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MICROBIOLOGICAL QUALITY OF EGG LIQUID PRODUCTS

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ABSTRACT

Egg liquid products are most commonly used as semi-products in different branches of food industry and catering industry. The main goal of this work was to assess the microbiological quality of egg liquid products (liquid pasteurized egg white, pasteurized egg yolk with sugar 33%, pasteurized whole egg-blend) sampled in a period from April until February, and evaluate whether parameters such as the total aerobic count (TAC), coliform bacteria (KFB), enterococci, staphylococci, moulds and yeast changed significantly during this period of time. Microbiological analysis detected the highest ($P < 0.05$) total aerobic count in egg yolk (2.8 log CFU.ml^{-1}). The incidence of coliform bacteria was also highest ($P < 0.05$) in egg yolk (1.7 log CFU.ml^{-1}) but very low in egg white and whole egg. The highest count ($P < 0.05$) of enterococci was detected in samples of egg white (1.0 log CFU.ml^{-1}). The counts of staphylococci, moulds and yeast were particularly high ($P < 0.05$) in egg yolk (1.2 log CFU.ml^{-1} for staphylococci and 1.1 log CFU.ml^{-1} for moulds and yeast). Statistical analysis showed ($P > 0.05$) no correlation between respective seasons and microbial counts for any of the egg substances investigated. It follows from our results that all analysed egg products comply with the TAC limit defined by Council Directive (EC) 89/437/EEC on hygiene and healthy problems affecting the production of egg products, however, all egg products analysed in our study would fail to meet the required criteria for other groups of microorganisms.

Keywords: egg liquid substances; total aerobic count; coliform bacteria; enterococci; staphylococci; moulds and yeast

INTRODUCTION

Demand for shell eggs has recently declined whereas egg liquid products have attracted increasing interest in food industry. Egg liquid products are most commonly used as semi-products in different branches of food industry and catering industry **(Simeonovová, 2013)** since they provide a rich source of high-quality proteins, vitamins and trace elements **(Patrignani et al., 2013; Rossi et al., 2010)**. The term 'egg products' means liquid whole eggs (blend), egg whites and enriched sweetened or salted egg yolks or blends **(Chmielevski et al., 2013)**. Only fresh, refrigerated, preserved, sorted or unsorted eggs are subjected to egg breaking and processing. Such eggs may not comply with the requirements for size and shape specified for the shell eggs intended for consumers but they are wholesome and of good quality **(Steinhauserová and Simeonovová, 2003)**. Microbial contamination on the shell eggs is one of the major factors to indicate egg quality, affecting the level of exogenous microbial contamination in the egg contents. It poses a fundamental problem in the production of eggs intended for consumers **(Dev et al., 2013; Englmaierová and Tůmová, 2007)**, particularly with regard to the total aerobic count and contamination with Gram-negative bacteria from the family *Enterobacteriaceae* **(Németh et al., 2011; Reu et al., 2005)**. Egg liquid products are responsible for a high number of foodborne illnesses **(Latimer et al., 2008)** every year. Psychrophilic microorganisms and moulds are most common species to grow on the contents of the frozen eggs. The growth of moulds arises mainly due to insufficient air exchange and high humidity. The most common moulds detected in an egg include *Cladosporium herbarum, Penicillium cyclopium, Alternaria humicola, Trichoderma viride* and *Aspergillus niger.* The optimum temperature for the growth of these species varies in a range of 18 – 28 °C, with relative humidity being at least 80% **(Hejlová, 2001)**.

MATERIAL AND METHODOLOGY

Analysis was performed with the samples of egg liquid products used for the production of baked goods and pastry in a bakery located in the surroundings of Brno. This included liquid pasteurized egg white, pasteurized egg yolk with sugar 33% and pasteurized whole egg. Respective samples of egg liquid products were transported in sterile bottles in a cool box into the laboratory. 3 representative samples from each egg substance were collected from a package unit (5 kg). Analyses were performed in the microbiological laboratory of the Institute for Food Technology, MENDELU in Brno, during one year (April – February). A total of 24 samples were analysed from each egg liquid products (overall 72 samples). The samples were storing at temperature 5°C and relative humidity of 70 – 80% by recommendation of producer.

Parameters such as the total aerobic count (TAC), coliform bacteria (KFB), coagulase-positive staphylococci, enterococci and moulds and yeast were determined in all samples.

