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Dedicated to Professor Liviu Literat, at his 80th anniversary

WATER ACTIVITY – INDICATOR OF FOOD SAFETY AND THE FACTORS THAT INFLUENCE THE BIOCHEMICAL STABILITY OF SOFT DRINKS

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ABSTRACT. The aim of this paper is to evaluate the water activity value of different food products, as well as to test the different functional ingredients and the best preserving conditions that inhibit the development of microorganisms in soft drinks – the analysed products that have the highest a_w value. Using the experimental data, a sensitivity analysis with respect to water activity was performed. According to this analysis, the paper suggests the using of water activity value as indicator that ensure the sanitation of soft drinks.

Keywords: *water activity, soft drinks, microbiology, HACCP*

INTRODUCTION

The moisture of foods is an important factor for the products stability or shelf-life. The limitations of water content measurement as an indicator of safety and quality are attributed to differences in the intensity which water associates with other components in the product [1].

The osmotic pressure of food products can be measured by their water activity (a_w) and is expressed as it follows:

$$a_w = \frac{p}{p_0} = \frac{ERH[\%]}{100} \qquad (1)$$

p – water-vapour pressure above the product surface at the given T temperature, [N/m^2];

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p_0 – water-vapour pressure above the surface of pure water at the product temperature, [N/m^2].

a_w reflects the amount of free water in food products, which is available to microorganisms for chemical reactions, biochemical reactions, transfer of metabolites through the cell's layers [1]. It corresponds to the equilibrium relative humidity, ERH [%] at the T temperature.

By measuring and controlling the water activity, it is possible to predict and identify the type of microorganism which could be a potential source of degradation and infection [2, 3].

The biological stability of soft drinks is influenced by: the level of initial contamination, the chemical composition of the drink, the thermal treatment, the preservatives adding and the storage temperature [4].

The decrease of the water activity value delays the growth of the microorganisms, it slows down the enzyme catalyzed reactions and eventually delays the non-enzymatic browning. Another possibility for decreasing a_w and thus for increasing the food shelf life is the use of additives with a higher capacity of keeping the water (sodium, sugar) [1].

Microorganisms develop within large pH limits. When the pH is acid (values of pH<4.5), a small number of microorganisms develops, which can be stopped by thermal treatment [5, 6].

The pH value of soft drinks influences the growing of the microorganisms. The acidifying process - by adding acids - is used in preserving many different types of foodstuffs. The citric acid is one of the acids used to reduce the pH level of soft drinks [7].

It has been identified that the three primary factors controlling the microbial growth are water activity, temperature and pH [8]. Starting from these findings mathematical models were developed in order to predict the combined effect of temperature, pH and water activity on the growth rate of bacteria [9, 10].

The FDA's Good Manufacturing Practice Regulations incorporates water activity guidelines in defining food safety regulation. In food industry, a science based system - Hazard Analysis and Critical Control Points (HACCP) identifies where hazards might occur in the process and puts into place actions to prevent the hazards from occurring.

The purpose of this study is to measure the water activity values and to correlate them with the growth of microorganisms in fruit juices and the presence of different additive substances with preservative proprieties.

EXPERIMENTAL SECTION

Foodstuffs present on the Romanian market were used (Fruttia orange nectar, Crimbo bacon, Miacarn salami, Unirea margarine, FCOJ MTrade Juice, white flour of 000 PF Brudar type, Mib Prodcom strawberry jam, Florisa honey, Price nuts, spices–Mix Vegetal, Cazan pasta, powder eggs, Mio Delicia cookies, toast and powder milk). For each product the a_w was determined.

In order to test the factors that can lead to the decrease of the water activity value, the product with the highest water activity was chosen from the list of the tested products (the soft drink with fruit content – Fruttia orange nectar).

