Original scientific paper
UDC 663.8

THE QUALITY OF NON-CARBONATED NON-ALCOHOLIC BEVERAGES DURING THE SHELF LIFE

Dragutin Djukic[1*], Slavica Veskovic Moracanin[2], Leka Mandic[1], Jelena Atanaskovic[1]

[1]Faculty of Agronomy Cacak, University of Kragujevac, Cara Dusana 34, 32000 Cacak, Serbia
[2]Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Serbia

[*]e-mail: lekamg@kg.ac.rs

Abstract

The manufacture of safe products that have important quality parameters and the preservation of these requirements during the specified shelf life are the main tasks of any manufacturer. To meet these requirements, it is necessary to implement appropriate manufacturing practices and adhere to the prescribed regulations.

The aim of this study was to investigate the microbiological safety and sensory properties of seven types of non-carbonated non-alcoholic beverages (I - VII), produced by the Knjaz Miloš AD Company, Arandjelovac, Serbia, containing different ingredients (I: peach flavouring, citric acid, a mixture of vitamin B, sugar, potassium sorbate and sodium benzoate; II: herbal extracts of guarana and lemon grass, apple, grape and lemon juices, citric acid, caffeine, vitamins and lemon flavouring; III: inulin and extract of *Aloe vera*, apple, orange, pineapple, mango, passion fruit, kiwi and papaya juices, citric acid, L-ascorbic acid, melon flavouring and preservatives; IV: concentrated apple, grape and lemon juices, citric and L-ascorbic acids, juniper, gentian and lavender flower extracts, cherry flavouring and preservatives; V: Cambogia Garcinia extract, fructose, L-carnitine, Mg-citrate, vitamins, lemon, lime juice, green tea, and caffeine flavourings; VI: sugar, Mg and Ca-lactate, citric acid, preservatives and flavouring agents; VII: concentrated apple juice, Mg and Ca-lactate, sugar, citric acid, preservatives and flavourings). Samples were kept in the original packaging, at different storage temperatures (20, 25, 30 and 42 ^{0}C). Microbiological tests were carried out according to the national legislation on safety and non-alcoholic beverages, which is in accordance with both ISO methodology (*Enterobacteriaceae*, yeasts, fungi, aerobic mesophilic bacteria) and internal control requirements set down by the company (coagulase positive staphylococci, sulphate-reducing clostridia, E. *coli*, acid-tolerant microorganisms). All analyses were carried out at succesive ten-day intervals, during three months.

At the end of the production, all samples were microbiologically safe. The presence of lactic acid bacteria was not recorded in the tested samples during the three months of storage. The number of aerobic mesophilic microorganisms significantly increased in beverage I (20 days at 25 ^{0}C), IV (20 and 50 days at 20 ^{0}C and 90 days at 25 ^{0}C), and V (50 and 80 days at 20 ^{0}C). The total numbers of mould and yeast, and acid-tolerant microorganisms significantly increased only in the samples of beverage I (90 days at 25 ^{0}C) and III (10 days at 25 ^{0}C). Other microbiological results were within the prescribed limits. Throughout the study, sensory test results for all samples showed normal quality.

***Key words**: Non-alcoholic beverages, Microbiological and sensory testing, Shelf life.*

1. Introduction

Given the markedly growing food and water requirements, the production of non-carbonated non-alcoholic beverages is increasingly gaining importance. Water is a major component of food. As a sort of original matter transferred into life, water is the main constituent of living organisms (Schmoll *et al*. [1]).

Daily water requirements for adults are about 2.5 L, serving to balance the loss of the same amount of water from the body (400 mL lost as water vapour through exhalation, 600 mL through the skin by evaporation, 800 - 1300 mL through urine output, and 200 mL through faeces). This, however, does not imply that the said amount of water comes solely from drinking water; more particularly, water is a component of the food consumed, and part of water is metabolic water produced within the body i.e. endogenic water (Veselinović *et al*. [2]).

