

Estimation of Some Chemical and Biochemical Characteristics in The Juices of four Local Orange Fruits Produced Using The Probiotic

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Abstract: *This study was conducted in the laboratories of Tikrit University/ College of Agriculture/ Department of Food Sciences. The experiment aimed to study the effect of bio stimulants on some chemical and biochemical characteristics in juices of four types of oranges (summer orange, Abu Surra, Al Khalili Red, Al Yafawi). These four juices were stored under 5 ° C for four weeks. Several chemical biochemical tests were conducted on the juices after the first day of production (Control) and the later four weeks (T1, T2, T3 and T4). The results of the study showed a gradual decrease in the percentages of total dissolved solids (TSS) and total solids (TS) for the studied juices. Gradual decreases in the viscosity, and (pH) values were also observed. percentage of total acidity (TA) was increased. One other, the concentrations of vitamin C and total phenolic compounds were estimated at the beginning and the end the experiment period. These two parameters were increased in all juices studied.*

Keys: *probiotics, orange juice, antioxidants, vitamin C, density, viscosity.*

1. INTRODUCTION

Bio-enhanced juices are considered one of the most important functional foods. They provide human body with vitamins, mineral salts and phytochemical components (Ashurst, 2016). They work to give the body health and therapeutic benefits in addition to nutritional benefits (Beristin et al., 2006). Natural orange juices are one of the most popular food in the world. Its consumption has increased in recent times due to its excellent sensory properties that evoke vitality and activity in the soul , Tiis ttype of food has high nutritional value as it is an important source of vitamins and sugars that provide body with energy needed for vital functions (Bassis, 2014), Most natural orange juices contain high percentage of essential minerals, especially potassium (Densupsoontorn et al., 2002; Hallabo et al., 2008). This in turn, reduces blood pressure and expands blood vessels. They juice is rich in many effective compounds such as vitamins, pigments, antioxidants, flavor compounds and organic acids (Al-Bayati, 2011).

2. MATERIALS AND METHODS

Production of Functional orange beverage: Orange fruits that came from the local markets of Diyala city Were used in this experiment. The damaged and immature ones were isolated and discarded. The selected fruits then were washed with cool water, peeled and placed in a

fruit-pressing machine. The juice was collected and filled in tightly packed glass bottles, pasteurized at 74°C for 20 seconds, and quickly cooled. Part of the juice was kept in cold storage at 5°C (Control). The remaining was inoculated with two types of lactic acid bacteria (*Lb. rhamnosus*, *Lb. casei*) under controlled sterilization conditions. They were kept in the refrigerator for conducting the study.

Chemical and biochemical tests:

- 1- **TSS** : Total dissolved solid estimated using Hand Refractometer according to (A. O. A. C. , 2004) .
- 2- **TS** : The total solids were determined using a drying oven (A. O. A. C. ,2004) .
- 3- **Total Acidity**: The total acidity was measured according to the method adopted by (Al-Janabi, 2018).
- 4- **pH**: Measured using a pH meter as reported in Akoma (et al., 2010).
- 5- **Determination Relative Viscosity**: The viscosity was determined as stated in (Egan et al., 1988).
- 6- **Determination of Relative Density** : Was estimated using a pycnometer vial (A. O. A. C., 2004).
- 7- **Estimation of vitamin C**: Was estimated using HPLC (A. O. A. C., 2004).
- 8- **Estimation of total content of polyphenols**: Was estimated using HPLC, (A. O. A. C., 2004).

Statistical Analysis :

Data of the study was analyzed by ANOVA to determine the significance of the differences between the different averages . The probability level (0.05) was used according to the statistical program Minitab (2010).

3. RESULTS AND DISCUSSION

1- Total dissolved solids and total solids: TSS and TS

Table (1) showed the results of the statistical analysis of TSS. It is noted that there were significant differences between orange juice of red Al-Khalili and Abu Surra, Summer oranges and Al yaffy, between Abu Surra and summer and Al yaffy orange juice, and between Abu Surra and yafawi. However, there were non-significant differences between Abu Surra and summer juice, . In contrast, no significant differences were observed between all averages of the studied treatments during the storage stages.

