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REGULAR ARTICLE

MICROORGANISMS IN CONFECTIONERY PRODUCTS

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ABSTRACT

The aim of this work was to determine microbiological quality of confectionery products. In confectionery products microbiological parameters: coliforms bacteria, microscopic filamentous fungi and yeasts, *Salmonella* sp. and staphylococci were observed. The confectionery products were evaluated: Kremes - honey cube, roll Arabica, roll Rona, roll stuffed with apricot cream, honey cube, pinwheel caramel, Sachovnica cut, Zora cut and curd cake. For microbiological tests 18 samples of confectionery products were used. Numbers of coliforms bacteria in confectionery products ranged from $<1x10^1$ to $4x10^2$ cfu.g^{-1}, the number of microscopic fungi ranged from 0 to $<1x10^1$ cfu.g^{-1}, the number of yeasts from $<1x10^1$ to $5.5x10^2$ cfu.g^{-1}, cells of *Salmonella* sp. were not detected and the number of staphylococci was from 0 to $<1x10^1$ cfu.g^{-1}. All investigated samples of confectionary products were in accordance with the Codex Alimentarius of the Slovak Republic.

Keywords: confectionery products, microbiological quality, coliforms bacteria, microscopic fungi and yeasts

INTRODUCTION

The confectionery industry is one of the fastest growing segments in the global food market. It represents a broad array of products that can be divided into four categories: jellied candies; aerated and/or grained confections; caramels, fudge and toffees; and chocolate and compound coatings. Of these, chocolates and chocolatecoated products account for nearly 50 percent of U.S. confectionery sales. Retail forecast sales for U.S. confections are expected to reach $29 billion. With the growing demand for confectionery products worldwide, it is critical for manufacturers to take concerted actions to improve product quality and ensure product safety. The confectionery industry, both here and abroad, boasts an outstanding safety record. From 1999–2003, less than 11 percent of 1,146 Food and Drug Administration food recalls were due to chocolates and nonchocolate candy products**(Williams et al., 2006)**.

Traditionally, chocolate and other confectionery products are regarded as being microbiologically stable and safe to eat. Owing to the inherent low water activity of chocolate it is unlikely to support the growth and proliferation of bacterial pathogens. Despite this, there have been occasional outbreaks of salmonellosis throughout the world associated with the consumption of chocolate and chocolate products contaminated with Salmonella. To date in the UK, outbreaks have been only associated with imported chocolate and confectionery products and these and reported outbreaks in other countries have been well documented. These have included outbreaks caused by *Salmonella enterica* sub-species enterica serovar Eastbourne in the United States and Canada in 1973–1974 **(Craven et al., 1975; D'Aoust et al., 1975)**, Salm. Napoli infection in England and Wales in 1982 **(Gill et al., 1983)**, Salm. Nima in Canada in 1986 **(Hocking et al., 1989)**, Salm. Typhimurium in Norway and Finland in 1987 **(Kapperud et al., 1990)** and more recently, Salm. Oranienburg in Germany in 2001–2002 **(Anon, 2002)**.

Theoretically, there are certain parallels that exist between the potential sources of contamination between these Gram-negative pathogens and the control measures that could be taken to prevent or reduce the risk of contamination. However, despite the concern over VTEC in foods, unlike Salmonella there are few, if any, published papers on the survival of VTEC, including E. coli O157:H7, in chocolate or other related products. Consequently, The United Kingdom Biscuit, Cake, Chocolate & Confectionery Alliance (BCCCA) commissioned research to determine the survival of E. coli belonging to serotypes O157:H7, O111:H and O26:H11, artificially inoculated at low levels into chocolate, mallow and biscuit cream under conditions that would mimic contamination during manufacture. Possible routes

of contamination of chocolate by Salmonella bacteria can include the use of contaminated raw materials, in-line and finished product contamination during manufacture and deviations from good manufacturing practice **(D'Aoust, 1977; Baylis et al., 2001)**.