Water is involved in all life processes within the human body, and is as important as air (Tenge and Geiger [3], Đarmati *et al*. [4]). Water acts as a superb solvent for food and plays a role in digestion and thermoregulation. Therefore, the production of bottled water and

functional non-carbonated beverages is gaining increasing importance (Lawlor *et al*. [5]).

Water loss results in an increase in body temperature, skin redness, slow heart rate, rapid breathing, muscle weakness, vertigo, fainting, headache and, even, death.

Therefore, the production of microbiologically safe non-carbonated non-alcoholic beverages supplemented with different functional ingredients has been of great importance, particularly in recent years when water-borne epidemics cause death of 26,000 children around the world every day (Đukić and Ristanović [6]). The most commonly used supplements include extracts of different fruits and other plants, vitamins, minerals, sugars, antioxidants and other functional compounds added to enhance flavour and nutritional quality (Tribst *et al*. [7]). In addition to being beneficial, these supplements can have an adverse effect once they become a substrate for microbial growth during product storage, particularly long-term storage under inappropriate conditions (Moore *et al*. [8, 9], Criado *et al*. [10], Korzeniewska *et al*. [11]). Storage temperature and light conditions are major factors contributing to preserving the quality and microbiological safety of bottled waters and functional non-carbonated beverages.

The objective of this study was to examine the effect of storage temperature and storage time on the microbiological safety and sensory properties of different non-carbonated non-alcoholic beverages manufactured by the Knjaz Miloš A.D. Company, Aranđelovac, Serbia.

2. Materials and Methods

This study examined the microbiological safety and sensory properties of seven types of non-carbonated refreshing non-alcoholic beverages produced by the Knjaz Miloš A. D. Company, Aranđelovac, Serbia and supplemented with different ingredients (I - citric acid, a vitamin B mix, sugar, potassium sorbate, sodium benzoate and peach flavouring; II - guarana and lemon grass extracts, apple, grape and lemon juices, citric acid, caffeine, vitamins and lemon flavouring; III - inulin and Aloe vera extract, apple, orange, pineapple, mango, passion fruit, kiwi and papaya juices, citric acid, L-ascorbic acid, melon flavouring and preservatives; IV - concentrated apple, grape and lemon juices, citric acid, L-ascorbic acid, juniper, gentian and lavender flower extracts, sour cherry flavouring and preservatives; V - Garcinia Cambogia extract, fructose, L-carnitine, Mg citrate, vitamins, lemon, lime, green tea and caffeine flavourings; VI - sugar, Mg and Ca lactate, citric acid, preservatives and flavouring; VII - concentrated apple juice, Mg and Ca lactate, sugar, citric acid, preservatives and flavouring).

The tested samples of non-carbonated non-alcoholic beverages were kept in the original packaging under different storage temperatures (20, 25, 30, 42 ^{0}C) over a period of three months. Microbiological tests were carried out in accordance with the national legislation on the safety of non-alcoholic beverages (Official Gazette of SFRY [12], and Official Gazette of SRY [13]), ISO methodology (*Enterobacteriaceae*, yeasts, moulds, aerobic mesophilic bacteria) and internal control requirements set down by the company (coagulase positive staphylococci, sulphite reducing clostridia, *E. coli*, acid-tolerant microorganisms). The beverages were also tested for their sensory attributes viz. colour, aroma, flavour and texture (Trajković *et al*. [14]). The analyses were carried out at successive ten-day intervals over a period of three months.

