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Identification of Microorganisms in Fresh and Dried Fruits Cultivated, Imported and Consumed in Tirana City

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Abstract

Fruits products contamination present a particular concern for human health, since many of these products are raw consumed without any prior treatment, which would eliminate or reduce biological, microbiological or physical risks. The aim of this study is to gather basic information on microbiological quality in fresh and dried fruits, which are traded currently in Tirana, as this city presents almost one third of Albania. This study was conducted during the period November 2010-March 2013 in Tirana's main markets. In total were collected 257 samples, 174 samples are dried fruit and 83 samples are fresh fruit. Each sample of fresh fruits was analyzed for bacteria, molds and yeast, but dried fruits were analyzed only for molds and yeast. In fresh fruits we didn`t found *Staphylococcus aureus* and *Bacillus cereus*, but we detected presence of Aerobic mesophilic count plate 1.2%, *Coliform total* 2.4% and *E. coli* 1.2%. Also we found presence of mold and yeast for potential health hazard in 4.8% and 2.4% respectivilly. The results for dried fruits were 22.4% of them have indicated potential health hazard with mold, while yeast in 8.6%. Mold and yeast were the most frequent contaminants of fresh and dried fruits sold in trades of Tirana. Although fresh fruits have a lower microbiological contamination, but the dried fruits resulted in a considerable pollution from mold and yeast.

Keywords: fresh and dried fruits, bacteria, mold, yeast, trade

1. Introduction

Fruits products are very rich in vitamins, minerals and fiber compared to other products. WHO-FAO recommends a minimum of 400 gr per day of fruits for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrients deficiencies, especially in less development countries (12). The increasing availability and consumption of these fresh products have lead to an expansion in global trade for fruits and also their strong presence in the markets (21, 23, 27). The developed countries imported 80% of fresh fruits. Europe is the largest country with 47 % of importing for 2005 (22). Annual growth of consumption of these products has the tendency to increase by 3-4% (14).

Fresh fruits contaminations present a particular concern for human health, since many of these products are raw consumed without any prior treatment, which would eliminate or reduce biological, microbiological or physical risks. Fruits can be contaminated at any stage of growth, processing, trading, handling or maintance of them. Bad practices of farmers are recognized as sources of microbial contamination of fruits and vegatebles (7). Microbiological pollution of fruits come directely or indirectly by water, soil, chemicals or insect (4, 8, 7).

Minimally processed of fruits go through different steps during their preparation. However, they must maintain the same quality as the fresh products because the dried fruits occupy an important place in use by consumers. Presence of molds and yeast in these products bring great risk

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to public health due to the potential exposure of mycotoxins. Molds presence in food samples does not means presence of mycotoxins, but their presence can increase the risk of mycotoxins producing.

Most of fruits contain enough nutrients to allow rapid growth of pathogen microorganisms. So, bacterial and mycotic contamination of these products will necessarily lead to a potential problem of food safety (3, 9, 11).

The aim of this study is to gather basic information on microbiological quality in fresh and dried fruits, which are traded currently in Tirana, as this city presents almost one third of Albania.

2. Material and Methods

This study was conducted during the period November 2010 - March 2013 in Tirana's main markets. In total were collected 257 samples, 174 samples are dried fruit and 83 samples are fresh fruit. The samples were packaged and collected according to the Codex Alimentarius (10). We did random products selection for further microbiologic analysis. Also during the sampling process, food sellers were asked about the origin.

Each sample was put in a sterile bag, sealed and transported immediately to the Laboratory of Food Microbiology at Institute of Public Health, Tirana. All the samples were kept in refrigerator at 4°C till analyses.

Each fresh fruit was been analyzed for bacteria, molds and yeast, and the dried fruits were analyzed for molds and yeast only. The results were expressed as average cfu/g. Identification of the microorganisms was done according (28) and the ISO methods.

