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COMPARISON OF MICROBIOLOGICAL QUALITY OF READY-TO-EAT DELICATESSEN PRODUCT WITH RESPECT TO STORAGE TEMPERATURE

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ABSTRACT

The aim of the present study was to evaluate microbiological quality of ready-to-eat (RTE) delicatessen food. Microbiological parameter were determined in 24 samples together. The safety of ready-to-eat food is an important issue. Improper handling of ready-to-eat food items may result in foodborne diseases outbreaks. Total Viable Count (TVC), coliforms bacteria (CB), microscopic filamentous fungi and yeasts (MFFaY) were determined. Plate dilution method with individual culture conditions was used for microorganisms cultivation. The minimal value of TVC in delicatessen products was 2.25 log CFU.g^{-1} in sample no. 4 after opening and maximal value was 5.61 log CFU.g^{-1} in sample no. 8 after 3 days of storage at 15 °C. The minimal value of MFFaY in RTE delicatessen products was < 1 log CFU.g^{-1} in sample no. 3 after opening and maximal value was 6.1 log CFU.g^{-1} in sample no. 7, after 3 days of storage at 15 °C. Sample no. 1 and 8 after storage of products at 4 °C weren't in accordance with Codex Alimentarius of Slovak Republic (2006). Only sample no. 2 after storage at 15 °C meets requirements of Codex Alimentarius of Slovak Republic (2006). The values of CB in all samples of delicacy products were lower than 1 log CFU.g^{-1} (10 CFU.g^{-1}) after opening, after storage at 4 °C and 15°C. All samples were in accordance with Codex Alimentarius of Slovak Republic (2006).

Keywords: RTE food, total viable count, coliform bacteria, microscopic filamentous fungi and yeasts, microbial quality

INTRODUCTION

Controlling and improving the quality and safety of chilled foods at all stages of the cold chain have always been among the main concerns in order to reduce food losses and health hazards. Microbial and physico-chemical quality changes may occur in food products according to their time–temperature history, but also according to their composition and properties **(Mataragas et al., 2008; Verbeke et al., 2010)**.
The incidence of foodborne illness is increasing worldwide (**Mead *et al.*, 1999; Nguz, 2007**). This may in part be attributed to a change in commercial food production such as minimal processing as well as changing consumer demands for ready-to-eat (RTE) meals (**Kaneko *et al.*, 1996**). The microbiology of RTE foods during preparation in factories, in domestic kitchens, in canteens and on street corners by street vendors has previously been investigated (**Ayçiçek *et al.*, 2004; Baş *et al.*, 2006; von Holy and Makhoane, 2006**). However, the contamination of RTE foods that occurs in retail delicatessens has received relatively less attention (**Angelidis *et al.*, 2006**). Ready-to-eat (RTE) foods are very attractive to consumers looking for convenient meals. Codex Alimentarius Commision defines the RTEs as: foods that include any food (including beverages) consumed in its raw state or any food handled, processed, mixed, cooked or otherwise prepared into a form in which it is normally consumed without further processing (**Codex, 2004**). As the demand for RTE foods increases, a greater variety of RTE foods are becoming available. RTE foods may vary according to different eating habits, availability, cold chain conditions and regulations in different countries (**Almualla *et al.*, 2010**). Occurrence of pathogens in RTE foods presents a greater public health threat than its presence in raw meat products because RTE' are not usually subjected to sufficient temperature/time combination to destroy these bacteria before consumption (**Osaili *et al.*, 2011**). RTE foods could contain the indigenous microflora of the raw materials from which they are prepared (**Almualla *et al.*, 2010**). Ready-to-eat food is highly subject to bacterial foodborne outbreaks. Various foodborne pathogens associated with ready-to-eat food have been found to contribute to foodborne outbreaks (**Castro-Rosas *et al.*, 2012**; **Seow *et al.*, 2012**).
Methods of storage, processing, handling, and display can affect the levels of microorganisms in ready-to-eat food. Monitoring of the level of bacteria in ready-to-eat food is important to ensure the safety of this type of high-risk food (**Christison *et al.*, 2008**; **Fang *et al.*, 2003**). Foodborne pathogens in salads or sandwiches may proliferate in the presence of low numbers of other microorganisms as a result of cooking of ingredients before production (**Jay *et al.*, 2005**).
The aim of this article was to evaluate the microbiological quality of RTE delicatessen products during storage at various temperatures.

