.0 g | 27.650 | 28.867 | 37.733 | 31.417 |
| Mean | c 21.275 | b 32.068 | a 36.957 | **30.100 C** |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 11.220 | 11.290 | 7.300 | 9.937 |
| 0.6 g | 15.110 | 11.317 | 7.557 | 11.328 |
| 0.8 g | 7.500 | 11.430 | 11.383 | 10.104 |
| 1.0 g | 15.343 | 11.327 | 15.037 | 13.902 |
| Mean | a 12.293 | a 11.341 | a 10.319 | **11.318 D** |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 1.4737 | 1.7013 | 1.7013 | |
| **LSD 0.05** | 2.9193 | NS | 3.3709 | |
| **LSD 0.01** | 3.8609 | NS | 4.4582 | |

**Table 6.** pH of orange juice as influenced by various concentration of sodium benzoate concentrations and packing materials for various storage periods

| **Sodium benzoate concentration** | **Packing materials** | | | **Mean** |
|---|---|---|---|---|
| | **Glass bottle** | **Pet bottle** | **Amber bottle** | |
| Fresh juice | 4.3417 | 4.3417 | 4.3417 | **4.3417 A** |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 3.7267 | 3.5967 | 3.6300 | c 3.6511 |
| 0.6 g | 4.1933 | 4.3400 | 4.4533 | a 4.3289 |
| 0.8 g | 4.1367 | 4.1267 | 3.87233 | b 4.0289 |
| 1.0 g | 4.2400 | 4.1367 | 4.1567 | a 4.1778 |
| Mean | 4.0742 | 4.0500 | 4.0158 | **4.0467 B** |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 3.8900 | 3.4567 | 3.7967 | b 3.7144 |
| 0.6 g | 4.1900 | 4.4667 | 4.2400 | a 4.2989 |
| 0.8 g | 3.5667 | 3.6967 | 4.4100 | b 3.8911 |
| 1.0 g | 4.3200 | 4.1700 | 4.3267 | a 4.2722 |
| Mean | 3.9917 | 3.9475 | 4.1933 | **4.0442 B** |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 3.7533 | 3.8500 | 3.5833 | b 3.7289 |
| 0.6 g | 4.1700 | 4.1967 | 4.1800 | a 4.1822 |
| 0.8 g | 3.3900 | 3.6500 | 4.1667 | b 3.7356 |
| 1.0 g | 3.8937 | 4.15.33 | 4.2200 | a 4.0900 |
| Mean | 3.8025 | 3.9625 | 4.0375 | **3.9342 B** |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 0.1041 | 0.1202 | 0.1202 | |
| **LSD 0.05** | NS | 0.2381 | 0.2381 | |
| **LSD 0.01** | NS | 0.3150 | 0.3150 | |

**Table 7.** TSS in orange juice as influenced by various concentration of sodium benzoate and packing materials for various storage periods.

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | 10.104 | 10.104 | 10.104 | 10.104 B |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 11.840 | 8.730 | 10.547 | b 10.372 |
| 0.6 g | 11127 | 11.217 | 11.527 | a 11.200 |
| 0.8 g | 11.150 | 11.133 | 11.297 | a 11.193 |
| 1.0 g | 11.153 | 11.360 | 11.820 | a 11.444 |
| Mean | 11.317 | 10.610 | 11.230 | 11.052 A |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 11.267 | 11.200 | 11.233 | a 11.233 |
| 0.6 g | 11.233 | 11.200 | 11.233 | a 11.222 |
| 0.8 g | 11.233 | 11.133 | 11.167 | a 11.178 |
| 1.0 g | 11.133 | 11.233 | 11.167 | a 11.178 |
| Mean | 11.217 | 11.192 | 11.200 | 11.203 A |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 5.427 | 5.293 | 5.430 | c 5.383 |
| 0.6 g | 11.227 | 11.840 | 11.156 | a 11.406 |
| 0.8 g | 11.130 | 10.697 | 10.350 | b 10.726 |
| 1.0 g | 11.530 | 11.657 | 11.223 | a 11.470 |
| Mean | 9.8283 | 9.8717 | 9.5383 | 9.746 B |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 0.2046 | 0.2363 | 0.2363 | |
| **LSD 0.05** | NS | 0.4682 | 0.4682 | |
| **LSD 0.01** | NS | 0.6192 | 0.6192 | |

