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MICROBIOLOGY OF RAW MATERIALS USED FOR CONFECTIONARY PRODUCTION

*Jana Petrová[1], Ľubomíra Juhaniaková[1], Margarita Terentjeva[2], Simona Kunová[3], Henrieta Blaškovičová[1], Miroslava Kačániová[1]**

Address(es):
[1] Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Microbiology, Trieda Andreja Hlinku 2, 949 76, Nitra, Slovakia.
[2] Latvia University of Agriculture, Faculty of Veterinary Medicine, Institute of Food and Environmental Hygiene, K. Helmaņa iela 8, LV 3007, Jelgava, Latvia.
[3] Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Trieda Andreja Hlinku 2, 949 76 Nitra, Slovak Republic.

*Corresponding author: miroslava.kacaniova@gmail.com

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ABSTRACT

The aim of our study was to evaluate the microbiological quality of raw materials used for preparation of confectionery products. For microbiological evaluation total count of bacteria, mesophilic aerobic bacteria, coliform bacteria, yeast and microscopic filamentous fungi in samples of raw materials used in the manufacture and creams of confectionery products were detected. In addition to these groups of microorganisms the presence of pathogenic microorganisms *Salmonella* spp. and *Staphylococcus aureus* in creams was monitored. Products are assessed according to the limit values of the number of microorganisms defined in the Codex Alimentary of the Slovak Republic. For microbiological analysis of confectionery products, sampling of components of confectionary products and cream was carried out according to current health regulations and altogether 65 samples of components and creams were collected: 10 samples of raw materials sugar, 10 samples of flour, 10 samples of butter and 10 samples of eggs, 5 samples of butter yolk from cream-filled disposable bag without rum addition, 5 samples of butter yolk from cream-filled disposable bag with rum addition, 5 samples of cream-filled multiple use paid bag, 5 samples of cream-filled newly purchased paid bag, 5 samples of Venček corpus and 5 samples of the French cubes corpus. From raw material the highest TBC (2.65log CFU) was in flour, but the lowest in sugar (1.35 log CFU), the highest years counts was found on flour (2.42), but lowest in butter (1.18), while wasn't in egg. In samples of creams and corpus were increased occurrence of yeast, coliform bacteria. *Salmonella* spp. and *Staphylococcus aureus* weren't isolated from any tested sample.

Keywords: Microorganisms, bacteria, filamentous microscopic fungi, raw material products

INTRODUCTION

The control of raw materials, processing and environment are critical factors in the prevention of microbial contamination of confectionery.
Food spoilage is an actual economic problem worldwide. Approximately one-fourth of the world's food supply is lost through microbial activity alone (**Huis in't Veld, 1998**). This is linked to properties of the several components used in production of confectionary as milk, flour and sugar. Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms (**Ruegg, 2003; Rajagopal, 2005**). The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions, and post-heat treatment contamination (**Richter *et al.*, 1992**). Undesirable microbes that can cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms, lactic acid bacteria, yeasts, and molds. In addition, various bacteria of public health concern such as *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, pathogenic strains of *Escherichia coli* and enterotoxigenic strains of *Staphylococcus aureus* may also be found in milk and dairy products. For this reason, increased emphasis should be placed on the microbiological examination of milk and dairy foods. Microbiological analyses are critical for the assessment of quality and safety, conformation with standards and specifications, and regulatory compliance (**Vasavada, 1993**).
White sugar has been used for ages in food production not only as a sweetener but also as a valuable ingredient contributing to the color, flavor and texture of food. Recently, world sugar production is about 160 Mt per year and it shows a general tendency to increase. Further increase of world sugar production is still expected and, according to prognosis, in the year 2019/2020 may exceed 200 Mt per year. World sugar production is based in 70% on sugar cane and in 30% on sugar beet, while in the European Union 98% of total sugar production comes from sugar beet (**Nolte and Grethe, 2011; OECD-FAO, 2010**).
Flour is generally regarded as a microbiologically safe product as it is a low water activity commodity. Although the growth of pathogenic bacteria may not be supported under such conditions, pathogens that contaminate flour may survive for extended period. There are few reported incidents of food poisoning resulting from contaminated flour. Australian, European and the USA studies indicate that *Salmonella* spp., *Escherichia coli*, *Bacillus cereus* and spoilage microorganisms are present in wheat and flour at low levels (**Berghofer *et al.*, 2003**). It is important to point out that *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 (**De Figueiredo, 2007**).
In a controlled experimental setting, **De Reu *et al.* (2005a, 2005b)** found that levels of total aerobic microorganisms on eggs was 5.5-6.0 log CFU.mL^{-1}. **De Reu *et al.*, 2009**). **Huneau-Salaun *et al.* (2010)** found similar but less pronounced differences when they compared numbers of total aerobic microorganisms recovered from eggs. Another study conducted on a research farm found the opposite of **De Reu *et al.* (2005b; 2009) and Huneau-Salaun *et al.* (2010)** and reported that total aerobic microorganisms on eggs were 90% lower (2.25 and 2.75 log10 CFU.mL^{-1}, respectively) during both winter and spring (**Jones *et al.*, 2011**).
The aim of our study was to evaluate the microbiological quality of raw materials use for preparation of confectionery products. For microbiological evaluation total count of bacteria, mesophilic aerobic bacteria, coliform bacteria, yeast and microscopic filamentous fungi in samples of raw materials used in the manufacture and creams of confectionery products were detected. In addition to these groups of microorganisms the presence of pathogenic microorganisms such as *Salmonella* spp. and *Staphylococcus aureus* in creams was monitored.

