

## MICROBIOLOGICAL CONTAMINATION OF CONFECTIONARY CAKES IN CROATIA

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### Summary

Confectionery cakes are products obtained by mixing, shaping, baking, or other suitable processing procedures of two or more ingredients, giving the characteristic sensory properties of the product. They can be filled or topped with fruit, chocolate and other creams or toppings. The chemical composition and high water and sugar content make confectionery cakes suitable for the growth and multiplication of various microorganisms. Since contamination can occur at all stages of the production process, conducting good hygiene practices is necessary to obtain a product safe for consumption.

The aim of this study was to provide insight into the contamination of confectionery cakes at the market in the Republic of Croatia by potentially pathogenic microorganisms and microorganisms as indicators of hygienic production. The cakes were sampled during a one-year period in 12 cities in Croatia. Samples were analysed according to the microbiological criteria prescribed by Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs and as recommended by the Guideline of Microbiological Criteria (Ministry of Agriculture, 2011).

Results of the study showed that no pathogenic bacteria whose presence could have adverse health effects, were identified in confectionery cakes.

*Keywords:* confectionery cakes, microbiological contamination, food safety

### Introduction

Some bacteria that contaminate food during its production are potential causes of diseases in humans. In accordance with the general requirements for food safety, which are prescribed by Article 14 of Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, food should not be placed on the market if it is not safe. Food safety is ensured by a preventive approach, such as implementing good hygiene practice (GHP) and controlling hazards and critical control points (HACCP). In terms of the presence of certain microorganisms in food, the microbiological food safety criteria are related to pathogenic bacteria, and the process hygiene criteria to those that are not, but their presence must still be monitored and in the case of unsatisfactory test results certain measures have to be undertaken (Regulation 2073/2005). The whole production process acceptability is estimated based on the process hygiene criteria (Regulation 2073/2005).

Due to their chemical composition and high-water content, confectionery cakes are suitable for the growth and reproduction of various microorganisms. Contamination of confectionery cakes can occur at all stages of the production process: due to the use of contaminated raw materials, especially if the cakes are not heat treated before consumption, after thermal processing, during inadequate storage and/or transportation and due to non-hygienic handling (Kumar et al., 2011; Shahbaz et al., 2013; Chaudhari et al., 2017; El-Kadi et al., 2018). Higher moisture content products are more likely to cause foodborne illness because they promote the growth of a wide range of bacteria, yeast, and other fungus species. Bakery products with high moisture content or low acid content provide an ideal habitat for pathogenic bacteria to thrive. The growth of spoilage organisms such as osmophilic yeasts and mould is usually limited to intermediate moisture products (Gill et al., 2020). According to the Official Gazette on cereal and cereal products (OG 81/2016), cakes are products obtained by mixing, shaping, baking or other appropriate processing procedures of the mixture of two or more ingredients, thus achieving the characteristic sensory properties of the product. They can be stuffed or overflowing with fruit, chocolate and other fillers or dressings.

The study was conducted in order to determine the occurrence of potentially pathogenic microorganisms and microorganisms' indicators of process hygiene in confectionery cakes in the Republic of Croatia.

## Materials and methods

The research was conducted over the one-year period in 12 cities in the Republic of Croatia. 230 individual samples of confectionery cakes of different types (with fruit and/or creams) that were offered in catering facilities, either for consumption on the spot or for sale, were sampled.

Samples were taken by certificated employees of the Teaching Institute for Public Health "Dr. Andrija Štampar" and the Food Control Center of the Faculty of Food Technology and Biotechnology of the University of Zagreb. Each sample was taken as one unit in an amount from 200 to 500 g. The sample was put in a PVC container (bag) containing information on the place and time of sampling, the product's name, and the product's temperature value while sampling. The sample was put in the portable cooler and within not more than two hours delivered to the laboratory for further analysis.

Microbiological analyses were performed using internationally recognized ISO methods as listed in Table 1.

**Table 1.** ISO methods used in microbiological analyses

Microorganisms	Method
<b>Aerobic mesophilic bacteria</b>	HRN EN ISO 4833-1:2013 or HRN EN ISO 4833-2:2013
<i>Enterobacteriaceae</i>	HRN ISO 21528-2:2017
<i>Salmonella</i> spp.	HRN EN ISO 6579-1:2017
<i>Listeria monocytogenes</i>	HRN ISO 11290-2:2017
<i>Staphylococcus aureus</i>	HRN EN ISO 6888-1:2004
<b>Moulds</b>	HRN ISO 21527-1:2012 and HRN ISO 21527-2:2012

Microbiological criteria prescribed by Regulation 2073/2005 were applied to the obtained results of the sampled cakes and were interpreted in accordance

with the recommendations (Table 2) from the Guide on Microbiological Criteria (Ministry of Agriculture, 2011).