juices promote detoxification in the human body (Deanna and Jeffrey, 2007). The constituents of processed juices are mainly water, sugar, chemicals preservatives, colours and fruit pulp. The most commonly used preservatives are benzoic acid, sodium benzoate, potassium metabisulphit, sorbic acid and sulphur dioxide. Acid is an essential universal constituent of juice and the most commonly used acid is citric acid.

Orange juice is valuable in terms of nutritional qualities. 100g edible portion of orange juice contains Energy (46 kcal), Carbohydrates (11.54 g), Sugars (9.14g), Dietary fiber (2.4g), Fat (0.21g), Protein (0.70g), Thiamine Vit. B1 (0.100mg), Riboflavin Vit. B2 (0.040mg), Niacin Vit. B3 (0.400mg), Pantothenic acid B5 (0.250mg), Vitamin B6 (0.051mg) Folate Vit. B9 (17µg), Vitamin C (45mg), Calcium (43mg), Iron (0.09mg), Magnesium (10mg), Phosphorus (12mg), Potassium (169mg) and Zinc (0.08mg) (USDA, 2010). Fruit juices are available in essentially the same form almost anywhere in the world. From polar bases to the tropics and from the largest developed countries, fruit juices are available in bottles, cans, laminated paper packs, pouches, cups and almost all forms of packaging. In recent years these juices have been included significantly in the diet of most people, irrespective of age. Therefore, maintaining the quality of processed fruit juices is an important concern (Mathooko and Njiru, 1994). In order to facilitate preservation and distribution, it is a technological practice to package juices in metal cans, glass bottles or plastic containers. Although, metal cans are expensive and require sophisticated machinery for container closure (Griffin *et al.* 1985), they have been shown to effect ascorbic acid retention better than other packaging materials (Maeda and Mussa, 1986). However, packaging alone cannot preserve the quality of juice (Mathooko and Njiru, 1994) and therefore juices are treated with chemical preservatives.

Preservation of fruit juices have become the business activity of great significance and countries with abundant fruit resources, and having short harvest season are emphasizing more for established storage to maintain quality of fruits, increase shelf life and preserve fruit juices for off-season use (Franke *et al.* 2005). Preservation of fruit juices with chemicals mainly to prevent microbial spoilage during storage, both in the retail stores and consumer homes is in common practice (El-Gindy and Shehata, 1974). Sulfites and metabisulfites of sodium or potassium are added to fruit juices as potential sources of sulfur dioxide, which acts as an antimicrobial agent and also stabilizes ascorbic acid (El-Ashwah *et al.* 1981). Moreover, benzoic acid in various forms is also used in fruit juices and other acid products

**Table 8.** Sensory score for colour of orange juice added with various levels of sodium benzoate and packed in different types of bottles at different storage periods

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | a 6.70 | a 6.70 | a 6.70 | 6.70 A |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 5.20 | 5.40 | 5.80 | d 5.47 |
| 0.6 g | 6.24 | 6.32 | 6.66 | c 6.41 |
| 0.8 g | 6.80 | 6.89 | 7.19 | b 6.96 |
| 1.0 g | 7.41 | 7.51 | 7.84 | a 7.59 |
| Mean | b 6.41 | b 6.53 | a 6.87 | 6.61 A |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 4.89 | 5.08 | 5.45 | d 5.14 |
| 0.6 g | 5.87 | 5.94 | 6.26 | c 6.02 |
| 0.8 g | 6.39 | 6.48 | 6.76 | b 6.54 |
| 1.0 g | 6.97 | 7.06 | 7.37 | a 7.13 |
| Mean | b 6.03 | b 6.14 | a 6.46 | 6.21 B |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 2.44 | 2.54 | 2.73 | d 2.57 |
| 0.6 g | 2.93 | 2.97 | 3.13 | c 3.01 |
| 0.8 g | 3.20 | 3.24 | 3.38 | b 3.27 |
| 1.0 g | 3.48 | 3.53 | 3.68 | a 3.57 |
| Mean | b 3.01 | b 3.07 | a 3.23 | 3.10 C |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| S.E. | 0.0083 | 0.0050 | 0.0050 | |
| LSD 0.05 | 0.0165 | 0.0190 | 0.0190 | |
| LSD 0.01 | 0.0226 | 0.0261 | 0.0261 | |