MATERIAL AND METHODS

Samples collection

For microbiological analysis of confectionery products, we take adequate samples of intermediate products are carried out according to current health regulations. For microbiological analysis were collected 10 samples of raw materials sugar, 10 samples of flour, 10 samples of butter and 10 samples of eggs. Below were taken for microbiological examination samples of creams: 5 samples of butter yolk from cream-filled disposable bag without rum addition, 5 samples of butter yolk from cream-filled disposable bag with rum addition, 5 samples of cream-filled multiple use paid bag, 5 samples of cream-filled newly purchased paid bag, 5 samples of Venček corpus and 5 samples of the French cubes corpus.

Determination of CFU counts

For microbiological analysis the confectionary samples were processed immediately after collection. The total count of bacteria (TCB), mesophilic aerobic bacteria (MAB), coliforms bacteria (CB), yeasts (Y), microscopic filamentous fungi (MF), *Staphylococcus aureus* (SA) and *Salmonella* spp. (SS) were assessed. Colony forming unit counting method was applied for quantitative determination of respective groups of microorganisms in 1g of confectionery component materials. Plate Count Agar was used for CFU counting isolation of TCB and after inoculation agar was incubated for 48-72 h at 30 °C applying aerobic cultivation method. Meat peptone agar was used for MAB CFU counting and inoculated agar was incubated for 48-72 h at 25 °C applying aerobic cultivation method). Violet Red Bile agar was used for CB CFU counting by incubation of inoculated agar for 24 h at 37 °C applying aerobic cultivation method). DRBC and DG18 agars were used for Y and MF CFU counting by incubation of inoculated agars for 5-7 days at 25 °C applying aerobic cultivation method). XLD agar was used for isolation of *Salmonella* spp. by incubation of inoculated agar for 18-24 hour at 37 C applying aerobic cultivation method) and Baird Parker agar was used for *Staphylococcus aureus* isolation by incubation of inoculated agar for 45-48 hour at 35-37 °C applying aerobic cultivation method). All cultivating medium were obtained from Biomark^{TM}, Pune, India.

Statistical analysis

For data from each replication the mean was calculated and all data were log transformed. Statistical analysis was done with STATGRAPHICS 5 software (UMEX GmbH Dresden, Germany). For number of total count of bacteria (TCB), mesophilic aerobic bacteria (MAB), coliforms bacteria (CB), yeasts (Y), microscopic filamentous fungi (MF), standard deviation (SD) and coefficient of variability (CV) were calculated.