The objective of our study was determining microbiological quality of confectionery products. In confectionery products microbiological parameters: coliforms bacteria, microscopic filamentous fungi and yeasts, *Salmonella* sp. and staphylococci were observed.

MATERIAL AND METHODS

Collection of confectionery samples

The samples of confectionery products of selected types were collected from Slovak production. For microbiological analyses samples of Kremes -honey cube (2 samples), roll Arabica (2 samples), roll Rona (2 samples), roll stuffed with apricot cream (2 samples), honey cube (2 samples), Pinwheel caramel (2 samples, Sachovnica cut (2 samples), Zora cut (2 samples) and curd cake (2 samples) were used. For microbiological tests all 18 samples of confectionery products were used before expiration date.

Determination of CFU counts

For microbiological analysis the confectionary samples were processed immediately after collection. The coliforms bacteria (CB), microscopic filamentous fungi (MF) and yeasts (Y), *Salmonella* sp. (SS) and staphylococci (S) were observed. Plate diluting method was applied for quantitative CFU (Colony Forming Units) counts determination of respective groups of microorganisms in 1 g of confectionery products. Petri dishes of gelatinous nutritive substrate were inoculated with 1 mL of confectionery samples (CB, MF, Y, SS, S) in three replications. Homogenized samples of confectionery were prepared in advance by sequential diluting based on decimal dilution system application. For microorganism cultivation three types of cultivating mediums were used, to segregate individual microorganism groups. Violet red bile agar was used for CFU segregation of CB (incubation 24 h at 37 °C, aerobic cultivation method). Chloramphenicol glucose yeast agar was used for CFU segregation of MF (incubation 5-7 days at 25 °C, aerobic cultivation method). Yeast extract agar was used for CFU segregation of Y (incubation 5-7 days at 25 °C, aerobic cultivation method). Xylose

lysine desoxycholate agar was used for CFU *Salmonella* sp. segregation 24 h at 37 °C, aerobic cultivation method) and Baird Parker agar was used for CFU segregation of staphylococci (incubation 24 h at 37 °C, aerobic cultivation method). Cultivating medium composition corresponded to producer introductions (Biomark^{TM}, Pune, India). Basic dilution (10^{-1}) was prepared as follows: 5 g of confectionery was added to the bank containing 45 mL of distilled water. The cells were separated from substrate in shaking machine (30 minutes). Prepared basic substance was diluted to reduce the content of microorganisms below 300 CFU level.

RESULTS AND DISCUSSION

Confectionery, because of its low a_w is generally free from microbial hazards and microbial spoilage problems. The main hazards, when they exist, may come from such ingredients as cocoa, coconut, milk powder, and egg albumen, all of which sometimes contain *Salmonella,* and nuts and cocoa, which may contain mycotoxins. The main microbial spoilage problems are explosion of enrobed preparations of fruit and fondant as a result of growth and gas production by osmophilic yeasts introduced with the fruit, explosion of marzipan from gas produced by osmophilic yeasts introduced with nuts or from the environment, and 'Wasserflecken' (globular areas with watery appearance) in marzipan resulting from growth of moulds **(Windisch et al., 1978)**. Moulds may also cause spoilage as a result of condensation of moisture in water-impermeable packages **(Jarvis, 1982)**.

Table 1 Microbiological quality of Kremes - honey cube 100 g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	$CFU.g^{-1}$	$5x10^2$	$4x10^2$	30 %	**STN ISO 4832**	meets
Microscopic fungi	$CFU.g^{-1}$	$1x10^2$	0	-	**STN ISO 7954**	meets
Yeasts	$CFU.g^{-1}$	$1x10^4$	$1.2x10^2$	30 %	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	$CFU.g^{-1}$	$1x10^2$	0	-	**STN ISO 6888**	meets