**Table 9**. Sensory score for flavour of orange juice added with various levels of Sodium benzoate and packed in different types of bottles at different storage periods

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | a 7.20 | a 7.20 | a 7.20 | 7.20 A |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 5.45 | 5.71 | 5.98 | d 5.71 |
| 0.6 g | 6.54 | 6.32 | 6.66 | c 6.51 |
| 0.8 g | 7.13 | 6.89 | 7.19 | b 7.07 |
| 1.0 g | 7.77 | 7.51 | 7.84 | a 7.71 |
| Mean | c 6.72 | b 6.61 | a 6.92 | 6.75 B |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 5.12 | 5.37 | 5.62 | d 5.37 |
| 0.6 g | 6.15 | 5.94 | 6.26 | c 6.12 |
| 0.8 g | 6.70 | 6.48 | 6.76 | b 6.65 |
| 1.0 g | 7.30 | 7.06 | 7.37 | a 7.24 |
| Mean | c 6.32 | b 6.21 | a 6.50 | 6.34 C |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 2.56 | 2.68 | 2.81 | d 2.69 |
| 0.6 g | 3.07 | 2.97 | 3.13 | c 3.06 |
| 0.8 g | 3.35 | 3.24 | 3.38 | b 3.32 |
| 1.0 g | 3.65 | 3.53 | 3.68 | a 3.62 |
| Mean | b 3.16 | b 3.11 | a 3.25 | 3.17 D |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| S.E. | 0.0228 | 0.0264 | 0.0264 | |
| LSD 0.05 | 0.0480 | 0.0554 | 0.0554 | |
| LSD 0.01 | 0.0657 | 0.0759 | 0.0759 | |

for its antimicrobial activity (Mathooko and Njiru, 1994). Fruit juices are preserved with chemicals mainly to prevent microbial spoilage during storage, both in the retail stores and consumer homes. Some of these chemicals are thought to have protective effects on ascorbic acid. While a large number of chemicals with preservative effects have been described, only a relatively small number is allowed for direct use in human

**Table 10.** Sensory score for taste of orange juice added with various levels of Sodium benzoate and packed in different types of bottles at different storage periods

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| Fresh juice | a 7.70 | a 7.70 | a 7.70 | 7.70 A |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 5.88 | 4.88 | 6.41 | d 5.72 |
| 0.6 g | 7.06 | 6.32 | 6.66 | c 6.68 |
| 0.8 g | 7.69 | 6.89 | 7.19 | b 7.26 |
| 1.0 g | 8.38 | 7.51 | 7.84 | a 7.91 |
| Mean | a 7.25 | c 6.40 | b 7.03 | 6.89 B |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 5.53 | 4.59 | 6.03 | 5.38 |
| 0.6 g | 6.63 | 5.94 | 6.26 | 6.28 |
| 0.8 g | 7.23 | 6.48 | 6.76 | 6.82 |
| 1.0 g | 7.88 | 7.06 | 7.37 | 7.44 |
| Mean | a 6.82 | b 6.02 | c 6.60 | 6.48 C |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 2.76 | 2.29 | 3.01 | d 2.69 |
| 0.6 g | 3.32 | 2.97 | 3.13 | c 3.14 |
| 0.8 g | 3.61 | 3.24 | 3.38 | b 3.41 |
| 1.0 g | 3.94 | 3.53 | 3.68 | a 3.72 |
| Mean | a 3.41 | b 3.01 | a 3.30 | 3.24 D |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 0.0537 | 0.0620 | 0.0620 | |
| **LSD 0.05** | 0.1128 | 0.1302 | 0.1302 | |
| **LSD 0.01** | 0.1545 | 0.1785 | 0.1785 | |