Table (2) showed the results of the statistical analysis for TS . The results showed that there were significant differences between summer orange and the Abu Surra and Al Yafawi, between Al Khalili Red and Al Yafawi. However, on significant differences were observed between Summer Orange and Khalili red between Al-Khalili red and Abu Surra and between Abu Surra and Al-Yafawi. On the other hand, significant differences were observed between treatments during the storage stages. Significant differences were observed between the control treatment and the treatments T1, T2, T3, T4, and between the treatment T1 and the treatments T2, T3, T4, And between treatment T2 and treatments T3, T4, while no significant differences were observed between treatments T3 and T4. The reason for the gradual decrease in the values of TSS and TS may be due to their consumption of probiotics added to the juices of components of the medium for growth and reproduction (Tamang and Kailasapathy, 2010; Al-Janabi 2018), and (Hamad et al., 2021) the results were consistent with (Khaled et al., 2013)

Table (1) TSS% in functional beverage models

juice	Storage periods (weeks)					
	control	T1	T2	T3	T4	Average
Abu Surra	1.06	1.06	1.05	1.05	1.04	1.052 B
Al Khalili Red	1.09	1.09	1.08	1.08	1.08	1.084 A
Yavawi	1.03	1.03	1.03	1.02	1.02	1.026 C
summer orange	1.06	1.06	1.06	1.05	1.05	1.056 B
Average	1.06 a	1.06 a	1.06 a	1.05 a	1.05 a	

Table (2) TS% for functional beverage samples

juice	Storage periods (weeks)					
	control	T1	T2	T3	T4	Average
Abu Surra	17.2	16.7	14.9	13	11.9	14.74 BC
Al Khalili Red	18.9	17.6	15.7	14.1	12.9	15.84 AB
Yavawi	16.9	14.7	12.9	11.3	10.7	13.30 C
summer orange	20.1	18.3	16.7	15.7	13.9	16.94 A
Average	18.28 a	16.83 b	15.05 c	13.53 d	12.35 d	

2- Acidity and pH Total

Table (3) showed the results of statistical analysis of total acidity. There was a significant difference between summer orange and Al yafawi and Al-Khalili red and Abu Surra, and between Al-Yafawi, and Al-Khalili red and Abu Surra, and also between Al-Khalili red and Abu Surra. No differences were observed between au studied treatments during the storage weeks. Table (4) showed the results of statistical analysis of pH. There were significant differences between the average orange juice of Abu Surra and the two types of Al-Yafawi and summer orange, and between the Al-Khalili red variety and the two types of Al-Yafawi and summer orange, and also between Al-Yafawi and summer oranges. No significant differences were observed between the two types of Abu Surra and Khalili red. In contrast, significant differences were observed between the averages of the treatments during the storage stages between the control treatment and the treatments T1, T2, T3, T4, and between T1 and T3, T4, No significant differences were observed between the tow T1, T2 and the two treatments T3, T4 during the cold storage stages. The reason for the gradual increase in total acidity and the gradual decrease in pH may be due to the action of probiotics (Paseephol and Sherkat, 2009; Al-Janabi 2018), As lactic acid bacteria have the ability to produce organic acids such as lactic and acetic acids. These two acids can increase TA ratio in fermented foods and decrease pH (Law et al., 2011). The results were consistent with Khalid et al. (2013) indicated through study of orange juices types .

Table (3) Total acidity percentage (TA) % in functional beverage samples

juice	Storage periods (weeks)					
	control	T1	T2	T3	T4	Average
Abu Surra	1.042	1.162	1.287	1.442	1.477	1.282 C
Al Khalili Red	0.879	1.012	1.101	1.199	1.207	1.079 D
Yavawi	1.232	1.369	1.421	1.469	1.511	1.400 B
summer orange	1.429	1.516	1.599	1.679	1.719	1.588 A
Average	1.146 a	1.265 a	1.352 a	1.447 a	1.479 a	

Table (4) pH values for functional beverages

juice	Storage periods (weeks)					
	control	T1	T2	T3	T4	Average
3.56 A	4.1	3.9	3.5	3.3	3	3.56 A
3.40 A	3.9	3.7	3.4	3.1	2.9	3.40 A
3.14 B	3.6	3.3	3.1	2.9	2.8	3.14 B
2.86 C	3.3	3	2.8	2.7	2.5	2.86 C
Average	3.73 a	3.48 b	3.20 bc	3.00 cd	2.80 d	

