doi: 10.17221/645/2014-CJFS

Microbial Contamination of Paper-based Food Contact Materials with Different Contents of Recycled Fiber

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Abstract

Hladíková Z., Kejlová K., Sosnovcová J., Jírová D., Vavrouš A., Janoušek A., Syčová M., Špelina V. (2015): **Microbial contamination of paper-based food contact materials with different contents of recycled fiber**. Czech J. Food Sci., 33: 308–312.

Recycled paper is commonly used in food packaging industry, especially for disposable products. The material coming into direct contact with food products must not represent a source of contamination for food in accordance with the Framework Regulation (EC) No. 1935/2004 containing the general requirements on all food contact materials. In the present study, the microbial purity of 31 paper-based materials with different contents of recycled matter (0–100%) was evaluated using a standard method based on the disintegration of paper. The results of the present study indicate the existing relation between the use of recycled fibers and the content of microorganisms. The increased amount of recycled fibers significantly increased the amount of bacteria in the paper samples. The highest content of microorganisms was observed in the case of paper with the highest recycled fiber content. Total counts of bacteria ranged from 5.0×10^1 to 1.2×10^5 CFU/g. Moulds were detected only in three paper samples. As the microorganisms present in food packaging may penetrate into foodstuffs (particularly those with high contents of water or fat) and have an adverse effect on end-consumers' health, microbial criteria for food packaging should be included into the EU legislation.

Keywords: bacteria; disposable materials; health safety; microbial purity; recycled paper

Disposable paper-based materials such as boxes, bags, plates, cups, sacks, containers, and napkins have been used in food packaging for many years. The greatest benefit of these materials in comparison to plastic materials is their comparatively minimal impact on our environment and biodegradability. Cellulose and hemicellulose contained in papers and boards are fast biodegradable products, while lignin is biodegraded more slowly by microorganisms because of its structure. Adding cellulosic packaging to traditionally composted products may improve the compost structure while no negative or inhibitory effects on the plant germination and growth have been observed (Ottenio *et al.* 2004). Paper recycling represents another advantage, as cellulosic fibres can be used several times in the paper production.

The recycled paper and paperboard products are made from used papers such as newspapers, magazines and milk cartons, pulped with water and also cleaned and deinked with surfactants (Ozaki *et al.* 2004). Food grade paper with different pulp percentages of the recycled matter is commonly used in direct contact with foods. Food contact materials, including recycled fiber-based paper, have to be in compliance with general safety principles set up in Article 3 of the Framework Regulation. This Article requires that food contact materials must not transfer their components into food in quantities

Supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic, Project No. NT 14375-3/2013, and by the institutional support of the Ministry of Health of the Czech Republic, Project No. 1RVO-SZÚ-2014.

doi: 10.17221/645/2014-CJFS

that could endanger human health, change the food composition in an unacceptable way or deteriorate its taste and odour. This means that recycled paper for the food contact should not allow the migration of hazardous components potentially endangering human health (Triantafyllou *et al.* 2007). The requirements on the food packaging have increased significantly over recent decades. In addition to the material-related and environment-related safety aspects of packaging that is in direct contact with the products, namely the transfer of xenobiotics to the foods, tightness, mechanical strength, and the ability to be resealed, increasing demands are also being put on the microbiological-hygienic safety of the food packaging (Hennlich 2011).

Monitoring of the microbial purity of the packaging materials, starting with the production of paper, is an essential step for the protection of foodstuffs from microbial contamination. Paper production takes place in an open unsterile environment, in which water content of the slurry in the headbox (tank in which the papermaking materials are mixed and fed on the wire), pH, and temperature are favourable for the microbial growth (Väisänen *et al.* 1991). The main sources of microbial contamination in paper mills are recycled water, raw materials, parts of the machinery and the factory environment (Blanco *et al.* 1996). The extreme temperatures in the mill dryers kill most, if not all, microbial cells, but the heat-resistant spores will likely survive and thus contaminate the machinery as well as the finished paper products (McCusky Gendron *et al.* 2012). Bacteria and fungi present in the paper and paperboard may penetrate from the packaging into the food where they find ideal conditions for the growth, causing serious health problems (Guzińska *et al.* 2012).

The aim of this research was to evaluate the microbial purity of paper-based food contact materials with different contents of recycled fiber.

MATERIAL AND METHODS

Test paper-based materials. A total of 31 paper samples with varied pulp percentages of recycled matter were used. The content of recycled matter in the samples varied between 0 and 100%.