In Kremes - honey cube samples (Table 1) the number $4x10^2$ CFU.g^{-1} of coliforms bacteria was found. Number of yeast was $1.2x10^2$ CFU.g^{-1}. Zero numbers of microscopic fungi and staphylococci and the absence of cells *Salmonella* sp. were found. Kremes - honey cubes samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

Table 2 Microbiological quality of Zora cuts 80g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	CFU.g^{-1}	$5x10^2$	$< 1x10^1$	-	**STN ISO 4832**	meets
Microscopic fungi	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 7954**	meets
Yeasts	CFU.g^{-1}	$1x10^4$	$5.5x10^2$	25%	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

In Zora cuts (Table 2) numbers of coliforms bacteria, microscopic fungi and staphylococci were less than $1x10^1$ CFU.g^{-1}. Number of yeasts was $5.5x10^2$ CFU.g^{-1}. Zero numbers of cells *Salmonella* sp. were found. Zora cuts samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

In Rona roll samples (Table 3) of coliforms bacteria number, microscopic fungi and staphylococci were less than $1x10^1$ CFU.g^{-1}. Number of yeast was $1.8x10^2$ CFU.g^{-1}. Zero numbers of cells *Salmonella* sp. were found. Rona roll samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

Table 3 Microbiological quality of Rona rolls 360g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	CFU.g^{-1}	$5x10^2$	$< 1x10^1$	-	**STN ISO 4832**	meets
Microscopic fungi	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 7954**	meets
Yeasts	CFU.g^{-1}	$1x10^4$	$1.8x10^2$	25%	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

In honey cube samples (Table 4) coliforms bacteria number was $1.5x10^2$, numbers of microscopic fungi and staphylococci were less than $1x10^1$ CFU.g^{-1}. Number of yeast was $7.7x10^2$ CFU.g^{-1}. Zero numbers of *Salmonella* sp. cells were found. Rona roll samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

Table 4 Microbiological quality of honey cube 40g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	CFU.g^{-1}	$5x10^2$	$1.5x10^1$	27%	**STN ISO 4832**	meets
Microscopic fungi	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 7954**	meets
Yeasts	CFU.g^{-1}	$1x10^4$	$7.7x10^2$	25%	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

In pinwheel caramel samples (Table 5) of coliforms bacteria number, microscopic fungi, yeasts and staphylococci were less than $1x10^1$ CFU.g^{-1}. Zero numbers of cells *Salmonella* sp. were found. Pinwheel caramel samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

In Sachovnica cut samples (Table 6) of coliforms bacteria number, microscopic fungi, yeasts and staphylococci were less than $1x10^1$ CFU.g^{-1}. Zero numbers of cells *Salmonella* sp. were found. Sachovnica cut samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

Table 5 Microbiological quality of pinwheel caramel 120g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	CFU.g^{-1}	$5x10^2$	$< 1x10^1$	-	**STN ISO 4832**	meets
Microscopic fungi	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 7954**	meets
Yeasts	CFU.g^{-1}	$1x10^4$	$< 1x10^1$	-	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	CFU.g^{-1}	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

Table 6 Microbiological quality Sachovnica cut 35g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	$CFU.g^{-1}$	$5x10^2$	$< 1x10^1$	-	**STN ISO 4832**	meets
Microscopic fungi	$CFU.g^{-1}$	$1x10^2$	$< 1x10^1$	-	**STN ISO 7954**	meets
Yeasts	$CFU.g^{-1}$	$1x10^4$	$< 1x10^1$	-	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	$CFU.g^{-1}$	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

In roll stuffed with apricot cream samples (Table 7) of coliforms bacteria number, microscopic fungi, yeasts and staphylococci were less than $1x10^1$ $CFU.g^{-1}$. Number of yeasts was $1.8x10^2$ $CFU.g^{-1}$. Zero numbers of cells *Salmonella* sp. were found. Roll stuffed with apricot cream samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