The following microbiological parameters were determined in egg products:

The total aerobic count (TAC). Culture on the growth medium Plate Count Agar (PCA, NOACK, France) according to CSN EN ISO 4833 at 30°C for 72 hours.

Coliform bacteria. Culture on the growth medium Violet Red Agar (VRBL, NOACK, France) according to CSN ISO 4832 at 37°C for 24 - 48 hours.

Coagulase-positive staphylococci: Culture on Baird-Parker Agar (NOACK, France) according to CSN EN ISO 6888, incubation at 37°C for 24 – 48 hrs. Confirmation was performed by adding rabbit fibrinogen (Fibrinogen Plasma Trypsin Inhibitor Supplement, Oxoid).

Enterococci. Culture on the growth medium Compass-enterococcus agar **(**NOACK, France) at 37°C for 24 – 48 hrs.

Moulds and yeast. Culture on the growth medium Chloramphenicol glucose agar (GKCH) according to CSN ISO 21527-1 at 25°C for 5 days.

Samples were collected and processed according to CSN ISO 7218 and CSN EN ISO 6887-1.

The following methods were used for statistical evaluation: the calculation of basic statistical parameters (mean, standard deviation, standard deviation of the mean) and the simple sorting method of analysis of variance. Evaluation was performed using the programme STATISTICA CZ, version 9.

RESULTS AND DISCUSSION

Liquid pasteurized egg white (Fig. 1)

The highest value of the total aerobic count was found for Sampling 4 (October) 3.1 log CFU.ml^{-1} whereas the lowest value (1.6 log CFU.ml^{-1}) was detected in November (Sampling 5).

Coliform bacteria were detected only once (in October, 1.3 log CFU.ml^{-1}). Coagulase-positive staphylococci were found in egg white in two samplings (June and October) 2.5 log CFU.ml^{-1}. Enterococci were detected at four occasions, the highest count was found in May (2.8 log CFU.ml^{-1}), whereas in April, November, January, and February no enterococci were detected in pasteurized egg white. Moulds and yeast were detected in pasteurized white twice, in June and October. The highest value detected was 1.3 log CFU.ml^{-1}. No yeast and moulds were detected in other samplings.

Pasteurized egg yolk with sugar 33% (Fig. 2)

The highest value of the total aerobic count was found in pasteurized egg yolk with sugar 33% in June 4.3 log CFU.ml^{-1}, whereas in January, no colonies on dishes were recorded.

The highest count of coliform bacteria was found in October (4.1 log CFU.ml^{-1}). Three samplings show no coliform bacteria at all. The highest value of coagulase-positive staphylococci was found in February (3.5 log CFU.g^{-1}). Findings at three other samplings were negative. Enterococci, resp. moulds and yeast were detected in samples three, resp. four times with the highest count being found in May (2.7 log CFU.ml^{-1} and 3.1 log CFU.ml^{-1}, respectively).

Pasteurized whole egg (Fig. 3)

The highest value of TAC in pasteurized whole egg was detected in October (2.8 log CFU.ml^{-1}), whereas in May (Sampling 2) and January (Sampling 7) the TAC was < 10. Coliform bacteria were only found at Sampling 1 (April), 1.0 log CFU.ml^{-1}. Coagulase-positive staphylococci were detected in June, October and December; with the highest value being 2.3 log CFU.ml^{-1}. The highest value for enterococci was 1.5 log CFU.ml^{-1}. No enterococci were present in pasteurized whole egg blend in five sampling events. Moulds and yeast were only detected in January at an amount of 1 log CFU.ml^{-1}.

It follows from the comparison of microbial quality of all three egg liquid products that the highest ($P < 0.05$) levels of the total aerobic count, coliform bacteria, coagulase-positive staphylococci and moulds and yeast were found in pasteurized egg yolk with sugar 33% (n=24). The highest ($P < 0.05$) count of enterococci was found in egg white (1.0 log CFU.ml^{-1}; n=24). Statistical evaluation showed no correlation ($P > 0.05$) between the period of sampling and the count of particular microorganisms, for any of the egg liquid products.