RESULTS AND DISCUSSION

In this study were analyzed raw materials for confectionary products as sugar, flour, butter and eggs. In next stage of microbiological butter cream samples and corpuses of Venček and French cubes were analyzed. Results of microbiological testing of raw materials - sugar, flour, butter, eggs, as well as samples of butter yolk from cream-filled disposable bag without rum addition, samples of butter yolk from cream-filled disposable bag with rum addition, samples of cream-filled multiple use paid bag, samples of cream-filled newly purchased paid bag, samples of Venček corpus and samples of the French cubes corpus are summarized in Fig. 1-10.

The microflora of sugar cane, depending on its type, is composed of many different kinds of microorganisms. Among them, the most often enumerated include the following microorganisms: bacteria: *Bacillus* spp., *Flavobacterium* spp., *Pseudomonas* spp., *Xanthomonas* spp., *Lactobacillus* spp. and *Enterobacteriaceae* ; yeasts: *Saccharomyces* spp., *Torula* spp. and *Pichia* spp.; and fungi: *Penicillium* spp., *Actinomyces* spp. and *Streptomyces* spp. (**Martini *et al.*, 2010; Wojtczak *et al.*, 2012**). The composition of the population of microorganisms is closely linked to the sugar content and pH of sugar cane. Immediately after cane sugar is cut, the microflora inside its stem is composed primarily of yeasts and bacteria: *Leuconostoc* spp., *Xanthomonas* spp. and *Aerobacter* spp. At the same time, rapid development of bacteria of the genus *Leuconostoc*, namely: *L. mesenteroides* and *L. dextranicum* may lead to the formation of sugar losses reaching up to 1.5% due to the production of acids, dextran and mucus (**Cerutti de Guglielmone *et al.*, 2002 and Eggleston *et al.*, 2001**).

Number of microscopic filamentous fungi, number of coliforms bacteria, mesophilic aerobic bacteria was not find in this study. All tested samples were according Codex Alimentarius **CA SR (2009)**. Similar samples of butter were according to **CA SR (2009)**. Average number of total count of bacteria in egg samples was 2.17 log CFU.g^{-1}, number of mesophilic aerobes bacteria was 1.85 log CFU.g^{-1} and microscopic filamentous fungi 0.23 log CFU.g^{-1}. Statistical significant differences of total count of bacteria were found among sugar, butter, sugar and flour, sugar and eggs, flour and eggs. Coliform bacteria, *Staphylococcus aureus* and *Salmonella* spp. were not found in these samples.

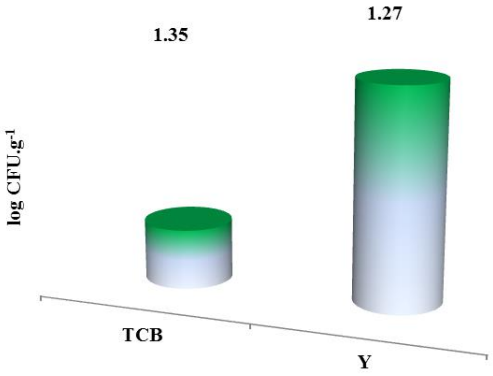

Figure 1 Microbiological quality of sugar samples

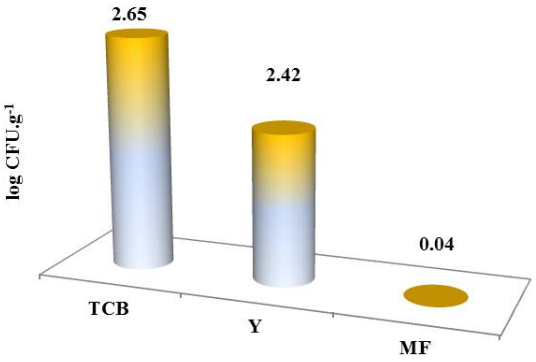

Figure 2 Microbiological quality of flour samples

Average number of total count of bacteria in flour samples was 2.65 log CFU.g^{-1}, number of yeast was 2.42 log CFU.g^{-1} and microscopic filamentous fungi 0.04 log CFU.g^{-1}.