Table 7 Microbiological quality of roll stuffed with apricot cream 60g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	$CFU.g^{-1}$	$5x10^2$	$< 1x10^1$	-	**STN ISO 4832**	meets
Microscopic fungi	$CFU.g^{-1}$	$1x10^2$	$< 1x10^1$	-	**STN ISO 7954**	meets
Yeasts	$CFU.g^{-1}$	$1x10^4$	$< 1x10^1$	-	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	$CFU.g^{-1}$	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

In Arabica roll samples (Table 8) of coliforms bacteria number was $1.6x10^2$ $CFU.g^{-1}$. Number of yeast was $1.5x10^2$ $CFU.g^{-1}$. Zero numbers of microscopic fungi and cells *Salmonella* sp. were found. Number of staphylococci was less than $1x10^1$ $CFU.g^{-1}$. Arabica roll samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

Table 8 Microbiological quality of Arabica rolls 60g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	$CFU.g^{-1}$	$5x10^2$	$1.6x10^1$	30%	**STN ISO 4832**	meets
Microscopic fungi	$CFU.g^{-1}$	$1x10^2$	0	-	**STN ISO 7954**	meets
Yeasts	$CFU.g^{-1}$	$1x10^4$	$1.5x10^1$	30%	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	absent	-	**STN ISO 6579**	meets
Staphylococci	$CFU.g^{-1}$	$1x10^2$	$< 1x10^1$	-	**STN ISO 6888**	meets

In curd cake (Table 9) of coliforms bacteria number $2x10^2$ $CFU.g^{-1}$ in samples was found. Number of yeast was $1.6x10^2$ $CFU.g^{-1}$. Zero numbers of microscopic fungi and staphylococci and the absence of cells *Salmonella* sp.cells were found. Curd cake samples were in accordance with the Codex Alimentarius of the Slovak Republic **(CA SR, 2009)**.

The control of raw materials, processing and environment are critical factors in the prevention of microbial contamination in confectionery. Salmonella has been found to be the major hazard in confectionery. Testing for this organism at specific control points provides the best means of quality control. Constant surveillance and good manufacturing practice are the best methods for prevention of contamination **(DeFigueiredo, 1979)**.

Table 9 Microbiological quality curd cake 80g

Indicator	Unit	Permitted value	Measured value	Measurement uncertainty	Test method	Evaluation
Coliforms bacteria	$CFU.g^{-1}$	$5x10^2$	$2x10^1$	30%	**STN ISO 4832**	meets
Microscopic fungi	$CFU.g^{-1}$	$1x10^2$	0	-	**STN ISO 7954**	meets
Yeasts	$CFU.g^{-1}$	$1x10^4$	$1.6x10^1$	30%	**STN ISO 7954**	meets
***Salmonella* sp.**	less	absent	Absent	-	**STN ISO 6579**	meets
Staphylococci	$CFU.g^{-1}$	$1x10^2$	0	-	**STN ISO 6888**	meets

A total of 49 dessert samples were collected from 35 restaurants in Guadalajara, and the same number of desserts from 33 restaurants in Houston. All the samples tested were negative for non–*E. coli* enteropathogens. Coliforms bacteria were found in 47 of the 49 (95.9%) samples from Guadalajara and in 10 of the 49 (20.4%) samples from Houston. The

average coliforms count was $5.8x10^4$ CFU.g^{-1} of dessert in Guadalajara compared with 0.2x 10^4 CFU.g^{-1} of dessert in Houston.

Escherichia coli were found in 6/49 (12%) desserts from Guadalajara compared with 0/49 (0%) from Houston. ETEC was detected in four Mexico desserts: one dessert was positive for both a heat stable toxin-producing strain (ST-ETEC) and a heat labile toxin-producing strain (LT-ETEC) and three others were positive for ST-ETEC strains. EAEC was identified in one dessert from Mexico. The sixth *E. coli* strain from Mexico was not identified as ETEC or EAEC, but was not further categorized to assure it was non-diarrheagenic.