Regulation (EC) 2073 (2005) on microbiological criteria

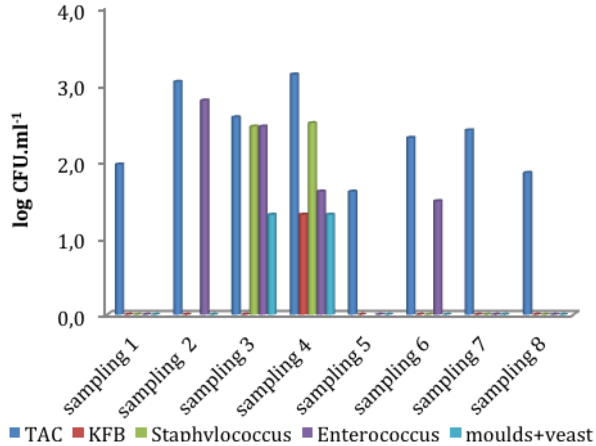

Fig. 1 The count of microorganisms (log CFU.ml^{-1}) in samples of liquid pasteurized egg white collected in 8 time points (April - February)

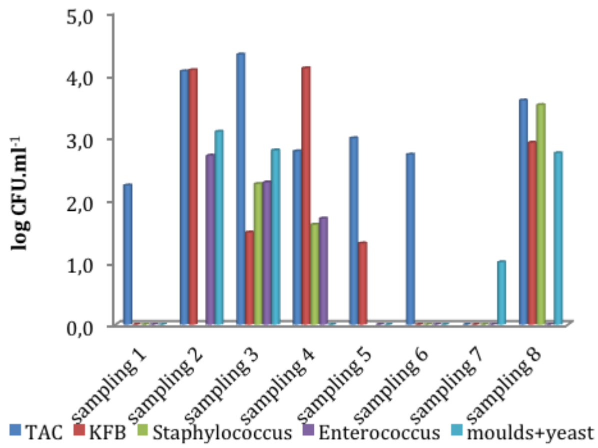

Fig. 2 The count of microorganisms (log CFU.ml^{-1}) in samples of pasteurized sweetened egg yolk, collected in 8 time points (April – February)

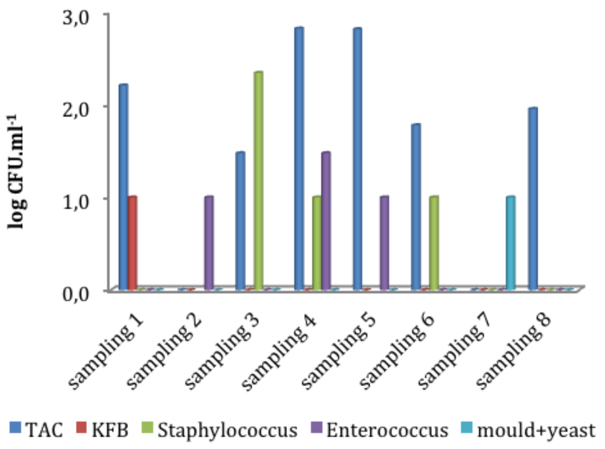

Fig. 3 The count of microorganisms (log CFU.ml^{-1}) in samples of pasteurized egg blend collected in 8 time points (April – February)

for foodstuffs does not specify the limits for the total aerobic count, the count of enterococci, coliform bacteria, staphylococci, moulds and yeast. **Regulation No. 287 (1999)** concerning veterinary requirements of animal products that is no longer in force defined the limit for the total aerobic count, which was 5.10^4 CFU per ml of a liquid egg product. According to this regulation, coliform bacteria, staphylococci, moulds and yeast should not be present in 1 ml of a particular pasteurized substance. The TAC values found in our analysis of egg liquid products would therefore meet the respective legal limit. However, egg liquid products analysed would fail to meet the counts of yeast, moulds, coliform bacteria and staphylococci. **Directive 89/437/EEC (1989)** on hygiene and health problems affecting the production and the placing on the market of egg products only specifies the limit for the total aerobic count 10^5 CFU.ml^{-1}, *Staphylococcus aureus* should not be present in 1 ml of any egg product. Our samples would therefore only comply with the TAC limit set in this directive. **Németh et al., (2011)** reported the TAC of 4.10^4 in the whole egg and 1.10^4 CFU.ml^{-1} in egg yolk after pasteurization. The TAC in the pasteurized egg white in the above mentioned experiment was <10. Unlike **Németh et al., (2011),** we found on the basis of our analysis that the mean total aerobic count after pasteurization was lower for whole egg (5.10^1 CFU.ml^{-1} , i.e. 1.64 log CFU.ml^{-1}) and egg yolk (6.8.10^2 CFU.ml^{-1}, i.e. 2.84 log CFU.ml^{-1}) and higher for egg white (2.5.10^2 CFU.ml^{-1}, i.e. 2.35 log CFU.ml^{-1}). **Mukhopadhyay et al., (2010)** reported that the value of the TAC in liquid egg products after pasteurization usually exceeds 10^3 CFU.ml^{-1}. **Rossi et al. (2010)** found in their experiment that the TAC was 1.10^2 CFU ml^{-1} in whole egg blend after pasteurization, which is in a good agreement with our findings (1.6 log CFU.ml^{-1}; n=24).