Microbial determinations were carried out using the standard methodologies described in Table 1. Twenty-five grams of each sample of fresh fruits were diluated in 225 ml of peptone saline solution (PS), (1 g peptone and 8.5 g NaCl per litre) and homogenized for 2 min at normal speed in a Stomacher. Serial dilutions of the suspension were made in PS and analyzed for aerobic mesophilic yeasts and moulds, Enterobacteriaceae, and *E. coli*. Another 25 g were diluted in 225 ml of buffered peptone water for the detection of Salmonella spp., for the *Bacillus cereus* inoculate 3 tubes with 10ml of initial suspension of double-strength TSPB (Tryptone soya polymyxin broth) with 1 gr of sample per tube and further diluetion (equal to 0,1g, 0,01g, 0,001g of sample per tube). The dried fruits, twenty-five grams of each sample were diluated in 225 ml of peptone saline solution and after were analyze for yeasts and moulds. After that we used specific media for identification of different microorganisms according to their methodology (Table 1).

In our country, information about microbiological quality of fresh and dried fruits cultivated, imported and consumed are limited and there is no Albanian microbiological standart about these food products. In our laboratory as the standart protocols for microbial quality was used the guideline Food Standards Australia New Zealand (FSANZ) and Food and Drug Administration (FDA) standard (15, 16). So all analyzed samples were compared with this two standards (Table 2 and 3).

3. Results

In this study were collected 257 samples in fourteen different trades of Tirana, capital city of Albania (Table 4). From them, 83 samples were fresh fruits and 174 samples were dried fruits.

The fresh fruits 83 samples we have analyzed six different products (Table 5). These products were analysed for presence of Aerobic mesophilic plate count, Coliform bacteria, *E.coli, Salmonella* spp., *S. aureus* and *B. cereus*, as well as for the detection of molds and yeast (Table 6 and 7). Most of products were in category "satisfactory", but based to the calculation of data we note that the category "satisfactory" has the statistically significant difference with other categories of results for $\chi 2 = 123.1$ we have $p < 0.01$. In fresh fruits we found presence of mold and yeast for potential health hazard in 4.8% and 2.4% respectivilly.

We analyzed 13 different products of dried fruits 174 samples in total (Table 8). Those products were analysed only for molds and yeast because this are the most frequent microbial contaminants.

Table 1. List of methodologies used to determine microbial quality

Determination	Methodology	Description
Standart Plate Count	ISO 4833:2003	Microbiology of food and animal feeding stuffs—Horizontal methods for the enumeration of microorganisms —Colony count thecnique at 30 degrees C
Coliform bacteria	ISO 21528-2:2004	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection and enumeration of Enterobacteriaceae—Part 2: Colony-count method
E. coli	ISO 6579:2002	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection and enumeration of presumptive Escherichia coli
Salmonella spp.	ISO 6579: 2002	Microbiology of food and animal feeding stuffs—Horizontal methods for the detection of Salmonella spp
Bacilus cereus	ISO 21871:2006	Microbiology of food and animal feeding stuffs—Horizontal methods for the determination of low numbers of presumptive Bacillus cereus- Most probable number technique and detection method
Staphylococcus aureus	ISO 6888-3:2003	Microbiology of food and animal feeding stuffs—Horizontal methods for the enumeration of coagulase-positive staphylococci (*S. aureus and other species)*
Yeasts and molds	ISO 21527-1:2008	Microbiology of food and animal feeding stuffs—Horizontal methods for the enumeration of yeasts and moulds

Table 2. Standard guidelines from FSANZ for microbial contamination for fruits ready to eat

Microbiological organisms test	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous
Standart Plate Count (Level 1)	$<10^4$	$<10^5$	$\geq 10^5$	
Enterobacteriaceae (Total coliform)	$< 10^2$	10^2-10^4	$\geq 10^4$	
Eschericia coli	< 3	3 – 100	> 100	
Coagulasepositive Staphylococcus /gr	$< 10^2$	$10^2 – 10^3$	$10^3 – 10^4$	$> 10^4$ SET +ve
Bacillus cereus/gr	$< 10^2$	$10^2 – 10^3$	$10^3 – 10^4$	$> 10^4$
Salmonella spp.	Not detected in 25g			Detected

Table 3. Standard guidelines from molds and yeast for fruits by FDA

Microbiological organisms test	n	c	m	M
Molds cfu/g	5	2	10^2	10^4
Yeast cfu/g	5	2	10	10^3

Legend: **n**-number of samples units selected from a lot of food to be examined.

m-accetable level of microorganism determined by a specified method; the values are generally based on levels that are achievable under GMP.

M-level which when exceeded in one or more samples wold cause the lot to be rejected as the indicates potential health hazard or imminent spoilage.

c-maximum allowable number of defective or marginally acceptable units.