In order to test the influence of the ingredients on the water activity, 7 soft drinks with 50% fruit content were prepared. In the composition of the 7 samples, along with 93.12 g/l orange concentrate, respectively deionized water, the ingredients presented in Table 1 were also added:

Table 1. Ingredients used in preparing the soft drinks with 50% fruit content

Sample	Ingredients	Quantity, g/l
1	(control sample)	-
2	Sugar syrup 65^{0}Brix	120.22 (crystal sugar 78 g)
3	Fructose	50
4	Lemon concentrate	1.2
5	Citric acid sol. 50%	1.6
6	Sodium benzoate	0.15
7	Potassium sorbate	0.15

The ingredients were added under stirring and pasteurized for 30 seconds at 85^{0}C. The each of 7 samples was divided in 4 fractions that were bottled in cold conditions in PET bottles. One fraction (which contains the 7 samples) was immediately analyzed after the bottling, t_{0h}, and the others were kept for 48 hours in closed bottles as it follows: refrigerating (+5^{0}C), at room temperature (+25^{0}C) and in a drying oven at +40^{0}C. All the samples were brought before the analyses at the room temperature.

The water activity (a_w) was determined with the Aquaspector apparatus AQS-2-TC [11] and the *pH* with the ph-metre WTW pH 340i and an electrochemical cell: Electrode SenTix 81. All the analyses were done three times, the final result being the average of the three analyses.

The yeast and the mould (Y&M) were determined through the following procedure: 1 mL of analyzed sample was transferred using a pipette into a Petri plate of 9 cm diameter. 15 ml of cultivation medium *Yeast-Glucose-Chloramphenicol-Agar* (Organics) melted and cooled to 45^{0}C were poured, mixing homogenously. Incubation took place at 25^0±1^{0}C for a period of 3-5 days. The yeasts and mould colonies grown on the cultivation medium could be easily detected on the entire surface of the Petri plate. Evaluation: absent/mL [12].

The total bacteria count (TCB) in the soft drinks with fruit content was calculated by introducing 1 ml of analysed sample into the Petri plate (9 cm in diameter), on which 12-15 ml of *Orange Serum Agar* (Merck) culture, melted and cold at 45^{0}C is added. The content is mixed up by stirring. The incubation is done at 30^{0}C for 72±2 hours. After incubation period all the colonies growth on the Petri plate are counted. Evaluation: maximum 30 CFU/ml [13].

The coliform bacteria (Colif.) were determined with the following procedure: 1 mL sample was transferred to a 9 cm-diameter Petri plate using a pipette. 12 ml of the cultivation medium *Violet red bile lactose agar (VRBL agar)* melted and cooled to 45^{0}C were poured, mixing homogenously. After the complete solidification of cultivation medium, 4 ml *VRBL agar* was added on the surface of existing medium. Incubation took place at 30^0±1^{0}C for 24±2 hours. The dark-red colonies with at least 0.5 mm diameter are counted. Evaluation: absent/mL [14].

RESULTS AND DISCUSSION

The values of the water activity for the studied food products are presented in Table 2, in a decreasing order of the water activity. This order was established on the basis of a qualitative sensitivity analysis [15].

Table 2. Values of the water activity index for different types of food

No.	Food	Water activity, a_w
1.	Fruttia orange nectar	0.951
2.	Crimbo bacon	0.942
3.	Miacarn salami	0.908
4.	Unirea margarine	0.887
5.	FCOJ MTrade Juice	0.813
6.	000 PF Brudar white flour	0.854
7.	Mib Prodcom strawberry jam	0.796
8.	Florisa honey	0.688
9.	Price nuts	0.639
10.	Mix Vegetal spices	0.504
11.	Cazan pasta	0.481
12.	Powder eggs	0.427
13.	Mio Delicia cookies	0.350
14.	Toast	0.336
15.	Powder milk	0.292

The effect of the ingredients used for preparing the soft drinks was studied both by analysing the physico-chemical parameters: a_w and pH (Table 3) and by microbiological control of the samples (Table 4).