The microflora of flour is composed of a variety of micro-organisms, including yeasts, moulds, psychotropic, thermophilic, and thermoduric bacteria, lactic acid bacteria, "rope bacteria", and pathogenic bacteria, more specifically *B. cereus*, *C. perfringens*, *C. botulinum*, and *Salmonella* spp. Although cereal grains and their milled products have rarely been implicated in foodborne disease (**Deibel *et al.*, 2001**), it is the large quantity of flour annually consumed and the associated significant exposure to these micro-organisms that prompted the retrieval of data on the frequency of pathogenic bacteria and microorganisms that would render the food unfit for the consumer. A number of studies on wheat flour report mean total aerobic counts in the order of 10^4 CFU.g^{-1} or below (**Berghofer *et al.*, 2003**). Although the number of samples exceeding total aerobic counts of 10^4CFU.g^{-1} in wheat flour can be very low (**Berghofer *et al.*, 2003**). Coliform bacteria and *E. coli* counts are important as these are indicative of the general hygienic properties of foodstuffs. Moreover, the presence of *E. coli* in a finished, ready-to-eat product can be a public health concern, as this finding may indicate deficiencies in process control (**Deibel *et al.*, 2001**). In our study there were no coliforms in analyzed samples that indicate good microbiological quality of the product. Mean mould counts are usually around 10^3CFU.g^{-1} (**Potus and Suchet, 1989**). Considerably higher levels of mould loads in cereal samples in Turkey, however, i.e. in the order of 10^5-10^6CFU.g^{-1}, have been reported by **Aran and Eke (1987)**. There are different sources for moulds present in flour, for example fungi prevailing in the grain, the mill machinery itself, and/or a lower quality of sanitary control (**Aydin *et al.*, 2009**).