3- Relative density and relative viscosity

Table (5) showed the results of statistical analysis of the viscosity characteristic. There were significant differences between Abu Surra and Al-Yafawi variety. and between Al-Khalili red and Al-Yafawi, and between the summer orange Al-Yafawi. No significant differences were observed between Abu Surra and Al-Khalili red. Significant differences were observed between the control treatment and the treatments T1,T2,T3,T4, and the treatment T1 and the treatments T2,T3,T4, and between T2,T4 and T3,T4. There were no significant differences between the two treatments T2. , T3 during storage periods. On the other hand, the results of the statistical analysis in Table (6) for the density showed there was a significant difference between Al-Khalili red and Abu Surra, summer orange and yafawi, and between Abu Surra and Yafawi, and between summer orange and Al-Yafawi significant difference was observed between Abu Surra and summer orange. On the other hand, it was noted that there were no significant differences between the averages of treatments for the studied juices during the weeks storage. Through the study, gradual decrease in the viscosity and relative density of the samples of bio-enhanced juices was observed. Several important factors may have led to these changes in these two characteristics. The type of bacterial strains used, duration of storage, high acidity, and chemical composition of the juice were same factors in clouded . (Espinoza and Navarro, 2010).

Table (5) Results of viscosity estimation/centiboy for the studied functional beverage models

juice	Storage periods (weeks)					
	control	T1	T2	T3	T4	Average
Abu Surra	5.11	4.93	3.8	4.69	3.43	4.39 A

Al Khalili Red	4.9	4.79	4.63	4.57	4.49	4.68 A
Yavawi	4.1	3.97	3.76	3.59	3.37	3.76 B
summer orange	4.75	4.51	4.36	4.19	4.01	4.36 A
Average	4.72 a	4.55 b	4.14 c	4.26 c	3.83 d	

Table (6) Results of estimating the relative density g/ml for functional beverage models

juice	Storage periods (weeks)					
	control	T1	T2	T3	T4	Average
Abu Surra	1.060	1.056	1.051	1.047	1.042	1.051 B
Al Khalili Red	1.088	1.086	1.084	1.081	1.078	1.083 A
Yavawi	1.033	1.03	1.027	1.024	1.022	1.027 C
summer orange	1.064	1.061	1.057	1.053	1.05	1.057 B
Average	1.061 a	1.058 a	1.055 a	1.051 a	1.048 a	

4- Vitamin C

Table (7) showed the results of the statistical analysis of vitamin C. There were no significant differences between the averages of orange juices and for all varieties during the study stages. However a significant difference was observed between the treatment T4 and treatment control during the storage periods. The results showed a high concentration of vitamin C in the models of bio-enhanced juices as a result of the action of the bio-stimulants. Fermentation processes can raise the concentrations of vitamins in the fermented products where it is considered a secondary metabolite (Al-Janabi 2018). It was been observed that some types of bacteria can increase the concentrations of vitamins, including vitamin C (Battcock, 1998), The results of the study agreed to some extent with (Al-Bayati, 2011 and Khaled et al. 2013).

Table (7) Vitamin C concentration in functional beverage samples during storage periods

juice	Control mg/100 ml	Fourth week: mg/100ml	Average
Abu Surra	36.62	172.93	104.775 A
Al Khalili Red	41.94	200.725	121.333 A
yavawi	38.16	189.14	113.650 A
summer orange	35.275	170.105	102.690 A
Average	37.999 b	183.225 a	

5- Total phenolic Compounds

It was shown in table (8) the results of the statistical analysis of the total phenolic compounds. Significant difference was observed between the average orange juice of Khalili red and summer orange, yaffy and Abu Surra. Significant differences were also observed between summer orange and yavawi and Abu Surra, and between Al-Yavawi and Abu Surra.

No significant differences were observed between the averages of the studied treatments during the storage stages. In a study conducted by (Battcock, 1998), it was shown that fermentation increased the concentration of phenolic compounds. There are factors explain the differences resulted from the characteristics and compositions. These factors included the type of probiotics used in production and chemical composition of the medium in which the fermentation process takes place (Espinoza and Navarro, 2010). Most of the phenolic substances are usually vitamins and pigments (Hamad et al., 2021). In a study to estimate some of the biologically active properties of juices of some orange, the result was consistent with (Al-Janabi 2018).

Table (8) Concentrations of total phenolic substances in functional beverage samples

juice	Control mg/100 ml	Fourth week: mg/100ml	Average
Abu Surra	243.75	243.75	243.75 D
Al Khalili Red	316.3	316.3	316.30 A
yavawi	279.205	279.205	279.21 C
summer orange	292.865	292.865	292.87 B
Average	283.03 a	283.03 a	

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