Among the various types of desserts studied in Mexico including cream-filled, those topped with frosting, and desserts with ice cream, items with ice cream were found to be the most frequently contaminated with *E. coli* compared to the desserts without ice cream. Four of the six dessert samples positive for *E. coli* contained ice cream. Additionally, desserts were also classified as being served hot, cold, or room temperature based on the temperature when served. The temperature differences were minimal and did not correlate with coliforms or *E. coli* counts **(Vigil et al, 2009)**.

Balyis et al., 2004 in their study determined, that the survival of *Escherichia coli* O157:H7 and other verocytotoxin-producing *E. coli* (VTEC) in chocolate and other confectionery products has not been fully established, unlike *Salmonella*, which have been responsible for occasional outbreaks of infection linked to contaminated chocolate and related products, although none of these outbreaks have been related to products produced in the United Kingdom. The United Kingdom Biscuit, Cake, Chocolate and Confectionery Alliance commissioned this study to obtain information on thedecline and potential survival of *E. coli*, particularly verocytotoxin-producing strains, in reduced a_w confectionery products chocolate, biscuit cream and mallow. These products were artificially contaminated with high (4 log10 CFU.g^{-1}) and low (2 log10 CFU.g^{-1}) levels of *E. coli* O157:H7, O111:H- and O26:H11 and their survival, as affected by storage temperature (10, 22 and 38 °C), was monitored over 12 months. Irrespective of sample type, rapid decline was observed in products stored at 38 °C and increased survival occurred in products stored at 10 °C. In chocolate (average a_w 0.40), these bacteria were detected for up to 43 days in samples stored at 38 °C. At 22 °C they survived for up to 90 days and in product stored at 10 °C they could still be detected after 366 days of storage. In biscuit cream (average a_w 0.75) they survived for 2 days at 38°C, 42 days at 22 °C and 58 days at 10 °C. Whilst mallow (a_w 0.73) was not stored at 38 °C, these bacteria could still be detected in samples stored for up to 113 and 273 days at 22 and 10 °C, respectively. The observed prolonged survival of these bacteria under conditions of reduced

a_w and lowered storage temperature in this study is supported by previous studies with *Salmonella* and *E. coli* O157:H7 in other foods. In the same way that *Salmonella* bacteria can survive for long periods, in excess of 12 months, in chocolate, this study provides evidence that *E. coli*, including pathogenic strains, can also survive for similar periods of time. Assuming the routes of transmission are similar, controls currently used by the confectionery industry to prevent contamination by *Salmonella* should also be effective against *E. coli*, including VT-producing strains, providing that all raw materials have been suitably processed, stored and handled before and during manufacture.

Understanding the nature of microorganisms (including their sources and growth characteristics) is key to microbial control in confectionery plants. Microorganisms gain access to food-processing areas through multiple routes (e.g., raw materials, personnel and equipment traffic, water leaks and pests). Failure to implement appropriate and effective process and sanitation controls could allow these microbes, including pathogens, to become established in the processing environment where they may be able to survive for extended periods of time and recontaminate product **(Williams et al., 2006)**.

CONCLUSION

For microbiological tests 18 samples of confectionery productswere used. Numbers of coliforms bacteria in confectionery products ranged from $<1x10^1$ to $4x10^2 CFU.g^{-1}$, the number of microscopic fungi ranged from 0 to $<1x10^1 CFU.g^{-1}$, the number of yeasts ranged from $<1x10^1$ to $5.5x10^2 CFU.\ g^{-1}$, cells of *Salmonella* sp.in either sample were not present and the number of staphylococci ranged from 0 to $<1x10^1 CFU.g^{-1}$. All investigated confectionery products samples meet the demands placed on these types of products.

The issue of safety and wholesomeness (safety)of food plays a special role in the prioritization of control during manufacture and handling of food. Questions of quality and wholesomeness of food, together with regard to environmental protection are becoming increasingly of concern not only among experts from various professions and disciplines, but also the general public.

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