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*Original scientific paper***MICROBIOLOGICAL PURITY TESTING IN FOOD PRODUCTION
AND MARKETING******Bojan GOLIC^{1*}, Tanja ILIC¹, Vesna KALABA¹, Milijana GOLIC¹, Slobodanka DELIC¹**

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Abstract: The Rulebook on microbiological purity criteria prescribes the criteria of microbiological purity and the frequency of control of equipment, devices, utensils, work surfaces, work clothes and workers' hands in food production and marketing as well as in facilities and means of transport that come in contact with food in which there is a risk of occurrence and the spread of infectious diseases. Food facilities, means of transport, items, accessories and equipment that come in contact with food must be regularly cleaned and disinfected to avoid any risk of contamination, and food and staff must be under regular supervision. Food handling staff must maintain a high level of personal hygiene and be trained in terms of food hygiene requirements. The aim of the study was to determine the state of microbiological purity in food production and marketing in facilities and means of transport that come into contact with food. As the test material we used swab samples from the surfaces of equipment, devices, utensils, work surfaces, work clothes and workers' hands originating from facilities for the production and marketing of food, restaurants and other catering facilities serving food, facilities in the field of education and social protection (facilities for accommodating persons) and means of transport that come into contact with food. A total of 3393 samples were examined in the course of self-control and official control during 2017. For microbiological examination of swab samples, standard BAS ISO methods were used. The percent of swab samples that did not satisfy the Rulebook on the criteria of microbiological purity in 2017 was 5.20%. In relation to the total number of samples tested, 4.70% of the samples were unsatisfactory due to an increased number of microorganisms, and 1.40% due to an increased number of enterobacteriaceae. Pathogenic microorganisms *Salmonella* spp. and *Listeria monocytogenes* are not detected in any swab sample. The largest percent of unsatisfactory samples was in the category "hands of food handlers" and amounted to 7.30%

Key words: microbiological purity, swab, food, production, marketing

INTRODUCTION

The Rulebook on microbiological purity criteria prescribes the criteria of microbiological purity and the frequency of control of equipment, devices, utensils, work surfaces, work clothes and workers' hands in production and marketing of food in facilities and means of transport that

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come in contact with food in which there is a risk of occurrence and the spread of infectious diseases. (Rulebook, 2014). The acceptance criteria for the microbiological purity of the sample is determined by comparing the obtained test results with the microbiological purity criteria, and the sample is of acceptable microbiological purity if it meets the microbiological criteria. Exceptionally, the sample may be of acceptable microbiological purity if it contains up to 50% more microorganisms, provided that it does not contain pathogenic bacteria. Sampling and microbiological examination is done with accredited methods, in authorized testing laboratories. The assessment of microbiological purity is done by the laboratory in which the samples were tested.

In the microbiological examination of swabs, the assessment of surface hygiene is mainly based on determining the number of microorganisms and enterobacteria per cm² (Aarnisalo et al., 2006). In addition to these, sometimes other microorganisms are also tested, since it is proven that infected food handlers can spread *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and faecal streptococci (Lawrie, 1998). In some cases, the presence of *Listeria monocytogenes*, as a causative agent of listeriosis, a serious infectious disease of humans and animals should be identified on food contact surfaces (Aguado et al., 2001; Lundén et al., 2002, Lundén et al., 2003; Suihko et al., 2002; Fonnesbech-Vogel et al., 2001). Some studies indicate that the environment in the production process is more involved as a source of *Listeria monocytogenes* than live animals and carcasses, and therefore special attention should be paid during the cleaning and disinfection process (Samelis and

Metaxopoulos, 1999). The absence of *Listeria monocytogenes* indicates an effective cleansing and disinfection program. It is considered that the remaining impurities on the equipment deposited on the meat during deboning are the primary source of *Escherichia coli*. (Gill and McGinnis, 2000).

One of the main risks of food contamination comes from the working process of food handlers and from microorganisms, the cause of the disease, which are present in or on staff, and then transmitted to food during the handling process (Gordon-Davis, 1998). The incidence of foodborne diseases in 81% of cases is due to contamination of food during the production when principles of good manufacturing practice are not respected (Raseta et al., 2012).