MATERIAL AND METHODS

The aim of this article was to evaluate microbiological quality of selected RTE delicatessen products. There were analyzed 24 samples of delicatessen products. These products were evaluated: paprika spread (samples 1, 2), cod salad (samples 3, 4), egg in aspic (samples 5, 6) and parisian salad (samples 7, 8). There were analyzed 8 samples immadietly after opening, 8 samples after three days of storage at 4 °C and 8 samples after three days of storage at 15 °C
The total viable count (TVC), coliform bacteria (CB) and microscopic filamentous fungi and yeasts (MFFaY) were evaluated. The values of microorganisms were compared with requirements of Codex Alimentarius of Slovak Republic (2006).

Determination of cfu counts

Plate diluting method was applied for quantitative cfu counts of respective groups of microorganisms in 1 g of product. Gelatinous nutritive substrate in petri dishes was inoculated with 1 ml of samples by flushing and on surface in three replications.

Dilution of the samples

Basic dilution (10^{-1}) was prepared as follows: 5 g of product was added to the test tube containing 45 ml of distilled water. Dilution plating method was used to determine the microorganisms.

Determination of TVC

Plate Count Agar was used for determine of Total Viable Counts in samples. Dilutions of 10^{-3} and 10^{-4} were used to determine of TVC. Petri dishes were cultivated upside-down in a thermostat at 30 °C for 48-72 hours under aerobic conditions.

Determination of CB

Violet red bile agar was used for determine of Coliform Bacteria in samples. Dilutions of 10^{-3} and 10^{-4} were used to determine of Coliform Bacteria. Petri dishes were cultivated upside-down in a thermostat at 37 °C for 24 -48 hours.

Determination of MFF

Chloramfenicol yeast glucose agar was used for determine of Microscopic Filamentous Fungi. Dilutions of 10^{-1} and 10^{-2} were used to determine of MFF. Petri dishes were cultivated upside-down in a thermostat at 25 °C for 5 -7 days under aerobic conditions.

Calculation of microorganisms

The number of microorganisms in1 g samples (N) were calculated using the following formula:

$$N = \Sigma C / [(n_1 + 0{,}1n_2) .d]$$

ΣC – sum of characteristic colonies on selected plates,
$\mathbf{n_1}$ – number of dishes from 1. dilutions used to calculate,
$\mathbf{n_2}$– number of dishes from 2. dilutions used to calculate,
d – dilution factor identical with 1. used dilution.

Statistical Analysis

Mathematical and statistical analysis (arithmetic mean, standard deviation, standard error, coefficient of variation) were performed using the program system Statgraffic.

RESULTS AND DISCUSSION

The Codex Alimentarius determines the maximal limit of coliform bacteria for salads with mayonnaise or without mayonnaise, spreads and products in aspic 10^4 CFU.g^{-1} (4.00 log CFU.g^{-1}), the maximal limit of yeasts for salads with mayonnaise or without mayonnaise is 5×10^4 CFU.g^{-1}, the maximal limit of yeasts for spreads and creams is 5×10^3 CFU.g^{-1}.