Examination of the total number of microorganisms in paper-based materials. The total number of colony forming units (CFU) of bacteria, yeasts, and moulds was estimated by the international standard method based on disintegration of paper (ISO 8784-1:2005). 1 g of the test paper was disintegrated in a volume of cooled sterile Ringer's solution to obtain a 1% fibre suspension free of clumps. Different dilutions of the fibre suspension were plated on nutrient agar on Petri dishes (TGEA for bacterial counts and PDA for yeasts and mould counts). Petri dishes were stored in an incubator at 37 ± 1°C for 48 ± 3 h for bacteria and 30 ± 1°C for 5 days for yeasts and moulds under aerobic conditions. After incubation, the total number of colony forming units was estimated and the concentrations of bacteria, yeasts, and moulds per 1 g of paper were calculated. The experiments were carried out in duplicate. The average values are presented in this study.

Statistical analysis. Statistical analysis was performed using statistical package SPSS 12 (SPSS Inc., Chicago, USA). The original data (4 groups of paper samples differing in the composition of raw materials: 0, 0–20, 50, and 90–100%) were analysed by nonparametric methods, namely by Friedman and Mann-Whitney tests. Further, the data were transformed to their logarithms and post hoc tests (Sidak method) were applied.

RESULTS AND DISCUSSION

The microbial contamination of paper-based food contact materials with different contents of recycled fibers was investigated.

Table 1 summarises the results obtained from the microbial analysis of the total numbers of bacteria and fungi in the paper samples. Total counts of bacteria ranged from 5.0×10^1 to 1.2×10^5 CFU/g. Filamentous fungi were detected only in three paper samples in concentrations of 5.0×10^2 to 1.0×10^3 CFU/g.

The results show a correlation between the recycled matter content and microbial contamination (Figure 1). Substantial differences were found in total counts of bacteria (CFU/g) within the 4 groups established on the basis of their percentage contents of recycle (0, 0–20, 50, and 90–100%).

The nonparametric Friedman test for comparing more than 2 groups of data was used. The differences between the groups compared were statistically significant ($P < 0.001$). To assess the significance of the pairwise comparisons of the groups, the nonparametric Mann-Whitney test was used. Only 3 groups were compared, the 50% group was omitted as it included only 1 specimen. The first two groups with

doi: 10.17221/645/2014-CJFS

Table 1. The number of bacteria, fungi and yeasts in test paper material with different content of recycled fiber

Test paper	Content of recycled fiber (%)	Bacteria number (CFU/g)	Number of fungi and yeasts (CFU/g)
1	0	–	–
2	0	6.0×10^2	–
3	0	–	–
4	0	–	–
5	0	–	–
6	0 max. 20	5.0×10^1	–
7	0 max. 20	9.5×10^3	–
8	0 max. 20	–	–
9	0 max. 20	–	5.0×10^2
10	0 max. 20	–	–
11	0 max. 20	–	–
12	0 max. 20	2.4×10^4	–
13	50	5.0×10^3	–
14	100 (min. 90)	7.0×10^3	–
15	100 (min. 90)	3.5×10^3	–
16	100 (min. 90)	7.0×10^3	–
17	100 (min. 90)	1.2×10^5	–
18	100 (min. 90)	9.5×10^4	–
19	100 (min. 90)	8.9×10^4	–
20	100 (min. 90)	8.2×10^4	–
21	100 (min. 90)	6.2×10^4	–
22	100 (min. 90)	2.5×10^4	–
23	100 (min. 90)	8.9×10^4	–
24	100 (min. 90)	9.8×10^4	5.0×10^2
25	100 (min. 90)	8.6×10^4	1.0×10^3
26	100 (min. 90)	9.2×10^4	–
27	100	1.4×10^4	–
28	100	6.5×10^3	–
29	100	2.5×10^3	–
30	100	4.0×10^3	–
31	100	1.9×10^3	–

a low recycle content did not differ from each other, but both of them were significantly different from the group with a high recycle content ($P < 0.001$).

Similarly, the differences between the groups were statistically significant when the data were transformed to logarithmic scale and a one-way analysis of variance was used ($P < 0.001$).

The results obtained are in line with the observations of other authors.

A similar study was carried out by Guzińska *et al.* (2012) who studied microbial contamination of various paper and paperboard materials for use in contact with food. Their study demonstrates that the number of the estimated microorganisms in paperboard was distinctly higher than in paper. The number of bacteria in paper determined by the defibering method was in the range of 10^2–10^3 CFU/g while the total amount of bacteria in paperboard was in the range of 10^5–10^6 CFU/g.

McCusky Gendron *et al.* (2012) investigated bacterial contaminants of unused paper towels made from recycled fiber (60–100%) and from 100% virgin wood pulp. According to their study, the concentration of bacteria in the recycled paper was between 100- to 1000-fold higher than in the virgin wood pulp brand. Test papers contained between 10^2–10^5 CFU/g.

Microorganisms isolated from paper-based food contact materials represented species of the genera *Bacillus.*

Suihko *et al.* (2004) studied the bacterial community in paper mills and their study indicates that many species adapted to the paper mill environment have not yet been described in other habitats. *Bacillus cereus*, *Bacillus licheniformis*, *Pseudoxanthomonas taiwanensis*, and *Paenibacillus* isolates were found in several mills, indicating a common contamination source such as paper making chemicals or wood raw material.

