

Studies Regarding the Use of Preservatives on Soft Drinks Stability

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Abstract: The shelf life of soft drink is limited by microbial alteration and chemical reactions. The aim of this paper is to test the action of commonly used preservatives on the soft drinks quality in different storage conditions. In the mean time, the appropriate concentration of citric acid used for product acidification was established. The samples microbiological analyses together with their physico-chemical parameters allow us to estimate the efficiency of sodium benzoate and potassium sorbate preservatives uses.

Keywords: soft drinks, microbiology, preservatives: sodium benzoate, potassium sorbate.

1. Introduction

Preservation is aimed at achieving the self life prolongation of foods. Present tendencies are based on the employment of certain methods which ensure qualitatively products, less preserved, with no additives, with nutritional value, but also safe from the microbiological point of view [1,2].

Preservatives are defined as substances able to inhibit, stop or delay the growth of microorganisms or any deterioration of aliments due to microorganisms.

The preservation methods used in the self life extension process include water removal, temperature control, freezing, drying, pH control, irradiation, vacuum packaging, modified atmosphere packaging, aseptic packaging, acidification, fermentation, heating (pasteurization and sterilization) and chemical preservatives addition. The preservation techniques are aimed to slow down the changes, which cause foods deterioration, due to a large number of physical, chemical, enzymatic or biological reactions [1].

Man uses natural preservatives and preservation methods without knowing how they help at food protection against alteration. The benzoic acid, acetic acid and sorbic acid together with propionate and sulphur dioxide are acid preservatives used in a large scale in foods and soft drinks preservation. The conditions met in many preserved aliments (small values of water activity, low pH, the presence of preservatives, carbon dioxide or ethanol, the lack of oxygen) are not just right environment for the growth of microorganisms. Yeasts and fungi play a major role in the alteration process of the foods preserved at a low pH, with low values of water activity, with or without preservative addition. The yeasts which produce alteration include *Zygosaccharomyces*, as well as *S. cerevisiae*. These species can grow in the presence of large quantities of acids used in food preservation at some pH values lower

than the value of pK_a of these acids. At low pH values (sorbic acid pK_a 4.76 and benzoic acid 4.19), these acids are in an undissociated form, a form in which they are potential inhibitors of microbial growth [3].

Soft drinks bottled at low temperatures have high values of the water activity, which allow microbial growth; the pH, the sugar content and the add of preservatives prevent the microorganisms growth in soft drinks. Some species of moulds *Aspergillus niger* and *Penicillium spinulosum* are resistant to chemical preservatives, as well as the sorbic acid and the benzoic acid and they can tolerate acid environments and low values of the water activity [4].

The factors which influence the microbial alteration of the foods include the number and type of contaminated microorganisms, humidity (and water activity), the pH (the level of acidity and alkalinity), the presence or absence of oxygen, the type and availability of nutrients, the temperature, the food physical condition [5,6].

The sorbic acid and the sorbates are the preservatives mostly used in the soft drinks industry. The sorbic acid acts efficiently against the yeasts and moulds growth, and of some bacteria, acting at a low pH, but however continues to be efficient at a pH of 6.5. The microorganisms growth inhibition is achieved through the interaction of the system of the two conjugated double bound in the aliphatic chain with cellular dehydrogenises which most of the yeasts and moulds cannot metabolize.

In fruit beverages preservation, the sodium salt of the benzoic acid is frequently used, as it is more soluble than the free acid; however, the undissociated acid, formed from the salt dissolved in solution, is responsible for the antimicrobial activity, which is optimum at a pH value ranging from 2.5 to 4. [7,8]

The researches described in this paper had in view the followings:

- To test the influence of the preservatives mostly used in the fruit beverages industry, to establish the optimum

concentration of citric acid used in the acidification of the fruit beverage and to deduce the efficiency of their addition by monitoring the organoleptic characteristics, microbiological analyses and by evaluating the physico-chemical parameters.

- The behaviour of the samples in different preservation conditions: at room temperature, under the action of the sun rays and by exposing at high temperatures (40°C) – (Accelerated shelf life determination, ASLD).

2. Experimental

The samples were prepared with demineralised and partly decarbonated water (with a content of carbon dioxide of 2g/L), fructose syrup 50g/L, ascorbic acid 0.1 g/L, citric acid solution 50%, potassium sorbate and sodium benzoate. The intended pH values were obtained by using as a buffer solution - sodium citrate 0.15g/L. The ingredients were added under stirring, and afterwards pasteurized and bottled at cold temperatures in PET bottles.