**Table 2.** Guide on Microbiological Criteria – criterion related to confectionary cakes

Food	Microorganisms/their toxins and metabolites	Sampling plan		Criterion
		<b>Recommendation</b>		
Pastries (pastry cakes) with fillings and ready-made creams	Aerobic mesophilic bacteria	5	2	m = 10 <sup>4</sup> cfu/g M = 10 <sup>5</sup> cfu/g
	<i>Salmonella</i> spp.	5	0	n.n. 25 g
	<i>Enterobacteriaceae</i>	5	2	m = 10 <sup>2</sup> cfu/g M = 10 <sup>3</sup> cfu/g
	Coagulase positive staphylococci / <i>Staphylococcus aureus</i>	5	1	m = 10 cfu/g M = 10 <sup>2</sup> cfu/g
	Moulds	5	1	m = 10 cfu/g M = 10 <sup>2</sup> cfu/g

m= minimal value for given criterion; M= maximal value for given criterion; cfu=colony forming units

Since only one sampling unit, instead of prescribed five, was taken, in case that the result for particular microorganism from Table 2 was above or equal to "m", between "m" an "M" or above "M", the sample was declared as "unacceptable".

Statistical analyses were done for minimum, maximum and average values of microorganism contamination, using Microsoft Excel.

## Results and discussion

Out of a total of 230 samples of confectioner's cakes, no pathogenic microorganisms were detected in a quantity that poses a risk to human health.

*Salmonella* spp. was not detected in 25 g in any sample, which corresponds to the results of a three-year study conducted in Canada (Canadian Food Inspection Agency, 2016-2018) and in Ireland (FSAI, 2018). *Listeria* (*L.*) *monocytogenes* was not detected in an amount above 100 cfu/g, nor in the Irish study. However, *L. monocytogenes* was previously found in cakes and pastry products collected from different hotels, restaurants and pastry shops in Croatia in 12 samples (4.27%) out of 283, with the result of 10<sup>6</sup>-10<sup>7</sup> cfu/g (Uhitil et al., 2004). In the Canadian study, it was pointed out that *L. monocytogenes* was found in only one case below 10<sup>2</sup> cfu/g. However, given that the sources of contamination for *L. monocytogenes* are

numerous, in the case of poor cleaning and sanitation, all of them, i.e. the product itself, the personnel who handle food, the environment (utensils and equipment) and the consumers themselves can be the source or carriers of pathogens. microorganisms (Lianou and Sofos, 2007). In the Canadian study in only one sample *S. aureus* was found for the criteria  $10^2 < X < 10^4$  cfu/g. In our research, *S. aureus* was found in 86.52% (199) cases up to  $10^2$  cfu/g, while in 23 cases it was found  $10^2$ , in 7 cases  $10^3$  and in one case  $10^4$  cfu/g (Table 2). In the Irish research, they found coagulase-positive staphylococci, which they found to be acceptable ( $10^2 < X < 10^4$  cfu/g) in 1.5% and unacceptable in 0.3% of cases. ( $\geq 10^4$  cfu/g). Given that all the samples that were declared as unacceptable were kept at a temperature of 5-10 °C, they concluded that in addition to proper handling, a very important factor is the appropriate temperature of cake storage that limits the growth of bacteria, and consequently the risk of gastrointestinal diseases. Their recommendation is that cakes should not be kept at a temperature above 5 °C (FSAI, 2018). Staphylococcal toxin production is expected in cases where *S. aureus* is present in  $10^5$  cfu/g, or higher (HPA, 2009), therefore, despite the presence of this bacteria found in our research, there will be no harmful effects on human health.

Aerobic mesophilic bacteria (AMB), *Enterobacteriaceae* and moulds are microbiological parameters used as indicators of production hygiene. AMB were determined in an amount greater than  $10^4$

in 35% of cases, to which fruit cakes (43%) contributed more than other cakes (32%). In cases where cfu/g was greater than  $10^4$ , for fruit cakes it ranged from  $1.9 \times 10^4$  to  $10^8$  cfu/g, with a mean value of  $5.1 \times 10^6$  cfu/g. In the other cakes, they were present in the range of  $1.2 \times 10^4$ - $10^8$  cfu/g, and the mean value was  $3.1 \times 10^6$  cfu/g, so slightly lower values than in fruit cakes. Our results are worse than the result of Meldrum et al. (2005), who found aerobic colony counts in 15% (121/808), of the samples of custard slices surveyed but similar to Kumar et al., (2011) who found aerobic colony counts, in contamination of bacteria ranging  $1.37 \times 10^6$  cfu/g to  $11.27 \times 10^6$  cfu/g. Kotzekidou (2013) reported on 13.5% of desserts oven baked with  $> 10^5$  cfu/g AMB, and in desserts with dairy cream AMB count ranged from  $10^3$  to  $10^9$  cfu/g, obtained from a 10-year inspection survey.

