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EVALUATION OF MICROBIOLOGICAL QUALITY OF FRUIT JUICE (FROM A SINGLE FRUIT)

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Abstract

Today fruit juices have become a part of our daily lives, because of the variety and convenience of the packaging in which they are offered. The safety of fruit juices is an important factor, as they are very susceptible to spoilage. The purpose of the study is to evaluate the microbiological quality of some samples of fruit juices in the market of Albania.

The study was based only on fruit juices taken from a single fruit (peach, orange, strawberry, banana and cherry), which were taken from three types of containers of different materials: polyethylene terephthalate (PET), aluminum cans and Tetra Pack. These samples were taken randomly and analyzed directly in the laboratory in June of last year. Microbiological analyses were performed directly on the fruit juices without dilution. Microbiological evaluation was performed in different media for bacterial total microflora, moulds and yeasts using classical methods. After the incubation period, enumeration of the colony forming units (CFU) and species identification were performed.

The total microbiological load in all analyzed fruit juices was less than 0 - 100 CFU/mL, which indicates their good quality. Although in some samples the presence of some bacteria was observed, such as: *Bacillus* spp., *Micrococcus* spp., *Pseudomonas* spp. and moulds as: *Cladosporium* spp., it is worth noting that the yeasts did not appear to be present in any of the samples taken for analysis in this study.

Results obtained show a good microbiological quality of the fruit juices that have been analyzed. However, the presence of such microorganisms in certain samples indicates that it is important to evaluate the microbial load of the raw material and/or hygiene of equipment after pasteurization, in order to ensure the microbiological quality of fruit juices.

***Key words**: Fruit juice, Safety, Microbiological quality, Bacteria, Mould, Yeast.*

1. Introduction

Fruit juice is the unfermented, but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or of fruit maintained in sound condition by suitable means including post harvest surface treatments applied in accordance with the applicable provisions of the Codex Alimentarius Commission [10]. Fruit juices are considered to be rich in diverse sources of vital nutrients which include vitamins like A, B, C, folate; minerals like iron, copper, potassium, iodine, zinc, selenium, iodine, sulfur, manganese, boron, molybdenum and magnesium; dietary fiber and antioxidants, amino acids, and bioactive compounds phytonutrients which are crucial for good nutrition, disease prevention and offer great taste and health benefits [6].

Quality of fruit juice is influenced by approaches exploited to: harvest fruits, store and deliver fruit raw materials, fruit selection, screening, washing, drying, peeling/deseeding, pulp maceration, pressing/squeezing, pasteurization, clarification, drying or concentration, storage, and packing. Whatever, the extraction method or conditions applied, the juice should retain necessary physical, chemical, sensory, organoleptic and nutritional characteristics of the fruit it comes from [4].

Most fruit juices contain sufficient nutrients that could support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in commercialized juices; the most important are: pH value, water activity (a_w), quality of raw material, hygiene practice, processing method presence of preservatives, packaging material, and storage conditions [1].

The type of microorganisms in fruit juice is mostly consisted by the microorganisms on/in the respective fruits. For unpasteurized fruit juices, presence of high level of microorganisms in the raw materials, i.e. the

use of inferior quality of fruits will results in early detection of spoilage. The quality of water added or used for reconstitution of fruit juice influence quality and safety of the juices. Fruits commonly carry mold, yeasts and bacteria [7].

Fruits processed as fruit concentrates, jellies, syrups or similar products have reduced water activity achieved by sufficient sugar addition and heating at 60 - 82 ^{0}C, which kills most of xerotolerant fungi as well as restrains the growth of bacteria. Thus, the normal microflora of such diligently processed fruit products may include highly osmophilic yeasts and certain endospore-forming *Clostridium*, and *Bacillus* spp. that withstand canning procedures. Similar flora may appear for processed and pasteurized fruit juices and nectars that loose most vegetative bacteria, yeasts, and molds while retaining heat-resistant ascospores or sclerotia producing *Paecilomyces* spp., *Aspergillus* spp., and *Penicillum* spp. or endospore-forming bacteria such as *Alicyclobacillus acidoterrestris* [3], or unless the finished product is contaminated after pasteurization due to inappropriate packaging [5].

In the industry, the aim of thermal pasteurization is not to kill all microorganisms in foods; the target is to destroy pertinent pathogens and lower levels of spoilage organisms that may grow during storage and distribution [8]. A 5-log reduction can be considered for all processes that aim to reduce the microbial count. Thus, the shelf life of thermally processed fruit juices can be extended for several months without safety concerns or important quality losses at low or room temperatures.