food. Moreover, even use of these chemicals is regulated by the maximum allowable effective levels (El-Gindy and Shehata, 1974). Sulfites and metabisulfites of sodium or potassium are added to fruit juices as potential sources of sulfur dioxide, which acts as an antimicrobial agent and also stabilizes ascorbic acid. Use of metabisulfite providing up to 400 ppm of sulfur dioxide in orange juice has been reported. However, such high levels of sulfur dioxide are likely to impart a characteristic pungent smell to fruit juices. The action of sulphur dioxide as an antimicrobial agent as well as a stabilizer of ascorbic acid depends on the pH of the food (El-Ashwah *et al.* 1981). It is therefore, important to test its efficacy in a high acid juice such as orange and lime juice. On the other hand, benzoic acid is also used in fruit juices and other acid products for its antimicrobial activity. Since the two chemicals exert either one or both of synergistic or concerted actions, it is possible that benzoic acid makes a significant contribution to protection of ascorbic acid (Mathooko and Njiru, 1994). This study was, therefore, designed to investigate the role of sodium benzoate as a chemical preservative in extending the storage/shelf life of orange juice.

## MATERIAL AND METHODS

Studies were carried out on the role of sodium-benzoate as chemical preservative in extending the storage/shelf life of orange juice. Fresh orange fruit was obtained from the local market and brought to the laboratory of the Institute of Food Sciences and Technology, Sindh Agriculture University Tandojam. The juice was extracted from oranges as per the standard methods of extraction. After performing the preservation methods of orange juice, the following levels of Sodium Benzoate and packing materials were applied.

### Sodium Benzoate levels

$P_1$ Control
$P_2$ 0.60g/litre orange juice
$P_3$ 0.80g/litre orange juice
$P_4$ 1.00g/litre orange juice

### Packing materials

$M_1$ Glass bottle
$M_2$ Pet bottle
$M_3$ Amber bottle

The preserved orange juice was packed in various types of packaging materials such as transparent glass bottles, pet bottles and amber glass bottles and kept at room temperature (22±3$^{o}$C). The experiment was replicated in triplicate for each treatment to adjust any uneven

**Table 11.** Sensory score for appearance of orange juice added with various levels of Sodium benzoate and packed in different types of bottles at different storage periods

| Sodium benzoate concentration | Packing materials | | | Mean |
|---|---|---|---|---|
| | Glass bottle | Pet bottle | Amber bottle | |
| **FRESH JUICE** | | | | |
| Fresh juice | a 7.10 | a 7.10 | a 7.10 | 7.10 A |
| **AFTER 15 DAYS STORAGE** | | | | |
| Control | 4.78 | 5.58 | 6.00 | d 5.45 |
| 0.6 g | 5.74 | 6.32 | 6.66 | c 6.24 |
| 0.8 g | 6.25 | 6.89 | 7.19 | b 6.78 |
| 1.0 g | 6.81 | 7.51 | 7.84 | a 7.39 |
| Mean | b 5.90 | c 6.57 | a 6.92 | 6.46 B |
| **AFTER 30 DAYS STORAGE** | | | | |
| Control | 4.49 | 5.25 | 5.64 | d 5.13 |
| 0.6 g | 5.39 | 5.94 | 6.26 | c 5.86 |
| 0.8 g | 5.88 | 6.48 | 6.76 | b 6.37 |
| 1.0 g | 6.41 | 7.06 | 7.37 | a 6.94 |
| Mean | c 5.54 | b 6.18 | a 6.51 | 6.08 C |
| **AFTER 45 DAYS STORAGE** | | | | |
| Control | 2.25 | 2.62 | 2.82 | d 2.56 |
| 0.6 g | 2.70 | 2.97 | 3.13 | c 2.93 |
| 0.8 g | 2.94 | 3.24 | 3.38 | b 3.19 |
| 1.0 g | 3.20 | 3.53 | 3.68 | a 3.47 |
| Mean | c 2.77 | b 3.09 | a 3.25 | 3.04 D |
| **Analysis of variance** | **Packing materials** | **Preservatives** | **Storage periods** | |
| **S.E.** | 0.0118 | 0.0136 | 0.0136 | |
| **LSD 0.05** | 0.0247 | 0.0285 | 0.0285 | |
| **LSD 0.01** | 0.0339 | 0.0391 | 0.0391 | |