Food facilities, means of transport, items, utensils and equipment that come in contact with food must be regularly cleaned and disinfected to avoid any risk of contamination, and food and staff must be under regular supervision (Regulation 2004). Food handlers must maintain a high level of personal hygiene and wear suitable protective clothing and be trained in terms on food hygiene requirements. Improper cleaning and disinfection is directly related to the various cases of foodborne illness outbreaks (Gill and Jones, 1999). Training of food handlers, with regard to the basic concept and requirements of personal hygiene, constitutes an integral part of measures to obtain a safe consumer product (Adams and Moss, 1997). The quality of food handlers depends on their health, hygiene and habits (Johns, 1991).

The aim of the study was to determine the state of microbiological purity in food production and marketing in facilities and means of transport that come into contact with food.

MATERIAL AND METHODS

Material

Swab samples from equipment, devices, utensils, work surfaces, work clothes and

workers' hands in production and marketing in facilities and means of transport that come in contact with food were used as testing material.

Categorization of tested samples and 2013; Rulebook, 2014) are presented in Table 1. microbiological purity criteria (Guidelines,

Table 1. Categories of tested samples and microbiological purity criteria

Sample category	Number of microorganisms	Enterobacteriaceae
	Satisfactory	Satisfactory
Porcelain, glass, smooth metal surfaces cfu/cm ²	≤10	0-1
Other surfaces (wooden, plastic, stone) cfu/cm ²	≤30	0-1
Plates, bowls, cutlery and small dishes; dishes and utensils that come in contact with food cfu/ml (cm ²)	≤100	0-1
Bottles or containers for liquid products cfu/ml	0-1	0-1
Hands of food handlers cfu/ml (cm ²)	≤200	0-1

cfu: colony- forming units

These are regular samples, delivered in the course of self-control and official control during 2017. Samples come from facilities for the production and marketing of food, restaurants and other facilities serving food, facilities in the field of education and social protection (accommodation facilities) and transportation means that come into contact with food. A total of 3393 samples were tested.

Methods

For the microbiological examination of swab samples, the following standard test methods were used:

- BAS EN ISO 4833:2006 (Microbiology of food and animal feeding stuffs, 2006) for determining the number of microorganisms
- BAS ISO 21528-2:2008 (Microbiology of food and animal feeding stuffs, 2013) for determining the number of enterobacteria
- BAS EN ISO 11290-1/A1:2005 (Microbiology of food and animal feeding

stuffs, 2005) for the detection of *Listeria monocytogenes*

Sampling and transport of samples were carried out in accordance with the standard *BAS ISO 18593:2008* (Microbiology of food and animal feeding stuffs, 2008).

Laboratory tests on samples were performed at the Public Veterinary Institute of Republic of Srpska "Dr Vaso Butozan" Banja Luka.

stuffs, 2005) for the detection of *Listeria monocytogenes*

- BAS EN ISO 6579/Cor2:2010 (Microbiology of food and animal feeding stuffs, 2010) for the detection of *Salmonella spp.*

We used descriptive statistical parameters, as basic statistical methods in our research and in the statistical analysis of the obtained results. The results of the research are presented in tabular and graphical form.

RESULTS AND DISCUSSION

Chart 1. shows the structure of the taken samples examined by categories.

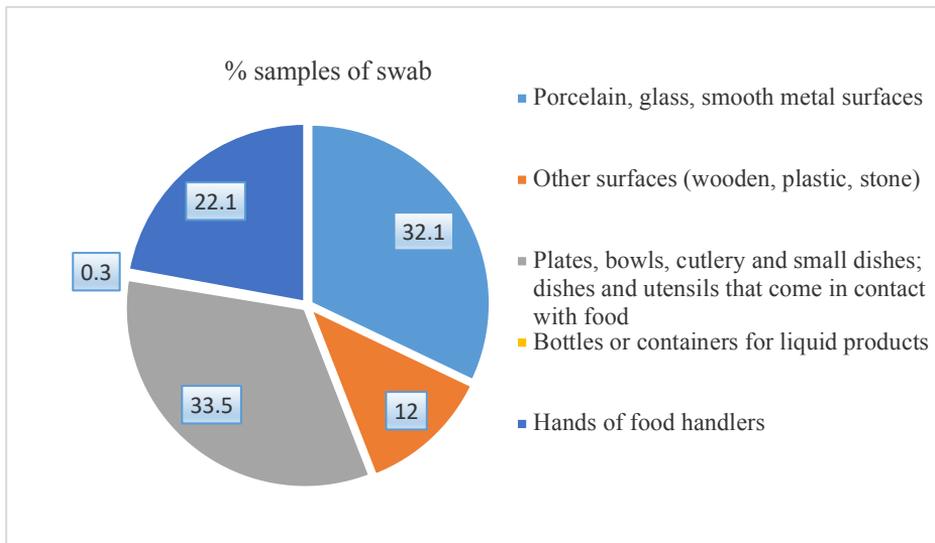


Chart 1. Samples of swabs by categories

Of the total number of sample swabs examined, the individual share of sample swabs of "porcelain, glass, smooth metal surfaces" and "plates, bowls, cutlery and small dishes; dishes and utensils that come into contact with food" amounted to over 30%, a total of 65.10%. A negligible number of swab samples referred to