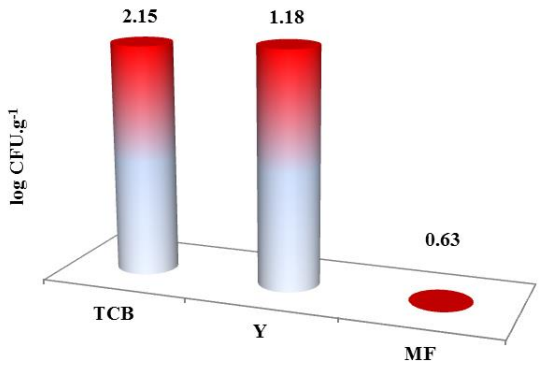

Figure 3 Microbiological quality of butter samples

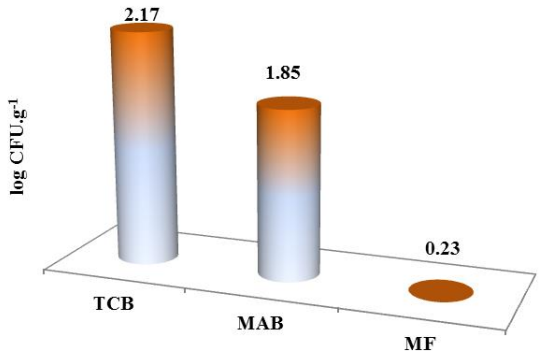

Figure 4 Microbiological quality of egg samples

Although the butter is not a highly perishable food, it does undergo spoilage by bacteria and molds. The main source of microorganisms of butter is cream, whether sweet or sour, raw or pasteurized (**Jay, 1996**). Yeast and molds are important spoilage microorganisms of butter and can result in surface discoloration and off-flavor. Psychrotrophic Gram negative bacteria may develop and result proteolytic and lipolytic changes (**ICMSF, 2005**). Microbiological analysis of butter for specific pathogens is not considered justified and testing is restricted to potential spoilage microorganisms; together with *Escherichia coli* and coliform bacteria (**Varnam and Sutherland, 1993**). The microflora of butter reflects the quality of cream, the sanitary conditions of equipment used to manufacture the butter and the environmental and sanitary conditions during packaging and handling of such product (**Richter *et al*, 1992**). In our study, all of the samples were contaminated by total count of bacteria, yeasts and microscopic filamentous fungi. In previous studies, level of psychrotrophic counts were recorded by **Ahmed *et al*. (1987) and Henin and Kaldes (1992)** with mean values of 3.06×10^4 and 3.01×10^4 cfu.g^{-1}, respectively. On the contrary, none of the examined cooking butter samples contained detectable level of psychrotrophic bacteria (<10g^{-1}) was reported by **El-Sherief (2007)**. Psychrotrophic Gram negative bacteria such as *Pseudomonas* spp. and *Flavobacterium* spp. may develop and cause off-odour formation and rancidity. Growth of *Alteromonas putrefaciens* or *Flavobacterium malodoris* may lead to surface taints very quickly affecting the mass of the product and accompanied by development of a putrid, decomposed or cheesy flavor that render the product unmarketable, leading to economic losses. The presence of molds and yeasts in butter are objectionable as they grow at a wide range of temperature and pH values resulting surface discoloration and off-flavor. However, of even more serious concern is that some molds are capable of producing toxic metabolites known as mycotoxins. Some of these toxins, such as aflatoxins, are known carcinogens (**Meshref, 2010**).

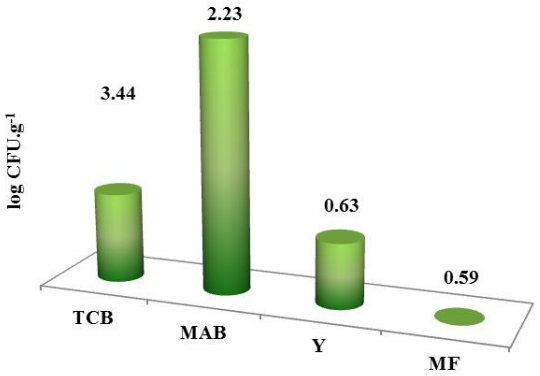

Figure 5 Microbiological quality of samplesbutter yolk from

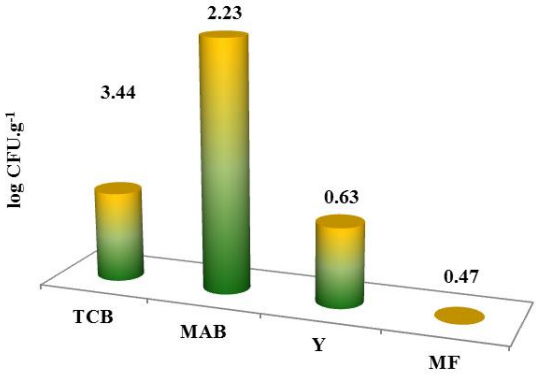

Figure 6 Microbiological quality of samples butter yolk cream-filled cream filled - disposable bag without rum addition disposable bag with rum addition

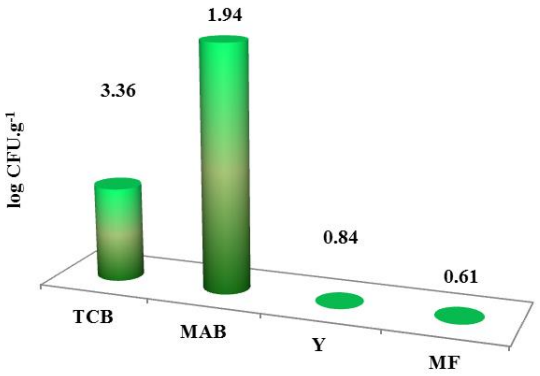

Figure 7 Microbiological quality of samples of cream-filled multiple

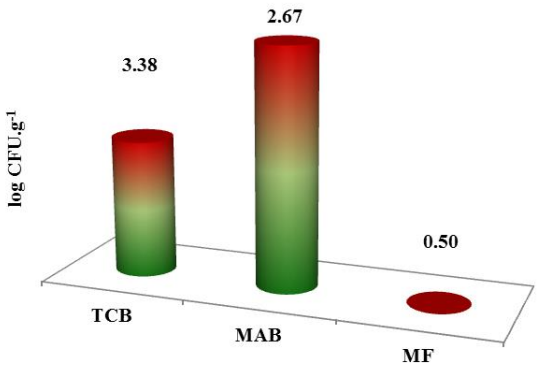

Figure 8 Microbiological quality of samples of cream-filled use of paid bagpaid bag newly purchased

