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Study of Accelerated Storage as Method of Predicting Orange Beverages Shelf-Life

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Abstract: The aim of present paper was to apply the accelerated shelf life method for shelf life determination of orange beverages with fruit juice content. The study compared changes in orange beverages properties due to storage of samples at 50°C for 7 days to changes caused by samples storage at 25°C during 270 days. The similar results suggested that the method can be successfully used to determine the shelf life of orange beverages with fruit juice content. The shelf life of orange juice drink with 12 % fruit juice content was about 300 days, 155 days, 42 days and 15 days for samples stored at 20, 30, 40 and 50°C respectively. Kinetics of ascorbic acid degradation in orange beverages with fruit juice content during storage was investigated. The loss of ascorbic acid at all studied temperatures followed a zero-order kinetic model.

Keywords: orange beverages, ascorbic acid, degradation, storage, kinetics.

1. Introduction

Orange juices quality and shelf life are mainly based on the stability of the ascorbic acid during storage. However, there are other specific parameters as well, such as colour and flavour, which are of paramount importance for a higher quality and a long shelf life. In time, ascorbic acid undergoes oxidation, the oxidation rate being related with the storage conditions. The spoiling is mainly caused by oxygen. Besides oxidation of ascorbic acid, oxygen also causes browning and promotes the developing of aerobic bacteria and yeasts [1].

Whenever a new or improved product is released on the market, the juice producers must first investigate the influence of internal and external parameters on the quality and shelf-life of the product [2,3].

The quality level of products is determined by physico-chemical parameters. The results obtained are reproducible and allow statistical analyses. This analytical approach can have many uses in the food system: a proper design of food products, development of a proper control and quality insurance system, determination of the shelf life of products based on statistical measurements [4].

The time period from the moment the product is created until it becomes unsafe or unaccepted by consumers represents the product maximum shelf life. This time period has to be determined in advance, before the product is released on the market [5]. In order to avoid excessive use of resources and significant delays in releasing a product on the market, researchers have initiated accelerated shelf life determination studies (ASLD) instead of monitoring the parameters of a product's quality in time, during its shelf life. These studies consist in subjecting the product to severe storage conditions. Thus, the most demanding requirements that juice and soft drinks usually have to meet are the high storage temperatures [6-9]. An example would be the orange juice made of fruit concentrate and packed in Tetra Pak bottles, whose quality changes in 6 months, at a temperature of 20°C as it would change in 13 days at a temperature of 40°C [10].

The kinetic of ascorbic acid degradation during storage of these products was studied by various authors. Among them opinions are divided: some studies described the degradation of ascorbic acid by a zero-order reaction model [4,11], others reported that the first-order reaction [12,13] or even second-order reaction [14] are suited for characterizing the ascorbic acid loss in beverages during storage.

In this context, the paper applies the ASLD method for the beverages industry, using the following storage conditions: temperature 50°C, time period 7 days. Investigations were carried out on natural orange juices that are highly acid and stable in time. Their pH level is between 2.5 and 3.5. The evolution of the physico-chemical and microbiological parameters for these types of beverages with a variable fruit content was monitored in time and a comparative analysis of results was carried out. The kinetic of ascorbic acid degradation in orange juices during storage was determined.

2. Experimental

Five types of beverages produced by a Romanian were used. These samples were divided in two categories according to the packaging material used:

Beverages aseptically packed in Tetra Pak bottles, with no added preservatives (sample A–orange soft drink with 4% fruit juice content; sample B–orange juice drink with 20% fruit juice content and sample C–orange nectar with 50% fruit juice content).

Beverages packed in polyethylene terephthalate (PET) bottles with added preservatives: sodium benzoate and potassium sorbate (sample D–orange soft drink with 5% fruit juice content and samples E–orange juice drink with 12% fruit juice content).

All those beverages had added antioxidant: ascorbic acid 0.3 g/L, allowed by the legislation *quantum satis*.

Ascorbic acid degradation was studied for sample E. This sample was divided into four sub-samples that were stored at different temperatures: 20, 30, 40 and 50°C. For each of the four sub-samples, the ascorbic acid content was monitored monthly. Measurements were performed until two consecutive results were below the lower limit allowed by the law - 90 mg/L.

Accelerated shelf life determination studies

Each of the studied samples were subjected to strict storage conditions (50°C) for 7 days. At the end of this period, the physico-chemical and microbiological parameters of samples were measured.

In order to compare the result of ASLD method, the samples were stored at 25°C for 270 days. The evolution of the physico-chemical and microbiological parameters were monitored monthly.