"bottles or packaging for liquid products" (0.30%). In relation to the number of samples tested in 2015 (Kalaba et al., 2017), this is an increase in the number of samples in 2017 by 2.3 times.

Chart 2. presents swab samples in relation to the control mode.

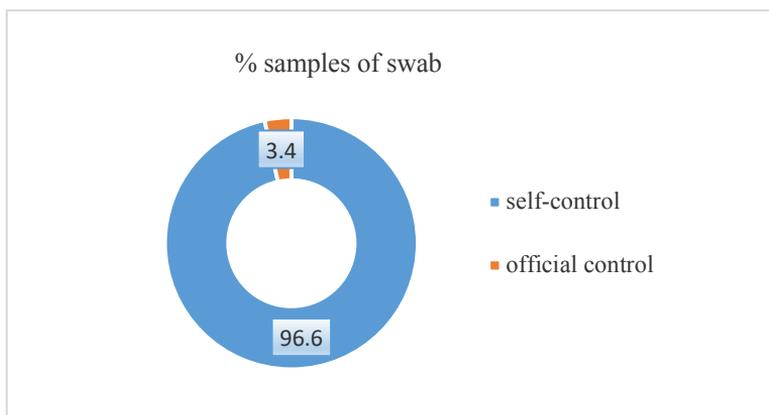


Chart 2. Samples of swabs in relation to the control mode

Most of the swab samples tested were submitted within the self-control (96.60%) undertaken by food businesses, while only 3.40% of the

samples were submitted by the inspection, within the framework of official controls. When it comes to swab samples examined for the

presence of *Salmonella spp.*, they all come from self-control, while in case of *Listeria monocytogenes* 99.50% comes from self-

control, and 0.50% from official control.

Chart 3. presents swab samples in relation to the test parameter.

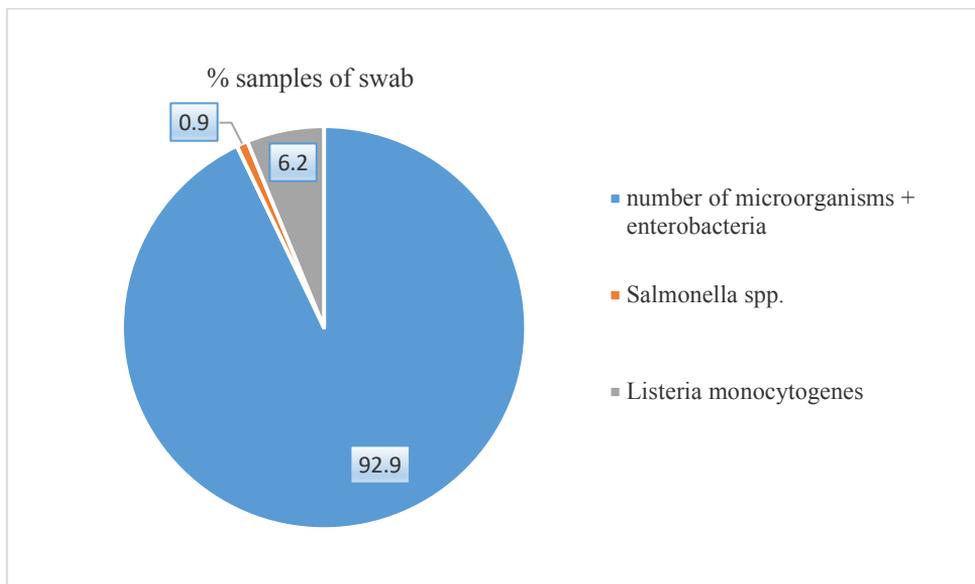
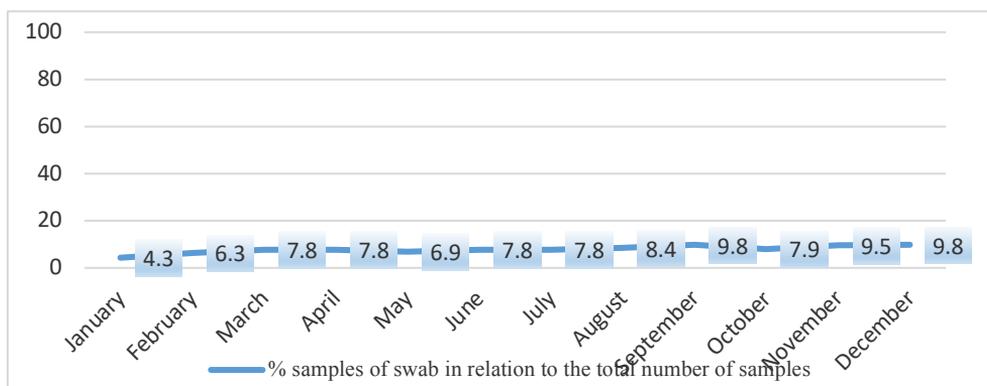