A number of *Enterobacteriaceae* above the value of  $10^2$  cfu/g were found in 35% of cakes, which was contributed more by fruit cakes where they were found in 39% of cases, than other cakes where they were found in 30% of cases. In cases where cfu/g was greater than  $10^2$  cfu/g, for fruit cakes it ranged from  $5 \times 10^2$  to  $10^5$  cfu/g, with a mean value of  $2.9 \times 10^4$  cfu/g. In the other cakes, they were present in the range of  $3 \times 10^2$ - $5 \times 10^6$  cfu/g, and the mean value was  $2 \times 10^5$  cfu/g. Kotzekidou (2013) found *Enterobacteriaceae* in 38.5% oven-baked desserts samples and highest *Enterobacteriaceae* contamination in desserts with dairy cream (76.7%) with distribution in the range of  $10^3$  to  $< 10^7$  cfu/g.

**Table 3.** Absolute and relative number of cakes contaminated with microorganisms in total, and divided in creamy/chocolate and fruit cakes

Microbiological parameters	Total		Creamy/chocolate cakes		Fruit cakes	
	N	%	N	%	N	%
cfu/g						
AMB* $\leq 10000$	149	65	110	68	39	57
AMB* $> 10000$	81	35	51	32	30	43
<i>Enterobacteriaceae</i> $\leq 100$	150	65	113	70	42	61
<i>Enterobacteriaceae</i> $> 100$	80	35	48	30	27	39
Moulds $\leq 10$	211	92	146	91	65	94
Moulds $> 10$	19	8	15	9	4	6
<i>Staphylococcus aureus</i> $\leq 10$	199	87	143	89	56	81
<i>Staphylococcus aureus</i> $> 10$	31	13	18	11	13	19

\*AMB= Aerobic mesophilic bacteria

Moulds above 10 cfu/g was found in a total of 8% (19), i.e. in 6% of fruit cakes and 9% of other cakes. In cases where cfu/g was greater than 10, for fruit cakes, it ranged from  $2 \times 10^2$  to  $8 \times 10^3$  cfu/g, with a mean value of  $2.9 \times 10^3$  cfu/g. In the other cakes, they were present in the range of  $10^2$ - $3.1 \times 10^3$  cfu/g, and the mean value was  $8 \times 10^2$  cfu/g,

thus lower than that found in the fruit cakes. Moulds and yeasts, can cause food spoilage. Mould spores are killed due to baking process, and contamination (O'Brien, 2004) is a consequence of cakes exposure to air and surfaces (Gill et al., 2020), but it can also occur, in production steps such as cooling, slicing, transporting, packing and storage.

Hassanzadazar et al. (2018) reported on 48,39% contamination of cream filled pastries with moulds and yeasts. They concluded that the cream-filled pastries containing fruit or nuts usually have higher mould contamination because of microbial contamination of used raw materials. The presence of mould in confectionary cakes was determined by Sharifzadeh et al. (2016) in 27.5% of samples, Pajohi-Alamoti et al. (2016) in 45% puff pastry and 30% jelly roll samples, and Kumar et al. (2011) reported on yeast and mould counts ranging from  $1.33 \times 10^5$  cfu/g to  $92.5 \times 10^5$  cfu/g, which suggests the conclusion that mould contamination is a ubiquitous problem and that not enough attention was paid to its prevention. Water activity plays important element for microbiological contamination, which survive or reproduce when water activity is high like in cakes/creamy or fruity based products (Abdullah et al., 2000; Syamaladevi et al., 2016). Particularly moulds but also some species of yeasts and bacteria may infect cakes because of increased sugar content which leads to cake spoilage (Gill et al., 2020).

## Conclusions

Cakes as RTE foods, can be contaminated with pathogens during production, handling, packaging and distribution. Since these products will not undergo any procedure that can inactivate bacterial pathogens their presence represents a potential risk to consumer for foodborne illnesses. However, in our research no foodborne bacterial pathogen was found in cakes samples, and for this type of food we can conclude that they are safe for consumption.

On the other hand, cakes were contaminated with microorganisms which represent hygiene parameters indicator and it is recommended for producers, retailers and consumer as well, to maintain safe and good hygienic practice while handling. Requirement for maintenance of the cold chain is mandatory prescribed in Commission Regulation (EC) No. 852/2004, and recommendation is to provide storage temperature from 0 °C to 5 °C (FSAI, 2018).

However, microorganism's indicator of good hygiene practice contamination number, no matter if higher than recommended, cannot be related with possible pathogen contamination. Reliable result indicating presence or absent of pathogen bacteria, can be only obtained with specific microbiological analytical method.

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