Two sets of samples were prepared: 7 samples contain as an acidifier solution citric acid in a quantity of 1g/L, the other 7 ones 1.6g/L citric acid. The quantities of preservatives varied for both sets, such as in table 1:

TABLE 1. The ratio (quantity) of preservatives used in the preparation of samples

Sample / Preservative	P1	P2	P3	P4	P5	P6	P7
Sodium benzoate, g	0	0.1	0.15	0	0	0.135	0.15
Potassium sorbate, g	0	0	0	0.15	0.3	0.135	0.25

The samples prepared as mentioned above were kept in different conditions for 14 days: at room temperature (T_{room}), under the action of the sun rays, at high temperatures, in drying chamber (40°C). The action determination of the preservatives was monitored by analyzing the samples physically and chemically (pH, dry substance D.S. [⁰Brix], acidity [g/L acid citric], water activity, a_w , the qualitative analysis of the preservatives: sodium benzoate and potassium sorbate), as well as microbiologically (yeasts and moulds Y&M, the total number of germs – TNG, coliform bacteria - C) [9-15]. The samples were previously brought at room temperature and only subsequently analyzed physically, chemically and microbiologically.

The *dry substance* was determined by means of the Abbe refractometer: *acidity* with an automatic titrator of the Mettler Toledo type and the *pH* with a WTW pH 340i pH-meter. The *water activity* for the analysed samples was measured with AQS-2-TC, NAGY Aquaspector apparatus, using kieselguhr as an adsorbing environment.

The *determination of the sodium benzoate*. 100 mL (V) sample and NaCl (30g/sample) are introduced in a volumetric flask of 200 cm³ (V1). The mixture is then alkalized with NaOH solution 10% in the presence of litmus, as indicator. It is brought to the necessary level with a saturated solution of NaCl (26.4 g/100 cm³ water at 20°C) and then homogenized. It is left to settle for 2-3 hours and then filtered through an average porosity filter paper.

100 mL (V) of the resulted solution are introduced in a separation funnel. The mixture is neutralized with a solution of HCl by adding 5 cm³ (5 mL) in addition. Successively some quantities of 35, 25, 20 and 15 mL of chloroform are added subsequently, stirring it each time and separating the chloroform-based extract. It evaporates drily. It is left for 12 hours in an exsiccator with sulphuric acid. The residue obtained as such is dissolved in 40 mL neutralized ethylic alcohol (ethylic alcohol 96% vol/vol neutralized with NaOH 0.1n in the presence of phenolphthalein), completed with 10 mL water, 0.5 mL phenolphthalein and then titrated with 0.05n NaOH (V₂). Determination: $C_{sodium\ benzoate} = 0,0072 \cdot V_1 \cdot V_2 \cdot 100 / V \cdot V_3$ [g/100mL].

The *determination of the potassium sorbate*. The oxidant solution is prepared as follows: 4.5 g sodium bicarbonate are introduced in a 1000mL volumetric flask. 400-500 mL distilled water are added and after the entire dissolution of the sodium bicarbonate, 0.0162 g crystallized CuSO₄ is added, and then brought to the necessary level and homogenized.

In 200 cm³ volumetric flasks the following are introduced: witness sample (contains 50mL oxidant solution and it is brought to the specified level with distilled water) and 3 mL juice to be analyzed, with the further addition of 50 mL oxidant solution, which is afterwards brought to the specified level by adding the necessary quantity of water. After 10-15 minutes, by means of a spectrophotometer the absorption is measured at 253 nm as compared to the blank sample.

The *determination of the standardization curve*: the standard solution of sorbic acid is prepared as follows: 0.1g sorbic acid is dissolved in 700-800 mL distilled water warmed at 70-80°C in a 1000 mL volumetric flask; after its cooling down, it is brought to the necessary level by adding water. In 6 - 200cm³ volumetric flask, the following quantities of standard solution – sorbic acid and oxidant solution are added according to the table 2.

TABLE 2. The preparation of sorbic acid standard solution

Number of volumetric flask	1	2	3	4	5	6
Standard sol. of sorbic acid, mL	1	2	3	4	5	6
Oxidant solution, mL	50	50	50	50	50	50
Water, mL	149	148	147	146	145	144
Sorbic acid conc., mg/200mL	0.1	0.2	0.3	0.4	0.5	0.6

The content of each volumetric flask is spectrophotometrical analyzed at the wave length of 253 nm as compared with the blank sample. Determination: $C_{potassium\ sorbate} = A \cdot ctg\ \alpha$ [g/L], where: A – the absorbance of the solution to be determined, ctg α – the gradient of the standardization curve, ctg $\alpha = 0.410$.