Physico-chemical and microbiological determinations

pH was measured with a pH-meter WTW pH 340i; dried substance (*DS*) was determined with the Abbe RFM800 refractometer [15]; sample acidity (*Ac*) was measured by titration with sodium hydroxide 0.1 n [16]; ascorbic acid content (C_{AA}) was establish by extraction with oxalic acid solution 1‰ and titration with 2,6-dichloroindophenol; water activity (a_w) was measured with Aquaspector AQS-2-TC; the microbiological measurements were performed for yeasts and molds (*YM*) [17] and Enterobacteriaceae (*E*) [18].

3. Results and Discussion

The shelf life of foods is subjected to changes due to the packaging material used and to the storage conditions [19]. Due to this fact, the accelerated shelf life determinations were made for orange beverages packed in Tetra Pak and PET bottles.

3.1. Soft drinks packed aseptically in Tetra Pak bottles

The analyses results of sample A packed in Tetra Pak bottles are presented in Table 1.

Five months after manufacturing date, the product A underwent changes in taste. There was an 'overripe orange' taste, slightly fermented. After 6 months, the product showed a pronounced ring on the bottle neck and a slight sediment deposit.

From table 1, it was noticed that sample A have registered a significant loss of ascorbic acid. After 7 days at 50°C, the vitamin C retention decreased below 50%. In the case of samples stored at room temperature, the ascorbic acid content decreased below 65% only after 240 days.

Under acid pH conditions (pH<4.5), a limited number of microorganisms are growing. This phenomenon can be stopped by thermal treatment. But, beverages modifications that occur during storage lead to the increase of pH. The decrease in time of sample A total acidity is favorable to microbial growth.

The physico-chemical analysis of samples subjected to 50°C for 7 days shows that the increasing of *DS*, a_w and the ascorbic acid degradation are accelerated by the direct action of temperature.

The analysis results of sample B packed in Tetra Pak are presented in Table 2.

TABLE 1. Variation of physico-chemical and microbiological parameters of product A

t (days)	T (°C)	DS (°Brix)	Ac (g/L)	pH	C_{AA} (mg/L)	a_w	YM/mL	E/mL
0	25	10.9	5.01	2.91	304.34	0.911	0	0
30		10.8	5.00	2.89	302.00	0.919	0	0
60		11.1	5.03	2.89	211.42	0.929	0	0
90		11.3	4.72	2.89	178.57	0.934	0	0
120		11.3	4.76	2.89	210.00	0.941	0	0
150		11.4	4.77	2.74	157.14	0.940	0	0
180		11.4	4.81	2.84	166.66	0.952	2	0
210		11.5	4.77	3.04	118.84	0.941	1	0
240		11.6	4.82	3.03	116.85	0.956	1	0
270		11.6	4.78	3.03	120.58	0.965	2	0
7	50	11.8	4.90	2.97	137.62	0.958	0	0

TABLE 2. Variation of physico-chemical and microbiological parameters of product B

t (days)	T (°C)	DS (°Brix)	Ac (g/L)	pH	C_{AA} (mg/L)	a_w	YM/mL	E/mL
0	25	10.2	2.75	3.46	618.57	0.945	0	0
30		10.1	2.82	3.44	625.00	0.954	0	0
60		10.3	2.69	3.40	533.33	0.957	1	0
90		10.2	2.73	3.40	534.33	0.956	0	0
120		10.3	2.77	3.41	535.29	0.958	4	0
150		10.3	2.67	3.44	492.85	0.962	1	0
180		10.3	2.74	3.39	482.35	0.948	2	0
210		10.5	2.99	3.45	473.52	0.965	3	0
240		10.3	2.99	3.46	449.25	0.969	2	0
270		10.4	3.02	3.44	438.25	0.971	3	0
7	50	10.2	3.01	3.42	430.17	0.980	2	0

TABLE 3. Variation of physico-chemical and microbiological parameters of product C

t (days)	T (°C)	DS (°Brix)	Ac (g/L)	pH	C_{AA} (mg/L)	a_w	YM/mL	E/mL
0	25	13.0	3.89	3.80	408.57	0.926	0	0
30		13.0	3.95	3.73	314.08	0.949	0	0
60		13.0	3.95	3.71	308.75	0.952	0	0
90		13.1	3.95	3.70	304.28	0.959	1	0
120		13.0	4.27	3.69	304.73	0.966	3	0
150		12.9	4.77	3.60	305.94	0.960	2	0
180		13.0	4.78	3.61	304.78	0.970	1	0
210		13.0	4.82	3.58	304.61	0.973	2	0
240		13.0	4.86	3.59	305.49	0.984	3	0
270		13.1	4.85	3.64	307.14	0.971	1	0
7	50	12.9	4.73	3.63	304.67	0.986	0	0