Table 3. The water activity value and the pH of the samples kept in different conditions

Sample	a_w				pH			
	$t_{0\,h}$	$t_{48\,h}$			$t_{0\,h}$	$t_{48\,h}$		
		+5^{0}C	+25^{0}C	+40^{0}C		+5^{0}C	+25^{0}C	+40^{0}C
1	0.945	0.947	0.997	0.950	3.802	3.761	3.714	3.608
2	0.934	0.953	0.986	0.980	3.737	3.587	3.612	3.400
3	0.925	0.932	0.983	0.963	3.734	3.694	3.445	3.413
4	0.910	0.916	0.947	0.924	3.387	3.390	3.328	3.232
5	0.907	0.909	0.922	0.913	3.314	3.245	3.293	3.155
6	0.911	0.914	0.918	0.917	3.701	3.640	3.681	3.464
7	0.906	0.910	0.914	0.910	3.694	3.461	3.440	3.406

The physico-chemical analyses performed immediately after preparation are presented comparatively to the ones carried out after 48 hours of keeping at different temperatures. According to the storage temperature, we can notice an increase of the a_w on the whole, but more emphasized at the samples kept at the room temperature. The pH values have a slight tendency to decrease in time.

Table 4. The result of the microbiological control of the samples kept in different conditions

Sample	$t_{0\,h}$			$t_{48\,h}$ (+5^{0}C)			$t_{48\,h}$ (+25^{0}C)			$t_{48\,h}$ (+40^{0}C)		
	Y&M	TCB	Colif.	Y&M	TCB	Colif.	Y&M	TCB	Colif.	Y&M	TCB	Colif.
1	>1000	>300	Abs	>300	4	Abs	>500	18	Abs	>1000	>1000	Abs
2	100	>300	Abs	25	Abs	Abs	64	>300	Abs	>300	>300	Abs
3	>300	40	Abs	48	Abs	Abs	100	50	Abs	>300	39	Abs
4	2	23	Abs	Abs	Abs	Abs	2	32	Abs	5	26	Abs
5	9	9	Abs	1	Abs	Abs	5	Abs	Abs	>300	1	Abs
6	4	18	Abs	Abs	2	Abs	4	3	Abs	>300	8	Abs
7	3	4	Abs	Abs	Abs	Abs	2	1	Abs	35	6	Abs

At room temperature and at higher temperatures (+40^{0}C) the enzymatic and microbiological reactions take place faster, which leads to the fast degradation of the samples, as compared to the refrigerated samples.

Sodium benzoate, potassium sorbate, citric acid or lemon concentrate delay the growth of the microorganisms. The experimental data show that pH and water activity values decrease when citric acid or lemon concentrate are added as acidifiants to the fruit juice (sample 4 and 5). The microbiological values, shown in Table 4, in correlation with water activity and pH values confirm that it's useful to add acidifiants and preservatives to the fruit juice.

The lemon concentrate (sample 4) - as a natural source of citric acid - can be used in juices with high content of fruit as a natural acidifier which also inhibit the microorganisms growth.

The soft drinks with fruit content have a reduced pH level and contain considerable amounts of carbohydrates (sample 2 and 3), so that they become favourable selective environments for yeast growth. The alterations can be due to the yeast and bacteria that can come from sugar or microbiota of soft drinks.

The quality management applications (TQM-total quality management) and the aspects related to food safety are at the moment a global concern that continues to increase due to their impact on international trade [16]. Thus, the HACCP system is based on preventing and reducing the risks and dependence on inspection and end-product testing [17]. A practical approach of the studies, involves a voluntary control of the stage corresponding to the storage of the end-product before bottling **by preparing the sheet that tests the water activity value a_w.** According to the obtained product and based on the literature and history data (antecedents) the limiting value of a_w can be established in the company (regarding the studied soft drinks and taking into consideration the thermal treatment carried out, the maximum value of a_w was set at 0.910).