For the microbiological analysis of the nectar *the yeasts and the moulds (Y&M)* are highlighted by increasing the *Orangeserum Agar* medium, and the incubation is made at the room temperature (20-25°C), for 2-5 days, followed by their counting.

The highlighting of the *total number of germs (TNG)*

in the juices with a certain fruit content is based on the insemination of that particular product by incorporating it in the *Standard Agar* culture medium, with an incubation at 37°C for a period of 48±2 hours and the counting of the colony-forming units developed under the given circumstances.

The analysis for *coliform bacteria* (C) uses the *Endo Agar* medium and the incubation is made at 37°C for 24 hours, followed by their evaluation.

3. Results and discussion

In both cases, variants P1 and P2 did not pose problems when being prepared, samples P3 have a faint foreign odour, while P4, P5, P6 and P7 have an accentuated odour and posed problems to dissolution.

Tables 3, 4, 5 and 6 present the results of the physico-chemical analyses of the samples with an addition of 1g/L citric acid.

TABLE 3. The results of the physico-chemical analyses for the freshly prepared samples

Sample	pH	D.S., °Brix	Acidity, g/L	a _w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.711	3.6	0.456	0.945	Ned.	Ned.
P2	3.697	3.5	0.450	0.952	0.0100	Ned.
P3	3.661	3.6	0.479	0.950	0.0142	Ned.
P4	3.954	3.5	0.442	0.954	Ned.	0.1488
P5	4.152	3.4	0.436	0.962	Ned.	0.2996
P6	3.923	3.6	0.396	0.956	0.0134	0.1345
P7	4.009	3.4	0.430	0.958	0.0149	0.2491

TABLE 4. The results of the physico-chemical analyses for the samples kept in sterilizer

Sample	pH	D.S., °Brix	Acidity, g/L	a _w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.518	3.5	0.461	0.962	Ned.	Ned.
P2	3.471	3.5	0.416	0.960	0.0038	Ned.
P3	3.433	3.5	0.467	0.961	0.0051	Ned.
P4	3.778	3.5	0.462	0.959	Ned.	0.1314
P5	3.980	3.6	0.441	0.963	Ned.	0.2890
P6	3.823	3.4	0.451	0.961	0.0067	0.1249
P7	3.924	3.4	0.450	0.970	0.0089	0.2126

TABLE 5. The results of the physico-chemical analyses for the samples kept at room temperature

Sample	pH	D.S., °Brix	Acidity, g/L	a _w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.548	3.4	0.460	0.949	Ned.	Ned.
P2	3.568	3.6	0.438	0.953	0.0085	Ned.
P3	3.51	3.6	0.471	0.960	0.0103	Ned.
P4	3.82	3.5	0.455	0.966	Ned.	0.1452
P5	3.981	3.5	0.426	0.971	Ned.	0.2899
P6	4.011	3.5	0.447	0.959	0.0116	0.1316
P7	3.848	3.5	0.470	0.973	0.0127	0.2413

TABLE 6. The results of the physico-chemical analyses for the samples exposed to sun rays

Sample	pH	D.S., °Brix	Acidity, g/L	a _w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.471	3.6	0.460	0.966	Ned.	Ned.
P2	3.517	3.6	0.493	0.967	0.0063	Ned.
P3	3.477	3.6	0.508	0.962	0.0095	Ned.
P4	3.862	3.6	0.498	0.964	Ned.	0.1423
P5	4.017	3.5	0.471	0.970	Ned.	0.2897
P6	3.769	3.5	0.486	0.968	0.0109	0.1281
P7	3.857	3.7	0.522	0.971	0.0114	0.2394

By comparing the physico-chemical analyses performed immediately after the preparation, they are presented as compared to those performed after 14 days from the preparation moment (kept in sterilizer, at the room

temperature and exposed to sun rays). Depending on the storage temperature, one can notice the decrease of the pH value (the lowest values were registered by the samples kept in the sterilizer, followed by the samples exposed to

sun rays and the highest values of the pH were registered by the samples kept at room temperature) with the consequential increase of the acidity (the highest values were registered by the samples exposed to sun rays) as compared to the preparation moment. At the same time, one can notice that in all the storage conditions, the pH values are higher in the samples containing potassium sorbate as compared to those containing sodium benzoate. The a_w values registered an increase generally depending

on the environment and temperature to which the sample is subjected. The quantity of the preservative present in the sample decreases in time, and this decrease is favoured by temperature or sun exposure, especially in the case of the sodium benzoate.