TABLE 4. Variation of physico-chemical and microbiological parameters of product D

t (days)	T (°C)	DS (°Brix)	Ac (g/L)	pH	C_{AA} (mg/L)	a_w	YM/mL	E/mL
0	25	11.2	4.64	2.94	257.97	0.908	0	0
30		11.1	4.72	2.79	250.00	0.921	0	0
60		11.0	4.84	2.89	207.14	0.942	1	0
90		11.3	4.69	2.90	220.00	0.935	0	0
120		11.2	4.65	2.89	195.71	0.954	1	0
150		11.3	4.68	2.80	142.85	0.953	0	0
180		11.4	4.85	2.86	139.42	0.969	1	0
210		11.5	4.78	3.03	121.73	0.968	1	0
240		11.6	4.73	2.99	112.84	0.974	3	0
270		11.6	4.74	3.01	108.82	0.979	2	0
7	50	11.9	4.88	2.96	118.54	0.961	1	0

Five months after manufacturing date, the product B presented an “overripe orange” taste, oily, “boiled taste”. From a microbiological perspective, the product was safe.

The variation of physico-chemical and microbiological parameters for the two studied situations was similar to that of the previous sample. After 270 days of storage at room temperature, the quantity of ascorbic acid in the sample was 70.84% from the initial one. In the case of sample subjected to 50°C for 7 days, the value of ascorbic acid was even lower: 69.54% from the initial value. Also, aw reveal a similar augmentation, for both situations, favorable to microbial growth.

The analysis results of sample C packed in Tetra Pak bottles are presented in table 3.

Six months after manufacturing date, the product C presented a persistent taste of orange peel, acid, oily, although the product was safe from a microbiological perspective.

In the two situations, the parameters that vary significantly in time are the acidity and the ascorbic acid values. The acidity increase of product C, with sugar and acidifiants added, promote the acidotolerant microorganisms growth–mould and yeasts–which will generate acids from carbohydrates. After 270 days of storage at room temperature and after 7 days of storage at 50°C, the ascorbic acid content of samples was reduced. The oxidation rate of ascorbic acid was accelerated by a higher storage temperature.

The results of the analyses of the three products have been revealed a similar evolution in time of physico-chemical and microbiological parameters.

3.2. Soft drinks packed in PET bottles

The analysis results of sample D packed in PET bottles are presented in table 4.

8 months after manufacturing date, the product D underwent changes in taste; slightly fermented, the product was safe from a microbiological perspective. The product showed a ring-shaped making on the container neck and a slight sediment deposition after 6 months.

The ascorbic acid quantity exhibit a significantly time variation, for both situations takes into account, the decreasing being accelerated by a higher storage temperature and by the material of PET bottles, which allows the access of light. Therefore, after 7 month of storage the quantity of ascorbic acid was one-half of the initial value. The increase of sample D water activity (aw) from 0.908 (fresh sample) to 0.979 (sample stored at 25°C for 270 days), and 0.961 (7 days at 50°C) respectively shows that the enzymatic reactions are similar for the two cases and the reaction rates are different according to the storage temperature.

The acidity of the Tetra Pak - packed orange beverages was lower than in the case of PET - packed drinks, while the pH values were higher. The advantage of aseptically packaging of products in Tetra Pak was the significantly reduction of vitamin C loss.

By comparing the values of physico-chemical and microbiological parameters, the results confirmed that after 7 days of storage at 50°C, the quality of products showed the same changes as after 270 days of storage at 25°C, thus validating the efficiency of the accelerated shelf life determination method.

Due to –OH groups present in the ascorbic acid molecule, part of water amount available to microorganisms is binded (hydrogen bonds) and the water activity values are generally growing in the same time with ascorbic acid degradation.

In the second part of this work the degradation of ascorbic acid from orange beverages was studied. For this purpose, the sample E was used. The fresh sample was divided into four sub-samples that were stored at different temperatures 20, 30, 40 and 50°C. For each of the four sub-samples, the C_{AA} was determined at equal periods of 30 days. Figure 1 show the time variation of C_{AA} of samples stored in the conditions mentioned above.

According to the Romanian Food Labelling Law no. 106/2002 that implement the Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, in order to declare on the product label the CAA, the product must contain a minimum amount of ascorbic acid, namely 15% of the recommended daily intake (RDI = 60 mg vitamin C in 100 mL), that is 9 mg in 100 mL at the end of its shelf life.