The data were analysed by SPSS version 16.

Table 4. Number of samples collected divided by the place of samples taken

Nr	Place of samples taken	Frequence for dried fruits	Percentage for dried fruits	Frequence for fresh fruits	Percentage for fresh fruits
1	Casa Italia	12	6.9	3	3.6
2	Citypark	11	6.3	4	4.8
3	Kristal center	19	10.9	2	2.4
4	Universal Trade Centre (QTU)	13	7.5	7	8.4
5	Trade "Avni Rustemi"	9	5.2	11	13.3
6	Trade "Dinamo"	12	6.9	12	14.5
7	Trade " Ish qyteti i nxenësve"	10	5.7	7	8.4
8	Trade "Xhamlliku"	8	4.6	5	6.1
9	Trade "21 Dhjetori"	10	5.7	6	7.2
10	Trade "Kinostudio"	11	6.3	3	3.6
11	Trade of "Kamza"	24	13.8	8	9.6
12	Trade of "Liqeni artificial"	21	12.1	4	4.8
13	Trade of "Lapraka"	10	5.7	5	6.1
14	Trade of "Shkolla e baletit"	4	2.3	6	7.2
	Total	**174**	**100.0**	**83**	**100.0**

Table 5. Kind of fresh fruits

Kind of products	Number of samples
Banana	13
Plump	14
Apple	16
Orange	12
Cheers	14
Raise	14
Total	83

Table 6. Microbiological contamination of fresh fruits

Microorganisms	**Satisfactory product**	Marginal	**Product unsatisfactory**	Potentially hazardous
Standart Plate Count (Level 1)	63 (75.9)	12 (14.5)	1 (1.2)	0
Coliform-bacteria	75 (90.4)	1 (1.2)	2 (2.4)	0
Eschericia coli	76 (91.6)	1 (1.2)	1 (1.2)	0
Staphylococcus spp.	37 (44.6)	41 (49.4)	0	0
Bacillus cereus	6 (7.2)	20 (24.1)	0	0

*Numbers in the parethenses represent Standard error

Table 7. Micotic contamination of fresh fruits

Microorganisms	m	M
Molds	74 (89.1)	4 (4.8)
Yeast	63 (75.9)	2 (2.4)

*Numbers in the parethenses represent Standard error

Table 8. Mycotics contamination of dried fruits

Kind of products	Number of samples	Mold (M)	Yeast (M)
Plum	16	5 (31.3)	3 (18.8)
Raisin	22	5 (22.7)	2 (9.1)
Dried apple	20	6 (30.0)	2 (10.0)
Bananas	13	2 (15.4)	1 (7.7)
Dried figs	6	1 (16.7)	1 (16.7)
Thai papaya	7	2 (28.6)	0
Pine-apple	6	1 (16.7)	1 (16.7)
Coco	2	0	0
Almonds	22	4 (18.2)	2 (9.1)
Different types of peanuts	31	6 (19.4)	2 (6.5)
Nuts	9	3 (33.3)	1 (11.1)
Hazelnuts	15	4 (26.7)	0
Stiks	5	0	0
Total	**174**	**39 (22.4)**	**15 (8.6)**

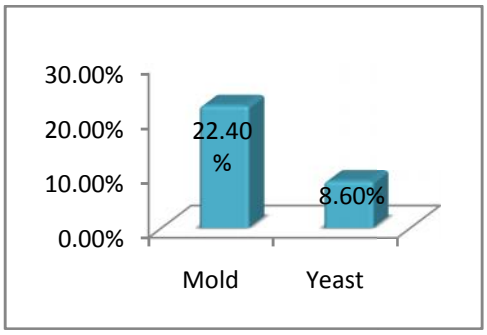

Figure 1. Percentage mycotic pollution of dried fruits

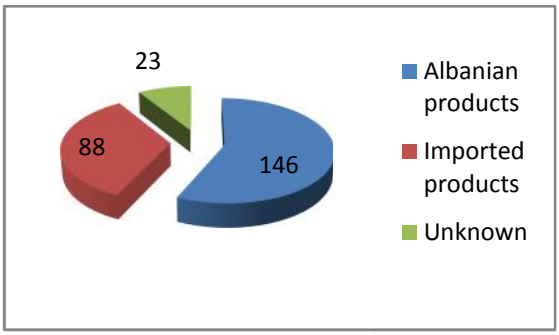

Figure 2. Number of fresh and dried fruit divided by Origin of products

For the dried fruits only 39 or 22.4% of them have resulted as products above the norm pollute with mold, while yeast we found only in 15 or 8.6% of them above the norm (figure 1).