Table 7 presents the results of the microbiological control of the samples with an addition of 1g/L of citric acid.

TABLE 7. The result of the microbiological analyses for the samples with 1g/L citric acid

Sample	After preparation			After 14 days at 40°C			After 14 days at T _{room}			After 14 days at sun		
	Y&M	TNG	C	Y&M	TNG	C	Y&M	TNG	C	Y&M	TNG	C
P1	154	4	0	1	0	0	> 300	14	0	> 300	6	0
P2	14	0	0	0	0	0	5	0	0	> 300	0	0
P3	54	54	0	0	0	0	> 300	50	0	> 300	0	0
P4	208	208	0	2	0	0	2	0	0	0	0	0
P5	9	9	0	0	0	0	> 300	0	0	0	1	0
P6	4	4	0	0	2	0	> 300	8	0	0	0	0
P7	0	0	0	0	0	0	0	0	0	2	0	0

The microbiological analyses performed immediately after the preparation are presented as compared to those performed after 14 days from the preparation moment (table 6). Depending on the storage temperature, after 14 days, the microbial development is slowed down in the samples containing the preservatives: P4-P7 (potassium sorbate) as compared to those which do not contain this type of preservative.

At room temperature, the enzymatic and microbiological reactions take place faster, and this fact leads to a faster degradation of the aliments and confirms the increased microbial potential (a large number of yeasts)

of the samples kept at the room temperature as compared to the remaining samples.

Once exposed to higher temperatures 40-50°C (sterilizer), the microbial development is slowed down, but due to the long storage time period (14 days) at high temperatures, the organoleptic characteristics (colour, taste, odour) of the aliment are affected, and thus, the variants containing potassium sorbate feature a foreign taste of cherry kernel.

The physico-chemical parameters for the samples with an addition of citric acid 1.6 g/L are presented in tables 8,9,10 and 11.

TABLE 8. The results of the physico-chemical analyses for the freshly prepared samples

Sample	pH	D.S., °Brix	Acidity, g/L	a_w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.449	3.6	0.718	0.943	Ned.	Ned.
P2	3.352	3.6	0.747	0.942	0.0100	Ned.
P3	3.368	3.6	0.740	0.941	0.0142	Ned.
P4	3.533	3.6	0.717	0.965	Ned.	0.1497
P5	3.745	3.6	0.711	0.954	Ned.	0.2990
P6	3.511	3.6	0.750	0.948	0.0131	0.1336
P7	3.675	3.5	0.689	0.960	0.0147	0.2496

TABLE 9. The results of the physico-chemical analyses for the samples kept in sterilizer

Sample	pH	D.S., °Brix	Acidity, g/L	a_w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.360	3.6	0.731	0.960	Ned.	Ned.
P2	3.301	3.6	0.753	0.957	0.0025	Ned.
P3	3.280	3.6	0.746	0.957	0.0027	Ned.
P4	3.457	3.6	0.729	0.968	Ned.	0.1327
P5	3.687	3.5	0.685	0.971	Ned.	0.2956
P6	3.413	3.6	0.758	0.956	0.0062	0.1279
P7	3.544	3.5	0.695	0.974	0.0088	0.2402

TABLE 10. The results of the physico-chemical analyses for the samples kept at the room's temperature

Sample	pH	D.S., °Brix	Acidity, g/L	a _w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.331	3.6	0.694	0.952	Ned.	Ned.
P2	3.156	3.6	0.759	0.950	0.0071	Ned.
P3	3.157	3.6	0.725	0.955	0.0129	Ned.
P4	3.347	3.6	0.781	0.967	Ned.	0.1473
P5	3.509	3.5	0.728	0.968	Ned.	0.2809
P6	3.255	3.6	0.766	0.959	0.0128	0.1319
P7	3.458	3.4	0.768	0.972	0.0132	0.2254

TABLE 11. The results of the physico-chemical analyses for the sun rays-exposed samples

Sample	pH	D.S., °Brix	Acidity, g/L	a _w	Sodium benzoate, g/100 mL*	Potassium sorbate, g/L*
P1	3.293	3.6	0.727	0.951	Ned.	Ned.
P2	3.225	3.6	0.783	0.950	0.0035	Ned.
P3	3.205	3.7	0.750	0.955	0.0044	Ned.
P4	3.312	3.6	0.709	0.967	Ned.	0.1381
P5	3.542	3.5	0.751	0.968	Ned.	0.2902
P6	3.242	3.7	0.777	0.959	0.0083	0.1293
P7	3.499	3.4	0.675	0.970	0.0113	0.2328