Chart 3. Swab samples in relation to the test parameter

Of the total number of tested swab samples, 92.90% was tested to determine the number of microorganisms and enterobacteria, 6.20% for the presence of *Listeria monocytogenes*, and

only 0.90% for the presence of *Salmonella spp.*

Graph 4. shows the dynamics of sampling or testing of swab samples by months.



Graph 4. Swab samples by months

When it comes to the month of testing, the number of samples tested ranged from 4.30 to 9.80% in relation to the total number of samples. A somewhat smaller number of samples in January and February is common for this period, as sampling at the beginning of the calendar year is of less intensity compared to other months of the year. This is linked to the end of the fiscal year for food business operators, when there is a

noticeable decrease in the number of samples submitted to the laboratory, regardless of sample type. Later, in the course of the year, in order to realize the self-control plan, the number of submitted samples increases.

Table 2. shows the results of examinations of swab samples in relation to the microbiological criteria established by the Rulebook on microbiological purity criteria (2014).

Table 2. Results of swab tests in relation to microbiological criteria

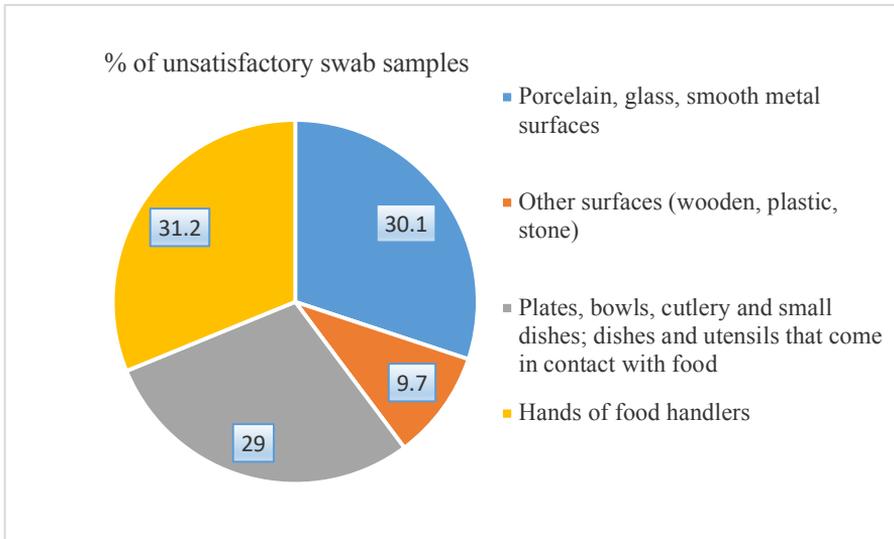
Total number of samples	Satisfactory samples		Unsatisfactory samples	
	number	%	number	%
3393	3217	94.80	176	5.20