Determination of total viable counts (TVC)

Ng *et al*. (2013) were examinated 115 samples of ready-to-eat products from supermarkets. They were tested for aerobic plate counts (APC), *Escherichia coli* and *Staphylococcus aureus* counts, and the presence of *Salmonella* spp. for assessing their safety level. Results showed APC ranging from 1.97 to 6.84 log CFU.g^{-1}, with a mean of 5.05 log CFU.g^{-1}; *E. coli* counts ranging from none detected to 3.10 log CFU.g^{-1}, with a mean of 1.78 log CFU.g^{-1}; and *S. aureus* counts ranging from none detected to 1.42 log CFU.g^{-1}, with a mean of 0.15 log CFU.g^{-1}.
The values of TVC after opening ranged from 2.25 log CFU. g^{-1} (1.81×10^2 CFU. g^{-1}) in sample no. 4 (cod salad) to 5.32 log CFU. g^{-1} (2.12×10^5 CFU.g^{-1}) in sample no. 8 (parisean salad) (fig. 1). Average number of TVC was 3.76 log CFU. g^{-1} (tab. 1).
Values of TVC after 3 days of storage at temperature 4 °C were in range from 3.19 log CFU. g^{-1} (1.58×10^3 CFU. g^{-1}) in sample no . 3 (cod salad) a to 5.37 log CFU. g^{-1} (2.38×10^5 CFU.g^{-1}) in sample no. 8 (parisean salad) (fig. 2). Average number of TVC was 4.47 log CFU. g^{-1} (table 1).

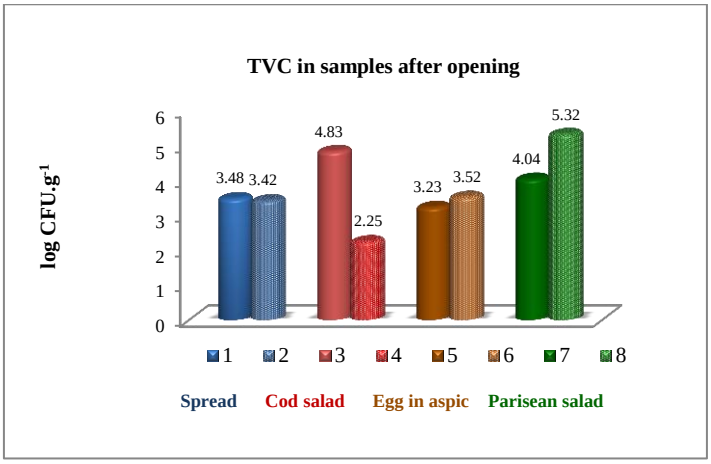

Figure 1 **Determination of TVC in delicacy products after opening**

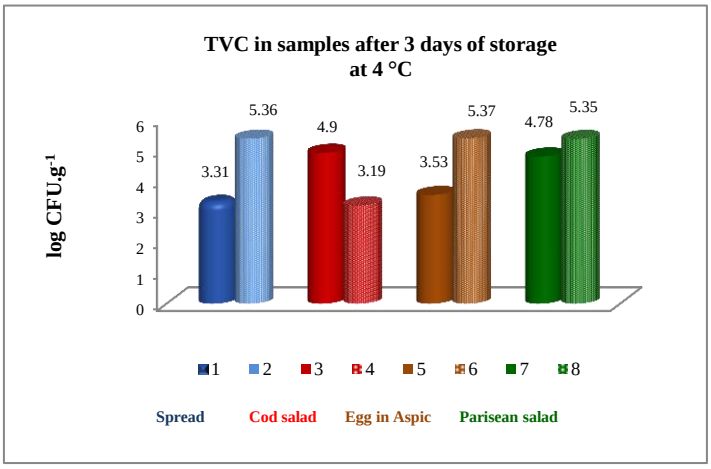

Figure 2 Determination of TVC in delicacy products after storage at 4 °C

RTE foods provide a source of readily available and nutritious meals for the consumers, but as these foods do not receive any heat treatment before consumption, the first priority should be their safety and microbiological quality. The initial microbiological load on RTE food ingredients is important, but the microbiological load of RTE foods at the point of sale is influenced by factors such as handling, processing, storage and display **(Smittle, 2000)**.