As compared to the preparation moment, the values of the pH decrease depending on the storage temperature (the lowest values were registered by the samples kept at the rooms' temperature and the highest ones were of the samples tested in the sterilizer) and the values of the acidity grow accordingly (the highest values were registered by the samples exposed to the sun rays). At the same time, one can notice that in this case, the usage of a greater quantity of citric acid when being prepared (1.6 g/L citric acid

solution 50 %) reduces the pH of the samples through their additional acidification as compared to the first case. The values of the a_w are lower in the samples with an addition of 1.6 g/L as compared to those with an addition of 1 g/L citric acid solution 50 %.

Table 12 presents the results of the microbiological control of the samples with an addition of 1.6 g/L citric acid.

TABLE 12. The result of the microbiological analyses for the samples with citric acid 1.6 g/L

Sample	After preparation			After 14 days at 40°C			After 14 days at T _{room}			After 14 days at sun		
	Y&M	TNG	C	Y&M	TNG	C	Y&M	TNG	C	Y&M	TNG	C
P1	7(+1000 bact)	1	0	3	18	0	25	>1000	0	100	0	0
P2	64	23	0	0	0	0	40	>300	0	100	>1000	0
P3	6	4	0	0	0	0	100	>500	0	100	0	0
P4	6	1	0	0	0	0	9	4	0	0	0	0
P5	9	4	0	0	0	0	3	0	0	0	0	0
P6	48	9	0	1	0	0	0	1	0	0	0	0
P7	>1000	>300	13	0	0	0	50	39	0	5	0	0

After 14 days, depending on the storage temperature, the microbial increase is slowed down in the samples containing preservatives (P4-P7 contains potassium sorbate) as compared to those which do not contain this preservative.

The results of the analyses confirm the higher microbial potential (see the large number of yeasts and TNGs) in the case of the samples kept at the room's temperature as compared to the remaining samples.

If we compare the results of the microbiological analyses of the samples kept at the room's temperature and exposed to sun rays, in the two cases with a different content of citric acid, one can notice the decrease of the microbial potential in the samples prepared with 1.6 g/L citric acid solution 50 % as compared to those prepared with 1.0 g/L citric acid 50 %.

Remarks:

Organoleptic characteristics: Colour and taste modifications can be noticed in all the samples tested in the sterilizer for a time period of 14 days at 40°C. All the samples preserved with potassium sorbate feature a foreign taste of cherry seed. As regards the colour modification, the most accentuated ones are the followings: P7, the most modified one, followed successively by P5, P6, P4, P3, P2, P1.

**Preservatives analysis:* In order to determine the content of preservatives in the samples, the obtained values are subject to discussion as the employed method does not ensure a satisfactory accuracy.

Under this study, the dry substance D.S. indicates only if the preparation manner of the samples was correct or not.

4. Conclusions

The content of the preservatives destined to human consumption allows to increase their self life by reducing the microbial growth .

At equal concentrations of 0.15 g/L the potassium sorbate (added in P4) has a powerful inhibiting effect than the sodium benzoate (added in P3) upon the development of microorganism in all the studied cases (room's temperature, sun exposure, storage at 40⁰C in a sterilizer). Also, the employment of the sodium benzoate in the preservation of soft drinks without an addition of carbon dioxide can only be performed together with the potassium sorbate in quantities established by the law.

By monitoring the pH values and the samples acidity evolution allows to predict the microorganism growth while the microbiological analyses confirm the efficiency of the preservatives usage and the importance of the foods aliments acidification (by adding citric acid) with a view to reducing or stopping the microbial growth.. By decreasing the pH values of foods ,during its acidification with citric acid (1.6 g/L) allows the usage of some smaller quantities of potassium sorbate and sodium benzoate (case P6) with an similar efficiency if they are used bigger quantities.

There is a close connection between the storage temperature of the aliment and its self life ,which is obvious in certain operations applied to it. Thus, one should avoid their prolonged exposure to sun rays and the aliments storage at high temperatures for longer periods. Thermal treatment applied to acid aliments, such as fruit juices, allow the destruction of microorganisms, but their

prolonged exposure for a long period of time, usually affects their organoleptic characteristics.

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