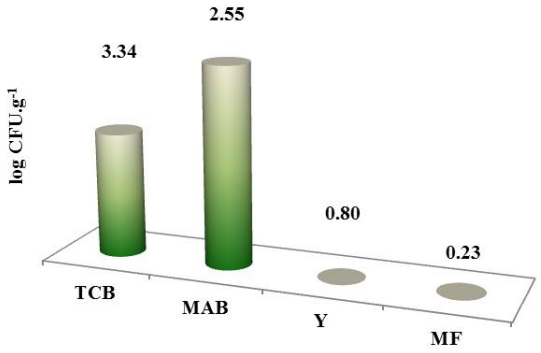

Figure 9 Microbiological quality of Venček corpus samples

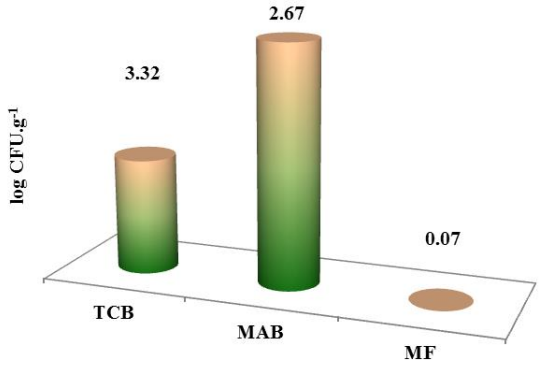

Figure 10 Microbiological quality of French cubes corpus samples

A high bacterial load present on the eggshell surface could increase the chance of eggshell penetration and contamination of internal contents (**Smith *et al*., 2000**). Spiking method indicated that the limit of detection for *Salmonella* Infantis by culture method was approximately 1 log CFU.ml^{-1}. In the present study, shell rinse and crush methods were used to recover *Enterobacteriaceae* from commercial shell eggs as described earlier by **Musgrove *et al*. (2005a,b)**. Limit of detection was not calculated for *Enterobacteriaceae* isolation/count from eggs. It is possible that the limit of detection of *Enterobacteriaceae* isolates from 11 different genera could be variable and further investigations are necessary to determine the detection limits of these different genera. **Jones and Musgrove (2007)** reported a higher *Enterobacteriaceae* count (3.40 log CFU.eggshell^{-1}) from eggshell wash. However, the study of **Jones and Musgrove (2007)** was conducted on restricted shell eggs which did not meet the quality standard for retail. As bacteria can move from eggshell surface into eggshell pores and further into egg internal contents, it is important to study the bacterial count in eggshell pores. **De Reu *et al*. (2005a) and Protais *et al*. (2003)** reported that there was no significant difference in eggshell contamination between beginning and end of the laying period in furnished cages or aviaries. **Huneau-Salaün et al. (2010)** found that eggshell contamination increased significantly with increasing age of flock but **Mallet *et al*. (2006)** reported that contamination decreased with age. However, both of these authors attributed the variation in their results to seasonal or environmental effects rather than flock age.

CONCLUSION

Comparing of the sugar microbiological quality with the requirements of the CA SR, we found that all of the sugar samples were with requirements of this type of raw material. Comparison of flour microbiological quality with CA SR, we found that all the samples were according with those requirements. Similarly, samples of butter were according with the requirements of the CA SR of this type of product. For creamy microbiology we don't have legislation, so we cannot compare this result. For future we recommended continue with research of products which are using for confectionary product production, because microbiological quality of this products can showing microbiological quality of confectionary product.

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