3. Results and Discussion

At the beginning of the production, all samples (I through VII) were microbiologically safe. The presence of lactic acid bacteria was not recorded in any of the tested samples during the 90 days of storage at different incubation temperatures (Table 1). The count of saprophytic mesophilic microorganisms significantly increased only in product I, at 20 days of incubation at a temperature of 25 ^{0}C; in product IV at 20 and 50 days of incubation at 20 ^{0}C and at 19 days of incubation at 25 ^{0}C; in product V at 50 and 80 days of incubation at 20 ^{0}C. The counts of saprophytic microorganisms in products VI and VII were low at all dates and at all incubation temperatures. Total numbers of moulds, yeasts and acid-tolerant microorganisms increased significantly only in product I (after 90 days of incubation at 25 ^{0}C) and product III (after 10 days of incubation at 25 ^{0}C).

Table 1. Effect of storage time and temperature on the count of some groups of microorganisms (SM - Saprophytic mesophilic microorganisms, MY - Moulds and yeasts, ATM - Acid-tolerant microorganisms and LAB - Lactic acid bacteria) in non-carbonated nonalcoholic beverages (products I through VII)

Storage time, day	Storage temperature, ^{0}C	Types of microorganisms	Microbial count in the tested samples, cell/100mL						
			I	II	III	IV	V	VI	VII
10	20	SM	0	0	0	0	0	0	0
		MY	2m	0	0	0	0	0	0
		ATM	4m	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0

10	25	SM	0	0	0	0	15	0	0
		MY	6m	5m	16m	14m	0	0	0
		ATM	5m	1m	28m	9m	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	1	0
		MY	1m	0	0	0	0	0	0
		ATM	0	0	0	1m	0	0	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	0	0	0	0	0	0
		MY	1m	1m	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
20	20	SM	0	0	0	20	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	0	0	0	0	0	0	0
		MY	1m	0	0	7m	0	0	0
		ATM	0	1m	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	0	0
		MY	0	2m	0	7m	0	0	0
		ATM	3y	0	3y	0	1m	0	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	2	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
30	20	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0

40	42	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	20	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	1m
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	1	0	0	0	0	0	1
		MY	0	0	0	1y	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	0	0
		MY	0	0	0	1y	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
50	20	SM	0	0	0	50	28	0	2
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	22	0	0	0	0	3	10
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	1	1
		MY	0	0	0	0	0	1m	0
		ATM	0	0	0	0	0	1m	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
60	20	SM	0	0	0	0	0	2	0
		MY	0	0	2y	1y	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0

60	25	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	1	2	0	0	0	1	5
		MY	0	0	20y	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	0	0	0	0	0	0
		MY	0	0	4y	0	0	0	0
		ATM	0	0	4y	0	0	0	0
		LAB	0	0	0	0	0	0	0
70	20	SM	0	0	0	0	0	1	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	0	0	0	0	0	1	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	1	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	0	0	0	0	0	1
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
80	20	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	0	0	0	0	30	0	1
		MY	0	0	0	0	1m	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	1m	0	0
		ATM	0	0	7y	0	0	0	0
		LAB	0	0	0	0	0	0	0

80	42	SM	0	0	0	0	0	0	0
		MY	0	2m	0	0	0	0	0
		ATM	3m	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
90	20	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	25	SM	0	0	0	50	0	0	0
		MY	20m	0	0	0	0	0	0
		ATM	20m	1y	0	0	0	0	1m
		LAB	0	0	0	0	0	0	0
	30	SM	0	0	0	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	0	0	0	0	0
		LAB	0	0	0	0	0	0	0
	42	SM	0	0	1	0	0	0	0
		MY	0	0	0	0	0	0	0
		ATM	0	0	2y	0	0	0	0
		LAB	0	0	0	0	0	0	0

y - Yeasts.

m - Moulds.