variation for chemical properties. After every two weeks, the samples of each bottle/treatment were taken for chemical and sensorial analysis. The chemical compositions were total sugars, reducing sugars, non-reducing sugars, total acidity Vitamin C and pH.

Whereas, sensorial parameters were general appearance, color, flavour and tastes were.

## Methods of Determination

### Determination of sugars

Sugars (total sugar, reducing sugar and non-reducing sugar) were carried out through Lane and Eynon Method as described by James (1995).

### Total sugars

Five g of orange sample was taken into a beaker and 100ml of warm water was added. The solution was stirred until all the soluble matters were dissolved and filtered through Whatman filter paper into a 250 volumetric flask. Pipetted 100 ml of the solution prepared into a conical flask added 10 ml diluted HCl and boiled for 5 minutes. On cooling, the solution was neutralized to phenolphthalein with 10% NaOH and make up to 250 ml in volumetric flask. This condition was used for titration against Fehling's solution and reading was calculated as follow:

$$\% \text{ total sugar} = \frac{\text{Factor (4.95) x dilution (250) x 2.5}}{\text{Titer x wt. of sample x 10}}$$

### Reducing sugars

Five grams of sample was taken into a beaker and 100 ml of warm water was added. The solution was stirred until all the soluble matters were dissolved and filtered through Whatman filter paper up to 250 ml in volumetric flask. Pipetted 100 ml of the solution into a conical flask and 10 ml diluted HCl was added and boiled for five minutes. After cooling, the solution was neutralized with phenolphthalein with 10% NaOH and make up to volume in a 250 ml volumetric flask. This solution was used for titration against Fehling's solution and reading was calculated as follow:

$$\% \text{ Reducing sugar} = \frac{\text{Factor (49.5) x dilution (250)}}{\text{Titer x wt of sample x 10}}$$

Non-reducing sugars: were estimated as the difference between the total sugar content and reducing sugar content on subtraction (total sugar-reducing sugar).

**Determination of total titratable acidity**

Total titratable acidity was determined according to the method of AOAC (2000). Each sample of the products was treated with 0.1N NaOH stock solution (reagent) using titration kit; where phenolphthalein 2-3 drops were used as an indicator. The acidity was determined in terms of malic acid. The volume of alkali used was noted and calculated using following formula.

$$\text{\% Titrable acidity} = \frac{1/10 \times \text{equiv. wt. of acid} \times \text{normality of NaOH} \times \text{titre}}{10}$$

**Vitamin-C**

Vitamin C (ascorbic acid) was determined by titration method as described by Mazumdar and Majumder (2003). 10 gram of sample was mixed with distilled water for 10 minutes and filtered through Whatman filter paper # 4. The 10 ml sample (fruit juice) was taken in 250 ml conical flash and 15 ml 21% oxalic acid was added. The sample was titrated with 2% dichlorophenol indo phenol till pink colour appeared. The results were calculated as per following formula and expressed in mg/100 grams fresh weight.

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre} \times \text{Dye factors} \times \text{Volume made up}}{\text{Volume of filtrate taken} \times \text{volume of sample}} \times 100$$

**pH**

The pH meter was calibrated with buffer solutions at pH 4 and 10 and electrode was dried and then inserted in orange juice solution. When the first reading was completed, the electrode was rinsed with distilled water and dried up with tissue paper; accordingly.