Of the total number of tested swab samples, 5.20% did not meet the provisions of the Rulebook (2014). Of this number, 91.50% of the samples are unsatisfactory due to the increased number of microorganisms, and 26.10% due to the increased number of enterobacteria. In relation to the total number of tested samples 4.70% of the samples are unsatisfactory due to the increased number of microorganisms, and 1.40% due to the increased number of enterobacteria. The obtained results indicate a significant improvement in microbiological purity in food production and marketing compared to 2015, when there were 12.45% of unsatisfactory samples (Kalaba et al., 2017), which is 2.4 times less unsatisfactory swab samples. Also, when it comes to unsatisfactory swab samples due to an increased number of microorganisms there is a significant reduction in 2017 compared to 2015, from 12.20% to 4.70% (2.6 times less). When it comes to enterobacteria, the difference is insignificant, the number of unsatisfactory samples is even increased (1.40%) compared to 2015 (1.18%). The obtained results differ significantly from the

results obtained by Ivanović and associates (2013), who found 15.36% of the unsatisfactory swab samples on the contact surfaces in the meat processing plant. This indicates that the general level of hygiene in food production and marketing is high, with a marked improvement, but that there is a constant risk of contamination with enterobacteria, mostly of faecal origin, which are hygiene microbiological indicators in food production. This is confirmed by the results of the research done by Gill and McGinnis (2000) and Lawrie (1998).

All samples tested for the presence of *Salmonella spp.* and *Listeria monocytogenes* satisfy the provisions of the Rulebook (2014), that is, these pathogenic microorganisms are not isolated in any single swab sample. These results are consistent with the results obtained by Kalaba et al., 2017. The absence of *Listeria monocytogenes* indicates an effective cleansing and disinfection program, as confirmed by the conclusions of Samelis and Metaxopoulos (1999).

Graph 5. shows the participation of unsatisfactory swab samples by category.

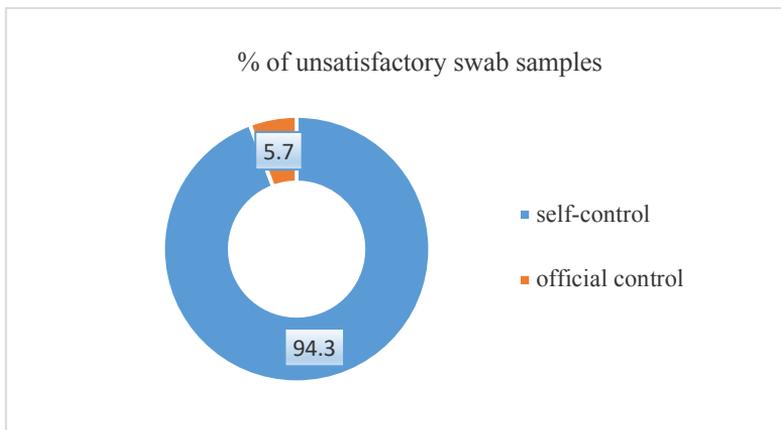


Graph 5. Participation of unsatisfactory swab samples by category

The smallest number of unsatisfactory swab samples were found in the category "other surfaces (wooden, plastic, stone)" and their participation in the total number of unsatisfactory samples was 9.70%, while there was no unsatisfactory samples in the category "bottles or packaging for liquid products". The

share of the other three categories in the total number of unsatisfactory swab samples is the same and together it amounts to over 90%, with the largest share of unsatisfactory samples from the category "hands of food handlers".

Graph 6. shows unsatisfactory swab samples in relation to control mode.

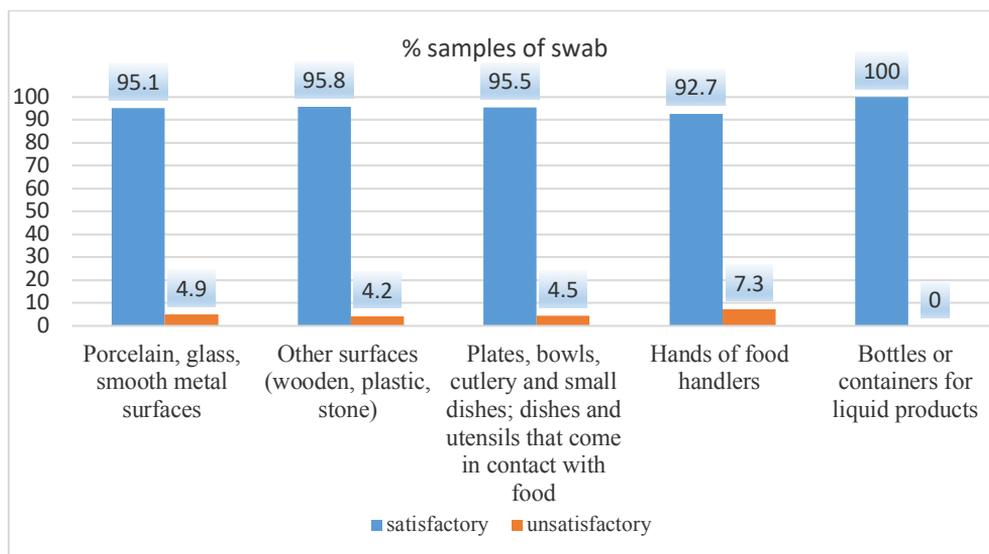


Graph 6. Unsatisfactory swab samples in relation to control mode

Out of the total number of unsatisfactory samples, 94.30% comes from self-control and