Comparing the pollution of fresh and dried fruits for presence of mold was not found statistically significant difference for plum (p=0.8), apple (p=0.7), bananas (p=0.4) and raisin (p=0.8). Also, comparing the pollution of fresh and dried fruits for yeast was not found statistically significant difference for plum (p=0.6), apple (p=0.8), bananas (p=0.6) and raisin (p=0.6).

After the information from the question done to whole sellers, we divided our samples (fresh and dried fruits) into three categories based on their: origin of products, so we have classificate in 1) Albanian products, 2) Imported products and 3) Unknown products (figure2). Based to our data we show that the most of the studied samples have been Albanian products. These were the most contaminated products compared with other category. About 27% of Albanian products resulted contaminated, imported products only in 14%. We note that the selection of samples was completely random and the origin of products were unknown by the inspector who was responsibility for sampling. In some cases the origin of products was not possible to determine, this for reason that in some times the seller don't know the origin of product or not wished to express any opinion on the matter.

4. Discussions

During recent years the presence of fresh and dried fruits in trades has increased significantly. The customer can find and consume these products regardless the time and season (1). Globalization of food products in general not only affects the distribution of various food products, but also we have the spread of the potential risks to human health from one region to another, thus enabling a wider spread of pathogens throughout the world. As result, increasing the consummation of fresh and dried fruits has also become an important tool in statistics of food borne diseases. During many years, there are many articles about occurrence of food borne pathogens in fresh products (13, 4, 5, 6, 7, 17, 24, 19).

Given the fact that fruits produced in natural environments or in greenhouses, we can say that these products are too fragile to contaminate from human pathogens. One big problem may be the wastes water that needed to irrigation of different fruits, which can also be a potential source of pathogenic bacteria, viruses and parasites. Usually, contamination of these waters is attributed to fecal contamination, due to the presence of livestock in the surrounding areas (20, 25, 26).

Regarding the microbiological quality of the fresh fruits, during laboratory testing and statistical processing of the data collected, it was observed that the microbiological quality of fresh fruits in general, were in category “satisfactory”. So for Aerobic mesophilic plate count 75.9% of those fruits were in category “satisfactory” for Coliform bacteria 90.4%, *E.coli* 91.6%, *Staphylococcus* spp. 44.6% and for *Bacillus cereus* 7.2%. The products in norms have the statistically significant difference with other categories for p <0:01.

For unsatisfactory products can be said that 1.2% resulted infected with Aerobic mesophilic plate count, 2.4% with Coliform bacteria and *E.coli*. *Staphylococcus* spp. and *Bacillus cereus* were not found in any products as unsatisfactory category.

Molds were found in fresh fruits for accetable level of microorganism (m) in 89.1% and for yeast in 75.9%. 4.8% and 2.4% of fresh fruits products indicates potential health hazard (M) for molds and yeast.

In dried fruits the presence of molds and yeast that indicates potential health hazard (M) were 22.4% with molds and 8.6% of them with yeast.

Alzamora et al have explain that the mechanical damage is caused by inappropriate methods used during harvesting, packaging, and inadequate transporting, which can lead to tissue wounds, abrasion, breakage, squeezing, and escape of fruits. Mechanical damage may increase susceptibility to decay and growth of microorganisms. Some operations, such as washing, can reduce the microbial load; however, they may also help to distribute spoilage microorganisms and moisten surfaces enough to permit growth of microorganisms during holding periods. Due to the acidity of raw fruits, the primary spoilage organisms are fungi, predominantly moulds and yeasts (2). During the questionnare of the sellers one of the point was what they do with the product that have not been sold. By the data 54% of the interviewers were answered; that for not wasted any of their products they have putted those products on drying process. Naturally, such processes can be a potential contamination. We thought that this was one of the reasons why we have found a high percentage of molds and yeast in our study.

5. Conclusions

As conclusion we can say that mold and yeast were the most frequent contaminants of fresh and dried fruits sold in trades of Tirana. Although fresh fruits have a lower microbiological contamination, but the dried fruits indicate potential health hazard for mold and yeast.

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