The plan for this new stage is presented in Table 5:

Table 5. The analysis and the evaluation of the hazards of the cooling/storage stage of the soft drinks

Hazard						Means of administration	Preventive/control measures	Document registration	Responsible
Type	Description	Acceptable level	G[4]	F[5]	R[6]				
B[1]	Y&M	30	Med	Low	2	PRP[7] Hygiene plan	-Respecting and monitoring the temperature conditions when it is cold and the water activity index ≤ 0.910 when it is stored -Sensor – specific calibration -Personal instruction	-Chart to monitor the water activity -PLC[8] memory	Production responsible
	Colif. bacteria	Abs	Med	Low	2				
	TCB	$2/cm^2$	Med	Low	2				
	Staphylococcus aureus	Abs	Med	Low	2				
C[2]	Traces of washing powders / disinfectants	Abs	Low	Low	1				
F[3]	Fitting remains	Abs	Low	Low	1	Maintenance plan			
	Hairs (from the operator)	Abs	Low	Low	1	Hygiene plan			
	Traces of vaseline / lubricant	Abs	Low	Low	1	Maintenance plan			
	Glass pieces	Abs	Med	Low	2				

B[1]-biological, C[2]-chemical, F[3]-physical, G[4]-gravity, F[5]- frequency of appearance, R[6]-risk, PRP[7]-preliminary programme, PLC[8]-Programme Logical Controller, Med-medium

The analysis of this plan leads to idea that the cooling/storage of soft drink constitutes PCC – critical control point with biological, chemical and physical risk for the safety of the food product, imposing monitoring at each charge. Based on this study, as well as on the analysis and evaluation of the hazards that may occur during the production of 50% fruit soft drinks, it has concluded that the cooling/storage stage of end-product must be integrated in the HACCP plan. Thus, a new phase of a_w monitoring is proposed in the flow sheet. It is presented in Figure 1.

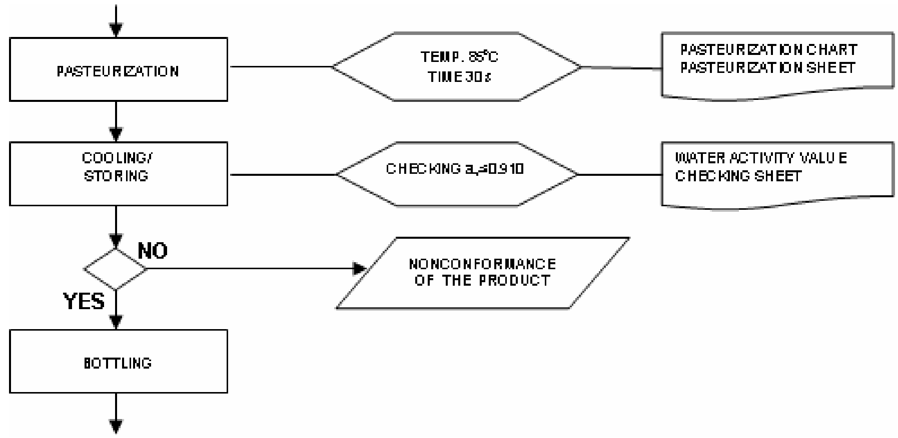

Figure 1. Flow sheet chart that include a_w monitoring.

The inconvenience of this flow sheet chart is that the final product that doesn't have the appropriate value of a_w is not recycled. The cyclicity of the process is not possible because the soft drink quality would be considerably reduced by another thermal treatment.

CONCLUSIONS

The acidifiers, such as citric acid and lemon concentrate, as well another known additives with preservative proprieties are inhibitors of the microorganisms growth. Their functionality is under the influence of product pH and the conservation temperature, factors which contribute to the stability of the drink. The water activity can be reduced by replacing sugar with fructose or artificial sweeteners.

The monitoring of the water activity value is favorable and adequate for using it as a general method for ensuring the sanitation of soft drinks. The control of free water amount constitutes the only practical mechanism for ensuring food safety.

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