According to Regulation (EC) No 852/2004 on materials and articles intended to come into contact with food (Section X of this regulation) stipulates that, the packaging must not be a source of foods contamination. Furthermore, some member states of the European Union have issued specific regulations or guideline values for microbial contamination in food packaging.

A work group of the Industrial Association for Food Technology and Packaging was also established at the Fraunhofer Institute for Process Engineering

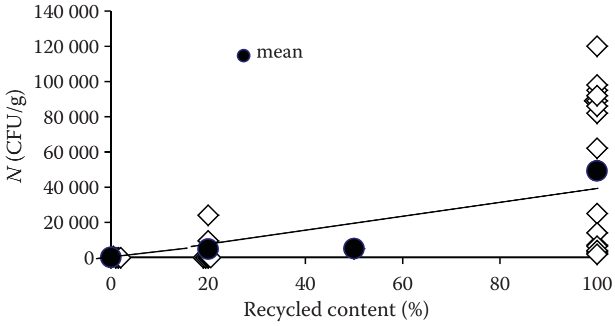

Figure 1. Correlation between content of recycled matter and increasing number of microorganisms in paper-based materials (N – number of microorganisms)

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and Packaging IVV in Freising. Since 2002, this group has established "Guideline values for microbiological contamination on food packaging". The values are a recommendation and do not represent are any legally binding limit values (Hennlich 2011). Another example of the microbiological regulation is the Food Code of the Slovak Republic Part 2, Chapter 4 (2006), which regulates the microbiological requirements on foodstuffs and their package materials and packaging. Polish standard PN-P-50430:1998 stipulates health standards for paperboard and paper packaging, but microbiological demands are not contained in the standard (Guzińska *et al.* 2012). However, the proportion of packaging made from uncoated and untreated paper and board coming into direct contact with food bought by the end-consumer was estimated to be below 3.5% of all direct contact food packaging in the EU 15 Member States in 2000. Direct contact takes place primarily with dry food (approximately 50%) and with food that is to be peeled or washed (approximately 30%), so only the remaining 20% gets into contact with moist and/or fatty food. According to the Industry Guideline for the Compliance of Paper & Board Materials and Articles for Food Contact (2012), uncoated and untreated paper and board are not suitable to pack food with a very high moisture content (for example liquid food or wet chilled products), since the exposure to high moisture will cause disintegration of the material. For these food types, coated paper and board are commonly used, and in the great majority of applications direct food contact takes place with a plastic layer.

Materials and articles, including active and intelligent materials and articles, shall be manufactured in compliance with good manufacturing practices so that, under normal or foreseeable conditions of use, they do not transfer their constituents to food in quantities which could endanger human health, bring about an unacceptable change in the composition of the food or bring about a deterioration in the organoleptic characteristics thereof (Regulation (EC) No 1935/2004, Article 3).

Johansson *et al.* (2001) studied microflora and the content of endotoxin in paper with various contents of recycled fibers. According to their results, recycled papers are carriers of microbial contaminants that affect the purity of the paper. Their findings showed that endotoxins were detected in all the samples tested and correlated with the amount of the recycled material and might pose an environmental health problem. Suominen *et al.* (1997) investigated microorganisms spreading inside paperboard for extended periods of time by non-destructive microscopic techniques. The study confirmed the need for effective and intact coating materials preventing the migration of microbes into the food. The development of active bio-packaging offering a better protection of the product in terms of quality (i.e. freshness and taste) and shelf-life, is expected (Lavoine *et al.* 2015).

CONCLUSIONS

Food safety and the related issues of food protection continues to be a public health priority that requires extensive research, education, and extension efforts focused on the prevention of microbial foodborne outbreaks.

The EU microbiological criteria for foodstuffs have been revised and certain important new criteria have also been set. Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, applicable from 1 January 2006, lays down food safety criteria for certain important foodborne bacteria, their toxins and metabolites, such as *Salmonella*, *Listeria monocytogenes*, *Enterobacter sakazakii*, staphylococcal enterotoxins, and histamine in specific foodstuffs. These criteria are applicable to products placed on the market during their entire shelf-life. In addition, the regulation sets certain process hygienic criteria to indicate the correct functioning of the production process.

The EU integrated approach to food safety aims to ensure a high level of food safety within the European Union through coherent farm-to-table measures, while securing the effective functioning of the internal market. The implementation of this approach involves the development of general and specific legislative requirements based on the risk assessment.

However, the microbial requirements for food packaging have not been included into the legislation of the European Union at all. The results of the present study confirmed that the microbial contamination of paper-based foodstuff packaging containing recycled matters may pose a health safety risk and should be dealt with in the future accordingly. It is very important to include microbial criteria for food packaging into the EU legislation in the nearest future.

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Receveid: 2014–11–11
Accepted after corrections: 2015–02–25

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