5.70% from official controls. This ratio is proportional to the ratio of the number of

samples in relation to the control mode. by category.
 Graph 7. shows the results swab examinations

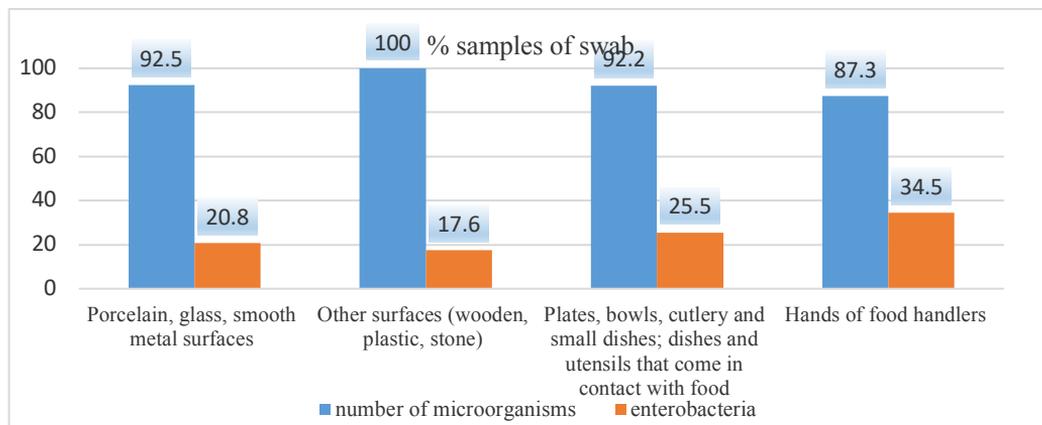


Graph 7. The results swab examinations by category

In the category of "bottles or packaging for liquid products" all samples were satisfactory. In the remaining four categories, the percentage of satisfactory samples was over 92%, while the percentage of unsatisfactory samples in the category "hands of food handlers" was the highest and amounted to 7.30%, while in the other three categories it was below 5%. When it comes to production and trade of food concerned, the obtained data indicate that the greatest risk is the human factor or the hygiene of people who come in contact with food, since most of the unsatisfactory samples relates to the hands of food handlers. Test results for categories "plates, bowls, cutlery and small

dishes; dishes and utensils that come into contact with food" and "the hands of food handlers" are consistent with the results obtained by Kalaba et al. (2017), while for other categories significant improvements in microbiological purity in relation to 2015 are noticeable. Also, the results obtained by Ivanović et al. (2013) for the category of "hands of food handlers" (5.55% of unsatisfactory samples) are close to the results we came up with in our research.

Graph 8 shows the results of unsatisfactory swab samples by categories in relation to the tested parameter.



Graph 8. Results of unsatisfactory samples by categories in relation to the tested parameter

When we observe the results of unsatisfactory swabs by categories in relation to the tested parameter, it is noticed that the vast majority of samples did not satisfy the provisions of the Rulebook (2014) due to the increased number of microorganisms, with this percentage ranging from 87.30-100% depending on categories. There was significantly smaller number of unsatisfactory samples due to the increased number of enterobacteria, ranging from 17.60-34.50%, depending on the category. All unsatisfactory samples from the category "other surfaces (wooden, plastic, stone)" were unsatisfactory due to the increased number of

microorganisms (100%), but with a minimum percentage of unsatisfactory samples due to an increased number of enterobacteria (17.60%). Contrary to this, the category of "hands of food handlers" had the least unsatisfactory samples due to the increased number of microorganisms (87.30%), but also the highest percentage of unsatisfactory samples due to the increased number of enterobacteria (34.50%). This suggests that the personal hygiene of food handlers pose a high risk, which is in accord with the conclusions of Gordon-Davis (1998).

Chart 9. shows the results of swab examinations by months.