**Total soluble solids**

The total soluble solids (TSS) were determined as per method described by Mazumdar and Majumdar (2003) using Digital Bench Refrectometer. Before use, the instrument was cleaned and adjusted to zero at $20^0$C using distilled water. An appropriate quantity of sample of each product was placed on the prism-plate of the refrectometer with the help of a glass rod and folding back the cover. For each sample, the instrument was calibrated by using distilled water. The reading appeared on the screen was directly recorded as total soluble solids (Brix).

**Sensory analysis**

Panel of ten judges was selected from the postgraduate students and teaching staff of the Institute of Food Sciences and Technology who were supposed to be organoleptically familiar with beverages assessment. Sensory evaluation (colour, flavour, texture, taste) of preserved orange juice was carried out using a 9 point hedonic scale. The hedonic scale was ranked as; like extremely to very much (8-9 scores), like moderately to like slightly (5-7 scores), neither like nor dislike to dislike slightly-dislike moderately (2-4 scores) and dislike very much to dislike extremely (0-1 score).

**Statistical analysis**

The data were analyzed using SAS procedures (SAS, 2005). The analysis of variance (ANOVA) tables were constructed using the GLM (General Linear Model) procedure. The mean separations were carried out by the least significant difference (LSD) method at 1 and 5% significance level.

## RESULTS AND DISCUSSIONS

### Results

**Total sugars**

The effect of sodium benzoate at various concentrations and packing materials of total sugars of orange juice was investigated at 0, 15, 30 and 45 days after storage. The results are presented in Table-1. The analysis of variance suggested that total sugars of juices were significantly affected by various concentration of sodium benzoate and also storage period was also affected ($P<0.01$).

**Reducing sugars**

The reducing sugars (%) in orange juice as affected by preservative (sodium benzoate) at various concentrations and packing materials were showed at 0, 15, 30 and 45 days after storage. The results are depicted in Table-2.The analysis of variance indicated that the effect of sodium benzoate concentrations, packing materials and the storage periods on reducing sugars was significant ($P<0.01$).

### Non-reducing sugars

The non-reducing sugars in orange juice as affected by various concentrations of Sodium Benzoate and packing bottles were examined at 0, 15, 30 and 45 days after storage. The data to this aspect are shown in Table-3. The analysis of variance illustrated that the effect of Sodium Benzoate concentrations and packing bottles on non-reducing sugars in preserved orange juice were non-significant (P>0.05) and significant (P<0.01) due to storage periods.

### Total acidity

The total acidity in orange juice as affected by different Sodium Benzoate concentrations and packing bottles was determined in the fresh juice and preserved juice after 15, 30 and 45 days of storage. The results on this characteristic of orange juice are presented in Table-4. The analysis of variance demonstrated that the effect of various Sodium Benzoate concentrations and packing bottles on total acidity in orange juice was statistically non-significant (P>0.05) and significant (P<0.01) due to storage periods.

### Vitamin C

The effect of Sodium Benzoate at various concentrations and packing materials on the vitamin-C in the preserved orange juice was examined at 0, 15, 30 and 45 days after storage. The data in relation to vitamin-C in orange juice are shown in Table-5. The analysis of variance indicated that the effect of various Sodium Benzoate concentrations on Vitamin-C in orange juice was statistically non-significant (P>0.05) and significant (P<0.01) for packing bottles and storage periods.

### pH

The pH of fresh as well as preserved orange juice was examined to investigate the effect of Sodium Benzoate levels, packing materials as well as storage periods. The data in relation to pH of fresh and processed orange juice are presented in Table-6. The analysis of variance showed that the effect of various packing bottles on pH of orange juice was statistically non-significant (P>0.05), while Sodium Benzoate concentrations and storage periods influenced the juice pH significantly (P<0.01).

### Total Soluble Solids (TSS)

The orange juice was processed and the effect of preservative (Sodium Benzoate) at various levels, packing materials as well as storage periods was investigated. The results pertaining to TSS in fresh and processed orange juice are presented in Table-7. The analysis of variance suggested that the effect of various packing bottles on TSS of preserved orange juice was statistically non-significant (P>0.05), while Sodium Benzoate levels and storage periods influenced the TSS in preserve orange juice significantly (P<0.01).