Specially yeast and molds are the dominant microorganisms in packed juice pasteurized because they can thrive the high acidic conditions of the juice. Some examples of them are species of the following genera: *Cladosporium*, *Candida*, *Dekkera*, *Pichia*, *Saccharomyces*, *Aspergillus*, *Zygosaccharomyces*, *Penicillium*, *Byssochlamys*, *Hanseniaspora*, *Paecilomyces*, *Mucor*, *Fusarium*, *Botrytis*, and *Neosartorya*, *Talaromyces*, etc. Some lactic acid and acetic acid bacteria may be present in fruit juices. Some pathogenic bacteria like *Escherichia coli* O157, *Salmonella*, and *Cryptosporidium,* fecal streptococci and some spore formers like *Clostridium pasteurianum* and *Bacillus coagulans* may be present in fruit juice if the juice is not processed adequately [9].

The aim of this study is to evaluate the microbiological quality of some samples of fruit juices in the market of Albania.

2. Materials and Methods

Fifteen fruit juice samples were collected from different markets of Tirana city. After collection, the samples were transferred immediately to the laboratory for analysis of microbial quality by following the procedures below. The study was based only on fruit juices taken from a single fruit (peach, orange, strawberry, banana, and cherry), which were taken from three types of containers of different materials: polyethylene terephthalate (PET), aluminum cans, and Tetra pack. Samples taken for analysis are pasteurized fruits juices that are written on their packaging.

Determination of microbial load of the samples was done based on the classic method as decimal dilution followed by quantitative determination of microorganisms. Exactly 1 mL of each fruit juice without dilution was evenly spread (in duplicates) on medium [2] and incubated at 37 ^{0}C for 24 hours and/ or 30 ^{0}C for 5 - 7 days. Plates were screened for the presence of colonies after the incubation period. We counted colonies formed and total numbers were estimated as colony forming units per mL (CFU/mL). The results were organized in the respective tables for each sample in the three types of containers from different materials.

The load of specific microorganisms was determined by plating on standard media as: plate count agar (PCA) for total aerobic mesophilic bacteria, potato dextrose agar (PDA) for yeasts, Czapek for moulds, and selective media Mc Conkey for coliforms bacteria and other enterobacteria.

Taxonomic identifications of different genera of microorganisms were made according to the microscopic criteria and observation of the morphological characteristics of the developed colonies and their main physiological characteristics. Microorganisms were identified visually and where applicable, by means of a magnifying glass or a stereomicroscope. Closer characterization was possible using a light-optical microscope. In this case, selected parts of colonies developed at Petri dishes were prepared various microscopic slides.

3. Results and Discussion

Results from microbiological analysis of all fruit juice are presented in Table 1. Also, this table presents the total number of microorganisms for all fruit juices in each package. Microbiological evaluation focused on determining: fungi, yeasts, and bacteria if they were present in the analyzed fluids.

From the fruit juices analyzed in three packages (Tetra Pack, Aluminum cans, PET) were taken different microorganisms colonies, dominated by bacteria and mould microorganisms, while yeast was not observed.

It is seen that fruit juices with the highest total number of microorganisms are the ones in the aluminium cans

Table 1. Mean microbial count (cfu/mL) of fruit juices in three packages

Fruit juice	Tetra Pack				Aluminum cans				PET			
	Czapek	PCA	PDA	Mac Conkey	Czapek	PCA	PDA	Mac Conkey	Czapek	PCA	PDA	Mac Conkey
Peach	n.d.*	n.d.*	n.d.*	22	n.d.*	1	1	57	n.d.*	n.d.*	1	n.d.*
Orange	9	13	n.d.*	n.d.*	1	92	n.d.*	n.d.*	n.d.*	3	n.d.*	n.d.*
Strawberry	n.d.*	n.d.*	1	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	1	n.d.*	n.d.*	n.d.*
Banana	2	n.d.*	n.d.*	n.d.*	1	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*	n.d.*
Cherry	n.d.	n.d.*	n.d.*	n.d.*	1	8	n.d.*	n.d.*	n.d.*	n.d.*	3	n.d.*
Total colonies	**11**	**13**	**1**	**22**	**3**	**100**	**1**	**57**	**1**	**3**	**4**	**0**
Total no. MO**	**47**				**161**				**8**			

Legend: *n.d. = not detected; **MO = microorganisms.

packaging. It is noted that for all three packages the highest number of total microorganisms is mesophilic aerobic bacteria in the PCA medium, followed by coliforms bacteria and other enterobacteria in the Mac Conkey medium.