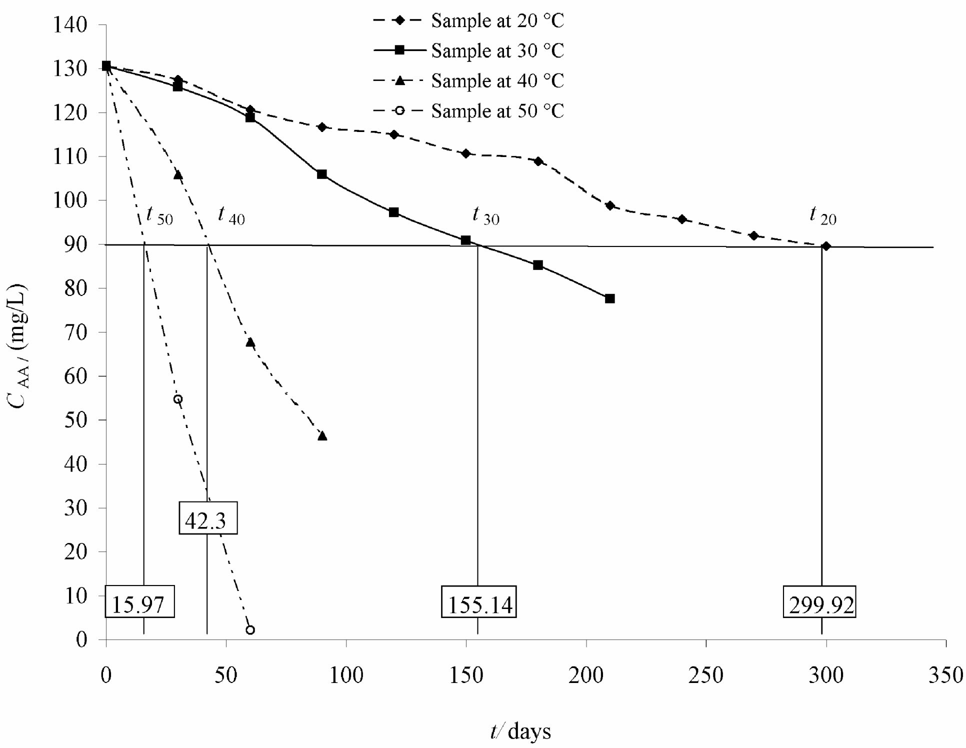

Figure 1. Variation of the ascorbic acid content of samples E at different temperatures

The variation of ascorbic acid content allows the determination of soft drink shelf life as the time period from the moment of product processing to the moment when the critical limit defined by the law is reached (90 mg/L). From figure 1 the shelf life of sample E was nearly 300 days in the case of samples stored at 20°C. The increase of storage temperature with 10°C decrease the shelf life of the product with half (155 days). Higher temperatures drastically reduced the shelf life of product (42 days for storage at 40°C and 16 days for storage at 50°C).

Using these experimental data, the thermal degradation kinetics of ascorbic acid from orange juice drinks was studied. The loss of ascorbic acid in samples during storage was calculated by using the equation of zero-order reaction kinetics (1) and the first-order reaction kinetics (2):

$$-\frac{dC_{AA}}{dt} = k \qquad (1)$$

$$-\frac{dC_{AA}}{dt} = k \cdot C_{AA} \qquad (2)$$

where C_{AA} (mg/L), t (days) and k - reaction rate constant.

The values of zero and first-order reaction rates constants (k) for ascorbic acid degradation in orange juice drink and the correspondents coefficients of determination (R^2) are presented in table 5.

TABLE 5. Reaction rate constants for ascorbic acid degradation and coefficients of determination

T (°C)	Zero-order kinetics		First-order kinetics	
	k (mg/L·days)	R^2	k (days)	R^2
20	0.1400	0.9852	0.0005	0.9786
30	0.2570	0.9874	0.0010	0.9843
40	0.9593	0.9885	0.0048	0.9746
50	2.2185	0.9870	0.0263	0.8805

The values of k indicate that the reaction velocity increases with increase of temperature. Comparing the values of R^2 for the two cases, it was noticed that the higher values were obtained for the case of zero-order kinetics. It means that the zero-order model fitted the data better than a first-order model. This is in agreement with others studies [4,11]. But opinions are divided and other studies have been reported that the loss of ascorbic acid during storage follows a first-order reaction [12,13] or second-order reaction [14].

4. Conclusions

The ascorbic acid content must be monitored in time so as to determine the shelf life of beverages with different orange juice content. To restrict the inevitable loss of ascorbic acid in time, it is advisable to use Tetra Pak and not PET containers for packaging.

Water activity is a predictive indicator for quality control of juices protected by antioxidant agents. Although the antioxidants molecules contain various free –OH groups, the amount of water available to microorganisms is reduced by hydrogen bonds formation and thus the water activity is decreased.

The results of this study have been confirmed the connection between alteration processes that take place due to the storage of soft drinks at a temperature of 50°C for 7 days and those that occur in 270 days at 25°C.

The loss of ascorbic acid in orange beverages at all storage temperatures was found to follow a kinetic reaction of zero-order.

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