Product I - contains water, sugar, a vitamin mixture (vitamin B complex), citric acid, potassium sorbate, sodium benzoate, peach flavouring; Product II - contains water, concentrated apple, grape and lemon juices, citric acid, preservatives, guarana and lemongrass extracts, caffeine, vitamins (pantothenic acid, B_6, B_{12}) and lemon flavouring; Product III - contains water, concentrated apple, orange, pineapple, mango, passion fruit, kiwi and papaya juices, inulin, citric acid, antioxidant L-ascorbic acid, preservatives, *Aloe vera* extract and melon flavouring; Product IV - contains water, concentrated apple, grape and lemon juices, citric acid, preservatives, antioxidant L-ascorbic acid, juniper, gentian and lavender flower extracts, sour cherry flavouring; Product V - contains water, fructose, L-carnitine, Garcinia Cambogia extract, magnesium citrate, preservatives, vitamins (niacin, pantothenic acid, B_6, B_{12}) and lemon, lime, green tea and caffeine flavourings; Product VI - contains water, sugar, magnesium lactate, calcium lactate, citric acid, preservatives (sodium benzoate, potassium sorbate), flavouring. Product VII - contains water, sugar, concentrated apple juice, Mg lactate, Ca lactate, citric acid, preservatives (Na benzoate, K sorbate), flavouring.

Data on the effect of different storage conditions on the growth of certain groups of microorganisms in bottled waters are in agreement with the findings of other authors (Tsai and Yu [15], Armas and Sutherland [16], Leclerc and Moreau [17]). The most common contaminants of these products include *Pseudomonas, Alternaria, Cladosporium, Paecilomices* and *Penicillium* species. Many researchers stressed the importance of temperature and storage time to the microbiological safety of non-carbonated nonalcoholic beverages (Criado *et al*. [10], Korzeniewska *et al*. [11]). Storage time has the strongest effect on the germination and growth of mould spores in bottled mineral waters (Criado *et al*. [10]). The authors also found that their growth can be promoted, inter alia, by dissolved compounds from plastic bottles developing during long-term storage. Moreover, an increase in the count of certain microorganisms in non-carbonated non-alcoholic beverages can also be due to the presence of different functional supplements (sweeteners, fruit juices, flavourings, colours, vitamins, antioxidants etc.) which can serve as their food or as regulators of metabolic processes (Statford and James [18], Ewe *et al*. [19]). Storage temperature is another parameter inducing an increase in microbial counts in bottled waters and non-carbonated non-alcoholic beverages (Nsanze *et al*. [20]; Korzeniwska *et al*. [11]). In general, low temperatures (4°C) reduce microbial growth, whereas the most rapid development of microorganisms occurs at storage temperatures in the range 25 - 37 ^{0}C.

The sensory properties of all tested samples during the three months of incubation under different temperatures (20, 25, 30, 42 ^{0}C) did not show significant changes. Slight changes were observed only in terms of turbidity in a few products, without any substantial loss of their quality.

4. Conclusions

The results of the study on the newly developed non-carbonated refreshing non-alcoholic beverages of the Knjaz Miloš Company suggest the following:

- All beverage samples were microbiologically safe i.e. no pathogenic microorganisms or microorganisms that can potentially affect consumer health were detected.

- Lactic acid bacteria were not detected in any of the products tested over a period of three months; in contrast, the counts of other acid-tolerant microorganisms, moulds and yeasts significantly increased in products I and III under room temperature, without degradation in their quality.

- Sensory properties (colour, aroma and flavour) during the three months of testing did not undergo substantial changes, thus suggesting microbial safety and good quality of the products tested.

- In order to keep the count of saprophytic mesophilic microorganisms and sensory attributes of the products within reference values, it is necessary to control the microbiological safety of air at critical stages in the technological process and the microbiological safety of water and inputs on a regular basis, and store end products under adequate conditions (low temperature).

5. References

[1] Schmoll O., Howard G., Chilton J., Chours I. (Eds). (2006). *Protecing Groundwater for Health*. World Health Organization (WHO).

[2] Veselinović D., Gržetić I., Đarmati Š., and Marković D. (Eds). (1995). *Physico-chemical basis of environmental protection* (in Serbian). Fakultet za fizičku hemiju, Beograd

[3] Tenge C., and Geiger L. (2001). *Alternative functional beverages*. MBAA Tech. Quart, 38, pp. 33-35.