The number of colonies of bacteria and moulds for each package and each fruit juice: peach, orange, strawberry, banana and cherry are shown in Figures 1 - 5.

In the peach juice, the highest microbiological load has been shown by sample in the aluminum can packaging, followed by the sample in the Tetra Pack packaging, and in both cases it is the bacteria of the *Enterobacteriaeriaceae* family that dominate. This may be related mainly to the lack of correctness in the application of hygienic measures during the stages of the process and to the microbiological quality of the raw materials from which this product is obtained.

From Figure 2 we can see that in the orange juices the highest microbiological load has been shown by the sample in the aluminum can packaging, which is the most contaminated (with the higher number of bacteria and mould) sample of all the analyzed samples. This sample is dominated by bacteria: *Pseudomonas* spp., *Bacillus* spp. and from molds as: *Cladosporium* spp. Bacteria of *Pseudomonas* spp. are part of the plant pathogens together with *Bacillus* spp., so we can understand that the raw material used in this case is not of good quality. This is reinforced by the presence of the fungi as *Cladosporium* spp., by which we conclude that the pasteurization process has not been realized as expected, given that this mold is sensitive to heat or is indicative of contamination after pasteurization or comes from the use of highly contaminated fruits as raw material.

From Figures 3 and 4 we can see that all the samples of strawberry and banana juices in all the packaging have an almost negligible microbiological load. It is noted that in strawberry samples we have only one mould colony as *Cladosporium* spp. to the liquid in the Tetra

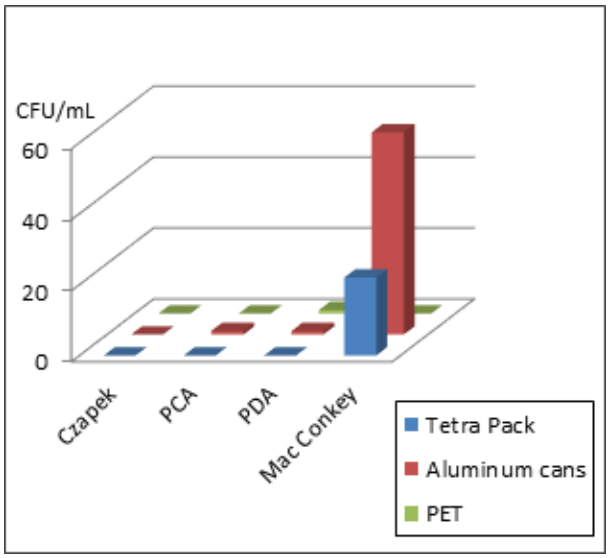

Figure 1. The number colonies of bacteria and moulds for each medium in peach juices

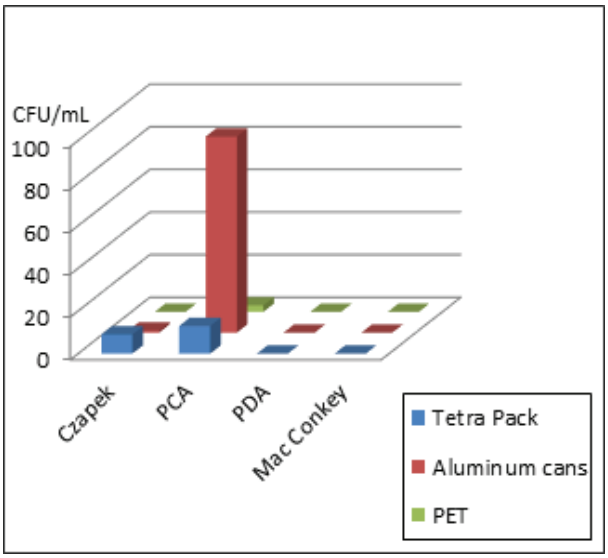

Figure 2. The number colonies of bacteria and moulds for each medium in orange juices