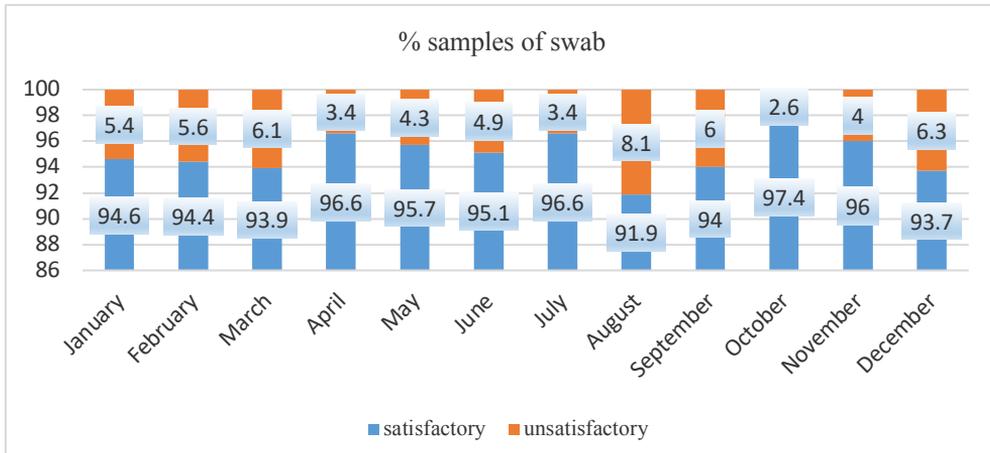


Chart 9. Results of swab examinations by months

Table 3. shows the average values of unsatisfactory samples according to seasonal periods during the year

Table 3. Average values of unsatisfactory samples according to periods during the year

Xav of unsatisfactory samples	Season			
	Spring (March, April, May)	Summer (June, July, August)	Fall (September, October, November)	Winter (December, January, February)
%	4.60	5.50	4.20	5.80

Xav- average value

During all 12 months in 2017, the percentage of satisfactory swab samples was over 91%. The highest percentage of unsatisfactory swab samples was in August (8.10%) and the lowest in October (2.60%). The obtained results do not indicate that there is a seasonal influence (seasonal effect) on the state of microbiological purity, or the percentage of unsatisfactory swabs, as expected in the spring and summer periods, given the increase in temperature, which has a favorable effect on the growth and increase in the number microorganisms. The obtained values indicate that the maximum average values of unsatisfactory samples were very close in the summer and winter season, and that minimal average values were also very close

during the spring and autumn. Observed by months, the highest percentage of unsatisfactory samples was in August (summer season), which points to the potential impact of high temperatures on the state of microbiological purity, or the risk of the microbiological quality of food produced and traded in this period of the year. However, this should not be taken for granted, since the month of August was the fourth month in relation to the number of tested samples during the year, so in order to have a realistic assessment of the influence of the seasonal period or temperature on the state of microbiological purity, it is necessary to observe these parameters and their interactions over a longer period of time.

CONCLUSION

Based on the obtained results, the following conclusions are drawn:

1. The tested swab samples are mostly derived from self-control (96.60%) undertaken by food business operators, while 3.40% of the samples were delivered under official control. Of the total number of swab samples tested, 92.90% was tested for the number of microorganisms and enterobacteria, 6.20% for *Listeria monocytogenes*, and 0.90% for *Salmonella spp.*
2. The percentage of swab samples that did not meet the provisions of the Rulebook on microbiological criteria in 2017 was 5.20%. Compared to the total number of samples tested, 4.70% of the samples were unsatisfactory due to the increased number of microorganisms, and 1.40% due to the increased number of enterobacteria. Pathogenic microorganisms *Salmonella spp.* and *Listeria monocytogenes* have not been proven in any swab sample.
3. Of the total percentage of unsatisfactory samples, 94.30% comes from self-control of food business operators, and 5.70% from official controls.
4. All samples from the category of "bottles or packaging for liquid products" were satisfactory.
5. The highest percentage of unsatisfactory samples was in the category of "hands of food handlers" and amounted to 7.30%. The percentage of unsatisfactory samples from this category due to the increased number of enterobacteria is 34.50%. The personal hygiene of food handlers poses the greatest risk in food production and marketing and in this regard, staff training should be carried out, hygiene measures should be prescribed and their implementation should be controlled. Also in the event of failure to comply with the measures, the possible consequences must be indicated.

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