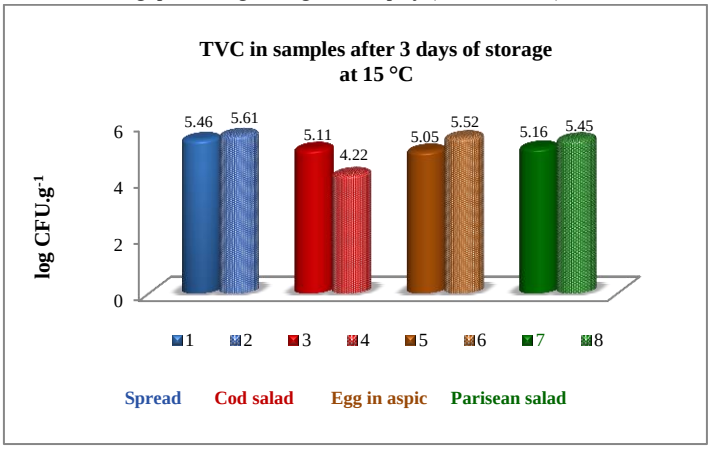

Figure 3 Determination of TVC in delicacy products after storage at 15 °C

Values of TVC after 3 days of storage at temperature 15 °C ranged from 4.22 log CFU.g^{-1} (1.67×10^4 CFU.g^{-1}) in samle no. 4 to 5.61 log CFU.g^{-1} (2.31×10^5 CFU.g^{-1}) in sample no. 2 (fig. 3). Average number of TVC was 5.2 log CFU.g^{-1} (tab. 1).
As the main constituents of these products were sliced products (either meat products or cheese) cross-contamination via equipment (i.e. slicer) could also contribute to the high population of aerobic colony count. The application of stringent hygienic practices during handling of vegetables is also necessary, as many of the above products contained vegetables. Commercial mayonnaise do not support the growth or survival of foodborne pathogens (i.e. *Salmonella, E. coli* O157:H7 and *L. monocytogenes*) due to low pH and water activity and the

presence of lysozyme in the whole eggs used in the production of commercial mayonnaise; but, food-service workers must use stringent hygienic practices to prevent microbial pathogen contamination during preparation, handling and storage **(Smittle, 2000).**

Table 1 Basic statistical characteristics of TVC in delicacy products

Parameter	TVC after opening	TVC after storage at 4 °C	TVC after storage at 15 °C
n	8	8	8
x	3.76	4.47	5.2
s	0.9	0.9	0.42
v%	23.96	20.13	8.07

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

A temperature of 8 °C is a realistic temperature abuse due to storage in canteens. But higher temperatures of storage, i.e. between 8 and 17 °C, should not be permitted for RTE products, unless the self life is about 20 h when they are placed in stores and kept at 18 °C **(Fang et al., 2003)**.

Determination of filamentous microscopic fungi and yeasts (MFFaY)

Results of our experiments showed the presence of MFFaY in delicacy products after opening in amount from < 1 log CFU.g^{-1} (< 10 CFU.g^{-1}) in sample no. 3 to 4,42 log CFU.g^{-1} (2.68×10^4 CFU.g^{-1}) in sample no. 4 (fig 4). Average value of MFFaY was 3.13 log CFU.g^{-1} (tab. 2). All samples of delicacy products were in accordance with Codex Alimentarius of Slovak Republic (2006).