Muchová (2004) assumes that the counts of microorganisms in egg liquid products depend on several factors such as the quality of eggs, the sanitation of an egg breaker and continuous inspection to ensure the cleanliness of egg liquid products separated from shell eggs in breaker tanks. According to the guideline Good Hygiene and Manufacture Practice for Egg and Egg products **(Míková and Zvárová, 2011),** properly pasteurized egg substances contain less than 10^3 CFU per g or ml of an egg substance. Egg yolk is more likely to become contaminated by microbes since it does not contain natural inhibitors of microbial growth such as lysozyme **(Görner a Valík, 2004)** or lecithin **(Németh et al., 2011)**. This is in a good agreement with our results. The increased number of microorganisms can be found in products where undesirable bacterial growth occurred due to insufficient cooling or extended storage **(Görner a Valík, 2004)**. Although coliform bacteria are unable to survive heat treatment during pasteurization, they pose a serious hygienic risk since they usually constitute accompanying microflora of pathogenic microorganisms. The incidence of coliform microorganisms in egg substances found in our study indicates insufficient heat treatment during pasteurization or subsequent contamination due to poor hygiene and sanitation practices. As reported by **Voldřich and Šotolová (2009)**, staphylococci can normally be found on the skin, skin glands, or the upper airway in man. Food processors are the major source of contamination. Bacterial transfer from devices and external environment should also be considered **(Komprda, 2004)**. The increased occurrence of enterococci in non-fermented foodstuffs indicates insufficient heat treatment and bacterial contamination on surfaces that have not been properly cleaned and decontaminated. Enterococci can grow in foodstuffs with increased salt levels and decreased water activity **(Görner and Valík, 2004)**. **Hejlová (2001)** have found the highest incidence rate of enterococci as heat-resistant bacteria in liquid egg products. This is in a good agreement with our findings where the highest count of enterococci was detected in egg white (according to the regulation, egg white is pasteurized at a lower temperature compared to other egg substances). As a result, these products must be processed in sanitary facilities under continuous inspection and pasteurized before distributed for consumption **(Unluturk et al., 2008)**.

CONCLUSION

Microbiological analysis revealed the highest ($P < 0.05$) total aerobic count 2.8 log CFU.ml^{-1} and the highest ($P < 0.05$) incidence rate of coliform bacteria (1.7 log CFU.ml^{-1}) in egg yolk. In contrast, egg white and whole egg contained coliform bacteria only at very low counts. The highest ($P < 0.05$) number of enterococci was found in egg white (1.0 log CFU.ml^{-1}). Staphylococci, moulds and yeast were present at highest levels ($P < 0.05$) in egg yolk (1.2 log CFU.ml^{-1} and 1.1 log CFU.ml^{-1}, respectively). Statistical analysis ($P > 0.05$) showed no correlation between the season and the counts of monitored microorganisms for any of the egg liquid products.

It follows from our results that all analysed egg products comply with the TAC limit defined by Council Directive (EC) 89/437/EEC on hygiene and healthy problems affecting the production of egg products, and correspond with other studies that deal with liquid egg substances. However, all egg products analysed in our study would fail to meet the required criteria for other groups of microorganisms. Bacterial contamination in egg products caused by staphylococci, KFB, enterococci, moulds and yeast probably results from insufficient heat treatment during pasteurization or subsequent contamination caused by poor hygiene and sanitation practices or storage at unsuitable conditions. Thermal pasteurization still represents the most available and best understood technique. Alternative pasteurization methods including ultrasonic wave treatment, high electric field pulses, high hydrostatic pressure or ultrapasteurization combined with aseptic packaging have been explored to extend the shelf life and minimize disadvantages of thermal processing of liquid egg products. Of course these methods does not cause substantial changes in the structure of liquid egg products by causing coagulation and denaturation of proteins **(Unluturk et al., 2008)**.

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