[4] Đarmati Š., Veselinović D., Gržetić I., and Marković D. (Eds). (2007). *The environment and its protection* (in Serbian). Životna sredina, Fakultet za primenjenu ekologiju Futura, Beograd (in Serbian).

[5] Lawlor K., Schuman J., Simpson P., and Taormina J. (2009). In: Sperber W. H., and Doyle M. P. (Eds.), Compendium of the Microbiological Spoilage of Foods and Beverages, Food Microbiology and Safety, Springer, New York, USA, pp. 245-283.

[6] Đukić A. D., Ristanović V. (eds). (2005). *Water chemistry and microbiology* (in Serbian). Stslos, Novi Sad, Serbia.

[7] Tribst A. A. Sant'Ana Ade S., and de Massaguer P. R. (2009). *Microbiological quality and safety of fruit juices-past, present and future perspectives*. Critical Reviews in Microbiology, 35, pp. 310-339.

[8] Moore J. E., McCalmont M., Xu J., Millar B. C., and Heaney N. (2002). *Asaia sp., an unusual spoilage organism of fruit-flavored bottled water*. Applied and Environ-mental Microbiology, 68, pp. 4130-4131.

[9] Moore J. E., Xu J., Heaney N., and Millar B. C. (2002). *Spoilage of fruit-flavoured bottled water by Gluconacetobacter sacchari*. Food Microbiology, 19, pp. 399-401.

[10] Criado M. V., Fernández Pinto V. E., Badessari A., and Cabral D. (2005). *Conditions that regulate the growth of moulds inoculated into bottled mineral water*. International Journal of Food Microbiology, 99, pp. 343-349.

[11] Korzeniewska E., Filipkowska Z., Domeradzka S., and Wlodkowksi K. (2005). *Microbiological quality of carbonated and non-carbonated mineral water stored at different temperatures*. Polish Journal of Microbiology, 54, pp. 27-33.

[12] Official Gazzete of SFRY. (1980). *Regulation on methods of performing microbiological analyzes and superanalyses on food* (in Serbian). Official Gazzete of SFRJ, No. 25/80.

[13] Official Gazzete of SRY. (2010). *Regulation on the general and special conditions of hygiene of food at any stage of production, processing and trade* (in Serbian). Official Gazzete of Republic of Serbia No. 72/10.

[14] Trajković J., Mirić M., Baras J., Šiler S. (1983). *Analysis of food* (in Serbian). Tehnološko-metalurški fakultet, Beograd, Serbia.

[15] Tsai G., and Yu S. (1997). *Microbiological evaluation of bottled uncarbonated mineral water in Taiwan*. International journal of food microbiology, 37, pp. 137-143.

[16] Armas A. B., and Sutherland J. P. (1999). *A survey of the microbiological quality of bottled water sold in the UK and changes occurring during storage*. International Journal of Food Microbiology, 48, pp. 59-65.

[17] Leclerc H., and Moreau A. (2002). *Microbiological safety of natural mineral water*. FEMS Microbiology Reviews, 26, pp. 207-222.

[18] Statford M., and James S.A. (2003). *Non-alcoholic beverages and yeasts*. In: Boekhout T., and Robert V. (Eds.), Yeasts in Food, B. Behr's Verlag GmbH & Co, Hamburg, Germany, Chapter 12, pp. 309-345.

[19] Ewe J. A., Wan-Abdullah W. N., and Liong M. T. (2010). *Viability and growth charasteris-tics of Lactobacillus in soymilk supplemented with B-vitamins*. International Journal of Food Science and Nutrition, 61, pp. 87-107.

[20] Nsanze H., Babarinde Z., and H. Kohaly H. A. (1999). *Microbiological quality of bottled drinking water in the UAE and the effect of storage at different temperatures*. Environment International, 25, (1), PP. 53-57.