## SENSORY ANALYSIS

### Colour

The orange juice was presented to panel of judges to offer score for liking of the preserved juice for assessment of the effect of Sodium Benzoate levels, packing materials and storage periods. The score offered by the judges is presented in Table-8. The analysis of variance showed that sensory quality of preserved orange juice differed significantly (P<0.01) for packing materials, Sodium Benzoate levels as well as storage periods.

### Flavour

The effect of preservative (Sodium Benzoate) at various levels and packing materials on flavour of preserved orange juice was assessed as fresh, 15, 30 and 45 days after storage according to the 9 points hedonic scale by a panel of judges; and the results are given in Table-9. The analysis of variance showed that flavour of preserved orange juice received significantly different score for packing materials, Sodium Benzoate levels as well as storage periods (P<0.01).

### Taste

The taste of orange juice preserved by adding Sodium Benzoate at various levels and packed in different types of bottles was examined at 15, 30 and 45 days after storage and compared with fresh juice samples; and scores were awarded by the judges according to the 9 points hedonic scale. The results (Table-10) and analysis of suggested that the differences in score of judges for taste of juice was significantly different (P<0.01) for packing materials, Sodium Benzoate levels and storage periods.

### Appearance

The orange juice was analysed by the judges for its appearance using 9 points hedonic scale and awarded score on samples preserved by different levels of Sodium Benzoate and various packing materials. The results finalized are presented in Table-11. It was observed that there was significant ($P<0.01$) variation in the opinion of the judges for packing materials, Sodium Benzoate levels and storage periods.

## DISCUSSIONS

The findings of the present study showed that the optimum storage period for orange juice remained 30 days after preservation. The preserved orange juice at optimum storage (30days) under $NaC_6H_5CO_2$ levels of 0, 0.6, 0.8 and 1.0 g had higher total sugars i.e. 4.7522, 4.6267, 4.4889, 4.5800%; higher reducing sugars 3.3633, 3.3056, 3.0989 and 3.2833%; Non-reducing sugars 1.4800, 1.3233, 1.4800 and 1.3000%; higher total soluble solids 11.233, 11.222, 11.178 and 11.178 % and lower acidity 0.1722, 0.1744, 1.1733 and 0.1733%, respectively; while Vitamin-C and juice pH was highest in the fresh orange juice. The total sugars in fresh orange juice (control) was 3.7378 percent which increased to 4.4297 percent after 15 days of storage, and total sugars reached to 4.612 percent after 30 days of storage of preserved orange juice. However, after 45 days of storage, the total sugars were reduced to 2.6533 percent, indicating deterioration occurred in the processed juice. The results showed that the total sugars in preserved orange juice increased significantly ($P<0.05$) with increase in the storage period. The preservation of fresh orange juice with Sodium Benzoate (without sugar) may be useable up to 30 days of storage and later bacterial growth was observed. However, the total sugars were almost similar in orange juice after 15 and 30 days of storage. The interactive effect showed that the total sugars were higher i.e. 5.3433, 5.1167 and 5.0867 percent in Amber bottles after 30 days storage at 0, 0.6 and 0.8g Sodium Benzoate concentrations respectively and lowest total sugars of 1.9230 percent in Pet bottle after 45 days of storage 0.8g Sodium Benzoate concentration. Results shows that Amber bottles could retain good quality composition during storage.