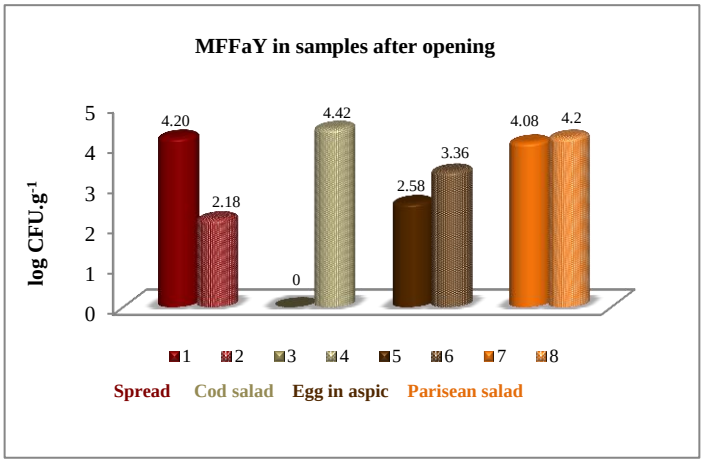

Figure 4 Determination of MFFaY in delicacy products after opening

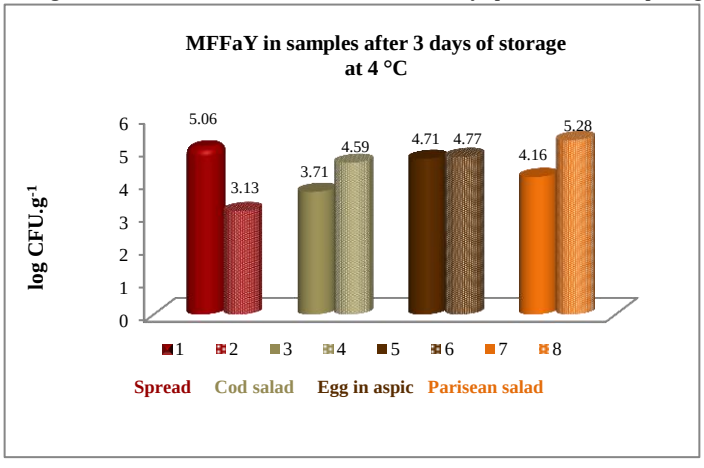

Figure 5 Determination of MFFaY in delicacy products after storage at 4 °C

Values MFFaY in delicacy products after 3 days of storage at temperature 4 °C ranged from 3.13 log CFU.g^{-1} (1.36×10^3 CFU.g^{-1}) in sample no. 2 to 5.28 log CFU.g^{-1}(1.92×10^5 CFU.g^{-1}) in sample no. 8. Average number of MFFaY was 4.43 log CFU.g^{-1} (tab. 2). Sample no. 1 and 8 weren't in accordance with Codex Alimentarius of Slovak Republic (2006).

Kanatt et al. (2006) found that the total viable bacterial count, Staphylococcus species and aerobic spore counts in samples of shrimp salad were in the range of 10^1–10^3 CFU.g^{-1}, 10^0–10^1 CFU.g^{-1} and 10^1–10^2 CFU.g^{-1}, respectively.

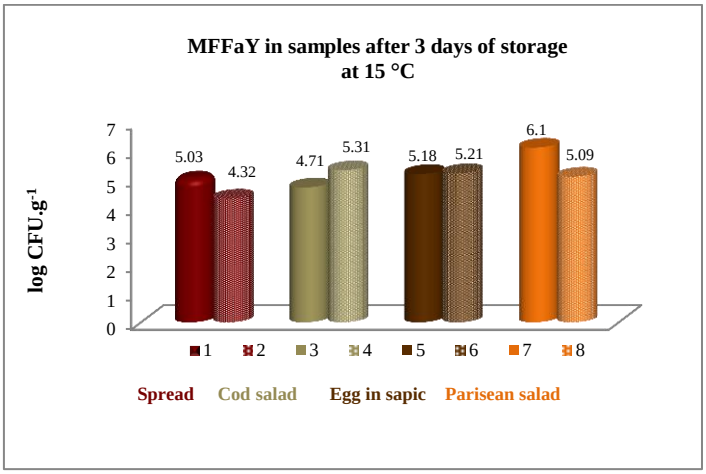

Figure 6 Determination of MFFaY in delicacy products after storage at 15 °C

Values MFFaY in delicacy products after 3 days of storage at temperature 15 °C were in range from 4.32 log CFU.g^{-1} (2.09×10^4 CFU.g^{-1}) in sample no. 2 to 6.1 log CFU.g^{-1} (1.26×10^6 CFU.g^{-1}) in sample no. 7 (fig. 6). Average number of MFFaY was 5.12 log CFU.g^{-1} (tab. 2). Only sample no. 2 meets requirements of Codex Alimentarius of Slovak Republic (2006).