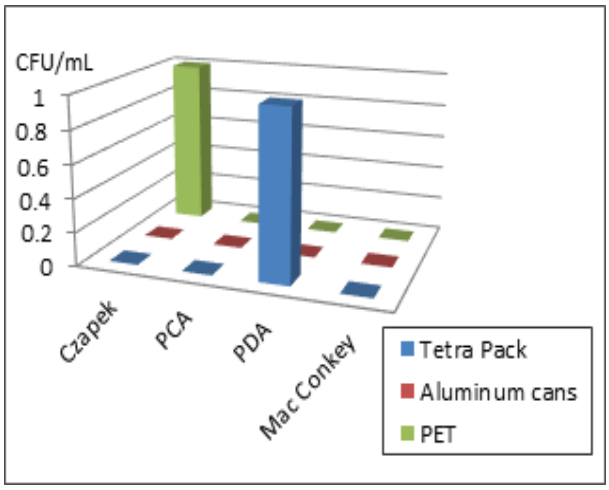

Figure 3. The number colonies of bacteria and moulds for each medium in strawberry juices

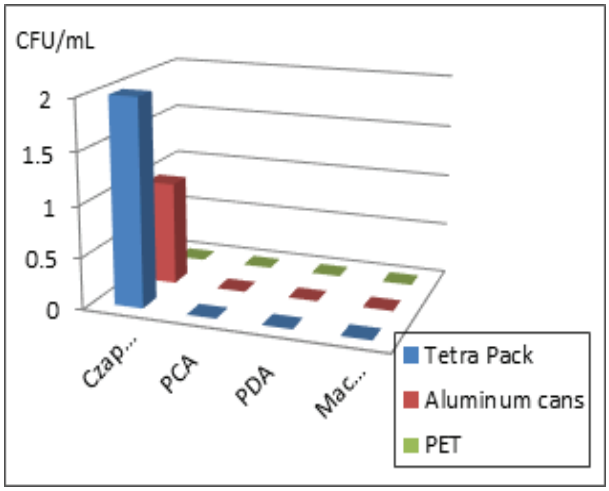

Figure 4. The number colonies of bacteria and moulds for each medium in banana juices

Pack package and a mold colony in the PET packaging.

Also in the banana juices were observed only colonies grown in the Czapek medium, so the 2 colonies that grew from the juice in the Tetra Pack packaging and the colony in juice in aluminum can, were moulds.

After analysis microbiologic of cherry juices, we see that cherry juice in the Tetra Pack results from free microbial load, while the cherry juice in aluminum can has one mould colony and *Bacillus* spp., and for cherry juice in PET results with three colonies of moulds.

These results can be affected by the composition of each fruit juice, starting from: pH value, pulp contained or clear juice extracted, the concentration of sugar because is different, referring to the product label of the fruit juice, and also by the presence of preservatives. These parameters that are different for each fruit juice can become a determining factor both for inhibition and in certain cases for the favor of

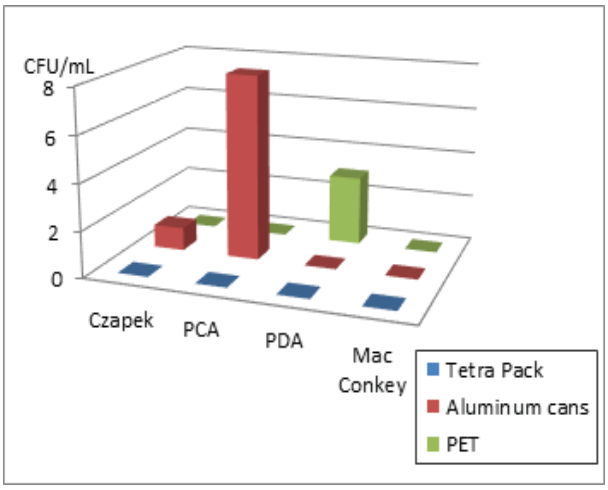

Figure 5. The number colonies of bacteria and moulds for each medium in cherry juices

certain microorganisms. Also, we can't leave without mentioning the packaging factor, or more precisely that each packaging has its own characteristics and affects the quality and shelf life of the final product.

Microbiological results of pasteurized fruit juices were evaluated based on microbiological criteria for foodstuffs, of ready- to eat foods, because there is no regulation specified for pasteurized fruit juices.

In regulation EC/2073/2005 microbiological criteria are divided into two types namely: food safety criteria - dealing with the presence of micro-organisms in the food, that represent a risk to human health, and process hygiene criteria - dealing with microorganisms that can be used as indicators of the level of hygiene present in the food business. For *Salmonella* spp., as an important human pathogen, where five samples of 25 g each are taken none of the samples will contain detectable levels of *Salmonella* is acceptable. For *E. coli* as an indicator of process hygiene the following apply: satisfactory if all values observed are less than 100 CFU/g, acceptable if a maximum of microbes are between 100 and 1000 CFU/g and the rest of the values are less than 100 CFU/g, unsatisfactory if one or more values observed are greater than 1000 CFU/g or more [11].