These results are confirmed by the findings of Polydera *et al.* (2003), they observed that ascorbic acid decreased whereas, sugars and TSS increased with preservation of orange juice. Tawfik and Huyghebaert (1998) reported that ascorbic acid content and rate of browning were significantly affected by the level of preservatives in the preserved juice. Similarly, Bahadur *et al.* (2006) reported TSS (17.47%), total sugar (11.83%), vitamin C (105 mg/100 g), pH (2.95), reducing sugar (8.17%) and acidity (0.88%) in citrus juice treated with 750 ppm of KMS. The variations observed in present study might be associated with the varieties of oranges used. Mathooko and Kiniiya (2002) reported that sodium benzoate concentration from 150 ppm to 300 ppm had significant increase in ascorbic acid stability. The study showed that preservation of fresh orange juice with Sodium Benzoate (without sugar) was useable up to 30 days of storage and later bacterial growth was experienced and suggested that amber bottles may be used for packing preserved orange juice; and Sodium Benzoate may be applied at the levels of 1.0 g; while under these packing and preservative level, the orange juice may be used up to 30 days after storage. In another study, Jalil *et al.* (2004) reported that ascorbic acid content decreased in all the samples during storage. Decrease trend was observed in acidity which was (0.516%); pH 4.01, TSS 3.33% with the increase of storage period. Shamsudin *et al.* (2007) reported TSS increased with storage of preserved juice; while pH of juice ranged from 2.77 to 2.90. The TSS and acid ratio of juice range was 12.32 to 18.60. Hussain *et al.* (2008) analyzed for physiochemical properties (ascorbic acid, acidity, pH reducing and non-reducing sugars) in preserved orange juice and found ascorbic acid (4.94), acidity (0.48), pH (4.00), reducing sugar (6.96) and non-reducing sugar (2.83) all the samples remain acceptable during storage period. Helali *et al.* (2007) analysed chemical composition, keeping quality and consumer's acceptability of the product at 15 days interval up to 120 days and found that except vitamin C content, no noticeable change was observed during the storage. Nitu *et al.* (2010) carried out the nutritive analysis of citrus juices and pH, TSS, acidity, reducing sugar, ascorbic acid and protein content were 3.22, 13%, 2.56%, 10.8%, 2.475% and 0.175%, respectively. Tasnim *et al.* (2010) analysed processed orange juice with highest total sugar (10.41%) and reducing sugar (2.24%) of different companies. The pH of samples ranged 3.50±0.10, TSS 10.50±0.01 percent and Acidity 0.59±0.011 percent and suggested that processed juices be prepared under hygienic conditions. The results of the present study were well comparable with most of the researches quoted above, and minor variation may be associated due to varieties and environmental variations.

In case of packing materials, the orange juice packed in Amber bottle had higher total sugars at 15, 30 and 45 days i.e. 4.4925, 4.9958 and 2.9042%; reducing sugars 2.9092, 3.4625 and 1.7158%; Non-Reducing Sugars 1.5217, 1.5983 and 1.2183%, acidity 0.2025, 0.1733 and 0.2808%, Vitamin-C 51.932, 36.957 and 10.319 mg/kg; pH 4.0467, 4.0442 and 3.9342; total soluble solids 11.052, 11.203 and 9.746%, respectively. In a similar study, Ayhan *et al.* (2001) evaluated orange juice aroma, colour and vitamin C in glass, PET, high-density polyethylene, and low-density polyethylene and found that packaging material had a significant effect on the retention of orange juice aroma, colour, and vitamin C and suggested Pet bottle packing. The present study further showed that total sugars, reducing sugars and total soluble solids increased after storage and reduced is preserved orange juice stored beyond 30 days. The quality of preserved orange juice packed in Amber bottles was relatively better than rest of the packing materials on the basis of various chemical properties of the juice.

Sensory analysis shows that colour, flavour, taste and appearance of preserved orange juice was acceptable for consumption up to 30 days of storage and later the experimental juice showed deterioration in these quality traits. Berlinet *et al.* (2003) packed orange juice in three different PET and glass packaging and indicated that PET packaging is the major factor detrimentally affecting the juice quality parameters. Chumillas *et al.* (2003) preferred PET and Amber packing for orange juice storage to increase the shelf life. The literature reviewed from the studies carried out in different parts of the world are well in agreement with the findings of the present research regarding the effect of packing materials on the chemical and organeoleptic parameters of processed orange juice. However, some variation in these results might be the result of variation in climatic conditions.

## CONCLUSIONS

On the basis of present research, it is suggested that Amber bottles may be used for packing preserved orange juice and Sodium Benzoate may be applied at the rate of 1.0g/liter. Besides, under these packaging and preservative level, the orange juice may be better used up to 30 days after storage.

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