Table 2 Basic statistical characteristics of MFFaY in delicacy products

Parameter	MFFaY after opening	MFFaY after storage at 4 °C	MFFaY after storage at 15 °C
n	8	8	8
x	3.13	4.43	5.12
s	1.41	0.67	0.48
v%	45.04	15.12	9.37

n – number of samples, x – average, s - standard deviation, v% - coefficient of variation

Mold growth was observed in samples of shrimps salads. Molds can grow even at a low $_{aw}$aw of 0.60 (**Jay, 2000**). Mold growth has been observed in foods having a $_{aw}$aw greater than 0.75 In mutton kababs having $_{aw}$aw of 0.85 mold growth was observed when stored at ambient temperature (**Chawla and Chander, 2004**).

Determination of coliform bacteria (CB)

The values of CB in all samples of delicacy products were lower than 1 log CFU.g^{-1} (10 CFU.g^{-1}) after opening, after storage at 4 °C and 15 °C (tab. 3). All samples were in accordance with Codex Alimentarius of Slovak Republic (2006). **Christisin et al. (2008)** showed, that aerobic plate counts were 10^9 CFU.g^{-1} for filled baguettes from retail delicatessens in South Africa. High aerobic colony counts alone do not make foods unsafe, but they indicate poor handling, storage or inadequate general hygiene **(Gillespie et al., 2000)**. But, samples are of unsatisfactory microbiological quality because of high aerobic colony counts (≥10^7 CFU.g^{-1} for cooked ham; ≥10^6 CFU.g^{-1} for other sliced meat samples) or high levels of *Enterobacteriaceae* (≥10^4 CFU.g^{-1}) **(Elson et al., 2004)**.

Table 3 Determination of CB in delicacy products

Sample	CB after opening		CB after storage at 4 °C		CB after storage at 15 °C	
	CFU.g^{-1}	log CFU.g^{-1}	CFU.g^{-1}	log CFU.g^{-1}	CFU.g^{-1}	log CFU.g^{-1}
1	< 10	< 1	< 10	< 1	< 10	< 1
2	< 10	< 1	< 10	< 1	< 10	< 1
3	< 10	< 1	< 10	< 1	< 10	< 1
4	< 10	< 1	< 10	< 1	< 10	< 1
5	< 10	< 1	< 10	< 1	< 10	< 1
6	< 10	< 1	< 10	< 1	< 10	< 1
7	< 10	< 1	< 10	< 1	< 10	< 1
8	< 10	< 1	< 10	< 1	< 10	< 1

The use of coliforms as indicators of cross-contamination in RTE foods has limited application as these bacteria may occur naturally on fresh produce, such as vegetables (**De Roever, 1998**). For food commodities, more importance is generally placed on the presence and numbers of *E. coli* as indicators of cross-contamination (**De Roever, 1998**). **Fang et al. (2003)** found, that *E. coli* counts were highest on meat- and cheese-based filled baguettes, and salads containing meat. It has been suggested that several factors may contribute to the presence of *E. coli* in RTE foods, including poor handling practices by food handlers, or cross contamination from food contact surfaces, or high storage temperatures. This

study did find that the core temperatures of both RTE food types were double that commonly recommended for RTE food storage, which indicated improper storage of these RTE food commodities.

CONCLUSION

The results for the aerobic colony count indicate that the general principles of good manufacturing programs and food hygiene as well as the temperature control should be enforced. Maintenance of correct refrigeration is fundamental for the safety of RTE foods. The results of the present study, can provide valuable information for the design of monitoring and surveillance programs for the official food microbiological control. Although the production of safe food is the responsibility of the producer, there is a need to verify and validate through inspection and end-product testing both at the site of production and at the point of sale. RTE foods should be sold to the consumer as bacteriologically safe products.

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