Based on the results of microbiological analysis for fruit juices that are present in Table 1, we see that the microbiological loads of all fruit juices analyzed for each package are below 100 CFU/mL.

According to Regulation EC / 2073/2005 for the microbiological examination of ready- to- eat food referring to Microbiological Quality all samples analyzed are considered of 'satisfying' category.

4. Conclusions

- All fruit juices analyzed are considered of 'satisfying'

category, because the microbiological loads were below 100 CFU/mL, according to Regulation EC/2073/2005 for the microbiological examination of ready- to- eat food referring to microbiological quality.

- Samples with the highest microbial load are peach and orange juice in aluminum cans package.

- Orange juice resulted in the highest microbiological load, as well as the variety of microorganisms found such as bacteria: *Micrococcus* spp., *Pseudomonas* spp. and *Bacillus* spp., as from molds as Cladosporium spp. In this case either the raw material used is not of good quality (there has been a high microbiological load) and/or the pasteurization process has not been carried out, or shows that the hygiene of the equipment after the pasteurization process is not good.

- Also, in the peach juice were cultivated some types of bacteria (*Enterobacteriaceae*) and some traces of mold. It is thought that this level of contamination is mainly due to the lack of correctness in the application of hygienic measures during the stages of the production process and in the microbiological quality of the raw materials from which this product is obtained.

- Strawberry and banana juices in all three packages resulted in lower microbial load than other juices.

- It should also be noted that yeasts are an important group of microorganisms, which are associated with the spoilage of fruit juice, but in samples that were taken for analysis in this study they weren't present.

- The results obtained show a good microbiological quality of the fruit juices that have been analyzed. However, the presence of such microorganisms in certain samples indicates that it is important to evaluate the microbial load of the raw material and/or hygiene of equipment after pasteurization, in order to ensure the microbiological quality and safety of fruit juices.

5. References

[1] Falguera V., and Ibarz A. (Eds.). (2014). *Juice Processing Quality, Safety and Value-Added Opportunities*. CRC Press, Taylor & Francis Group, Abingdon-on-Thames, UK.

[2] Goldman E., Green L.H. (Eds.). (2008). Practical Handbook of Microbiology. CRC Press, Taylor & Francis Group, Boca Raton, USA.

[3] Kalia A., Gupta R. P. (2006). *Fruit microbiology*. In: Hui Y. H. (Ed.), Handbook of fruits and fruit processing. Blackwell Publishing, New Jersey, USA, pp. 3-28.

[4] Rajurja G., Tiwar B. K. (2018). *Fruit Juice: Extraction, Composition, Quality and Analysis*. Academic Press, Cambridge, USA.

[5] Vasavada P. C. (2003). *Microbiology of fruit juice and beverages*. In: Foster T., Vasavada C. P. (Eds.), Beverage Quality and Safety. CRC Press, Boca Raton, USA, pp. 95-123.

[6] Bhardway, R.L., Nandal, U., Pal, A., Jain, S., 2014. *Bioactive compounds and medicinal properties of fruit juices*. Fruits Journal, Vol. 69, No. 5, pp. 391-412.

[7] Hariyadi R. D. (2013). *Microbiological Quality and Safety of Fruit Juices*. Food reviews International, Vol. 1, No. 1, pp. 54-57.

[8] Silva F. V., Gibbs P. A. (2010). *Non-proteolytic Clostridium botulinum spores in low-acid cold-distributed food and design of pasteurization processes*. Trends in Food Science Technology, Vol. 21, pp. 95-105.

[9] Tasnia A., Kamal D., Uddin M. (2018). *The Microbiological Quality of Commercial Fruit Juices-Current perspectives.* Bangladesh Journal Microbiol, Vol. 35, No. 2, pp. 128-133.

[10] Codex Alimentarius. (2005). *Codex stan 247-2005: Codex general standard for fruit juice and nectar*. <URL: www.fao.org/nput/download/standards/10154/CXS_247e.pdf. Accessed 01 June 2017.

[11] EU Commission. (2005). *Regulation No 2073/2005 on microbiological criteria for foodstuffs*. OJ., L 338.