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Investigation in the Microbiological Purity of Paper and Board Packaging Intended for Contact with Food

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Abstract

Paper is one of the main materials in the manufacture of packaging for contact with food. Microbes that are present in the paper or board may penetrate into the food- health hazard for the consumers. Consistent and unified regulations have not been enacted yet in the UE to prevent health hazards which may arise from infected packaging. Examination was carried out of the microbiological contamination in various paper and paperboard materials designed for uses in food packaging. The following three methods were applied in the testing: defibering-, agar flooding- and smear method. It has been proven that the defibering method yields best results in the testing of microbes' concentration in paper and board. In some of the commercial materials tested a high level of contamination was found raising concern whether the products can be used in contact with food. No clear criteria are presently defined to qualify the material in respect of microbiological purity.

Key words: microbial purity, paper, board, packing, food.

In the European Union the annual per capita consumption of plain paper used in direct contact with alimentary products is estimated at about 0.9 kg while consumption in coated and laminated paper products for liquid and wet food is as high as 4.5 kg [1].

Paper and paperboard is in 99% composed of natural materials: cellulose fibres, calcium carbonate and natural polymers primarily starch. The packaging is to protect the food against the action of hazardous factors of the environment thus prolonging the useful life of the packed food [3], however, it may carry microbiological impurities [4].

Paper packaging material may be infected with microorganisms by many ways. Pathogens can be found in raw materials, chemicals, air, operation surfaces and protective clothing of the operators. A serious source of infection is the circulation of technological water. With a high content of nutritive substances and temperature within the range of 25 - 45 °C, the water is a comfortable abode for the growth of microbes [5].

Starch is the crucial factor affecting the microbiological purity of the paper products. It is commonly used in the paper industry as reinforcement, surface glue and adhesive means [6]. Starch, an easy accessible source of carbon, is conducive to the growth of microorganisms. It is only a very intensive growth of the microorganisms within the range of about 10⁸ u/ml that causes a decrease in the starch viscosity. This is the reason why at lower

contents, the microbial infection is not easy detectable.

Microorganisms may attack on many ways, they cause decay of food (*Enterobacter cloacae*, *Bacillus subtilis*), deliver volatile odorous compounds generated in the course of microbe metabolism (eg *Clostridium* spp, *Desulfovibrio* spp, *Actinomyces*), produce mucus (e.g. *Burkholderia* spp, *Klebsiella* spp, *Bacillus* spp). They also may badly affect human health (e.g. (*Bacillus cereus*, coliformy, *Staphylococcus* spp.). Bacteria and fungi present in the paper and paperboard, can penetrate from the packaging to the food where they can find ideal conditions for growth, causing serious health problems [7, 8].

Products of paperboard and paper designed for contact with food are one of the factors determining safety of alimentary products. It is therefore why legislation has been set up placing quality demands before the materials [9 - 12]. Producers are required to meet the demands in order to put the products on the market [13]. The regulations unfortunately disregard microbiological purity.

All documents anticipate issuing of detailed decrees concerning individual groups of materials. The European Union has not issued any specific demands concerning paper and paper board designed for contact with food. Individual countries have enacted national regulations, or use related EU legislation. The Confederation of European Paper Industries (CEPI) has published guidelines concerning methodology of estimating the

■ Introduction

Paper is one of the the main packaging materials; plastic, glass and metal being the other ones [1]. Paper and paperboard have a long history in the food industry in a multitude of uses. The materials are often applied in close contact with alimentary products, let only give the instances of teabags, backing paper and coffee filters. As a packaging material, paper comes often in direct contact with food like butter, sugar, flour etc. Paperboard boxes serve as containers for dry food and frozen products. Candies, sandwiches and many other foods are wrapped in paper.

Paper and paperboard are also widely used in collective packaging in transport and distribution.

suitability of paper and paperboard to contact with food (Industry Guideline for the Compliance of Paper & Board Materials and Articles for Food Contact, 1 March 2010) [2]. The guidelines could form a basis for a relevant universal law in this field. Commonly applied are recommendations XXXVI (plus parts of I, II and III) of the German Bundesinstitut für Risikobewertung (Federal Institute for Risk Assessment) which quote the allowable limits of composition and form a base to the CEPI guidelines. The guidelines concern paper and paperboard designed for contact with food, or if the contact is conjectural and it might be expected that constituents of the material penetrate into the food.

Some European countries have issued specific national regulations which dominate over the CEPI guidelines.

In Poland in compliance with the decree 1935/2004 art. 3, materials and products of paper and paperboard are to be produced according to GMP principles in a way that prevents the material constituents to penetrate into the food at normal or foreseeable conditions in amounts which could:

- pose threat to human health or
- cause unacceptable change in the food composition or
- cause deterioration of the organoleptic properties of the food

Point 5 of the decree stipulates paper and paperboard to be of an adequate microbiological purity.

Polish standard PN-P-50430:1998 which stipulates health standards of paperboard and paper packaging for food sets demands concerning organoleptic features and content of heavy metals only. Microbiological demands are not contained in the standard. No other Polish standard deals with microbiological purity of paper and paperboard.

The aim of the work was to assess the microbiological infection of various paper and paperboard materials for uses in contact with food. A review of the legal regulations and available testing methods was made in order to select the best suited one which would enable the estimation of total number of microorganisms in the packaging. The results of the total number of microorganisms in paper and paperboard obtained by three methods were compared.

Materials

Microbiological contamination of paper and paperboard samples received from producers of packaging and acquired on the market was estimated. Materials in close contact with food like bags for sugar and flour, candy wrappings, pizza boxes as well as packaging which does not come in direct contact with food like boxes for confectionary (1) and (2), and collective packaging for chocolate bars (1), (2) and (3) were tested (see *Table 3*).

Examination of the total number of microorganisms in paper materials by defibering method

1 g of a sample was disintegrated in Ringer liquid [composition: NaCl - 2.5 g, KCl - 0.105 g, CaCl₂ - 0.12 g (POCh), NaHCO₃ - 0.05 g, water - 1000 ml] in a homogenizer at sterile conditions till a homogeneous mixture was obtained with concentration of 1%. The operative surface of the homogenizer was before hand sterilized with a 70% ethanol followed by rinsing with sterile distilled water. Then, the 1% suspension was 10-fold diluted for the measurement of the bacteria and fungi number. 2 ml of each of the dilutions were put onto 5 Petri dishes and flooded with nutrient medium TGEA [composition: bovine extract (BTL) - 3.0 g, tripton - 5.0 g, glucose (Chempur) - 1.0 g, agar (BTL) = 15.0, water 1000 ml, pH = 7.0 for bacteria and with glucose-starch agar (PDA) for fungi. For each medium a separate series of 7 dishes were prepared. The dishes were incubated at 37 ± 1 °C for 48 ± 2 hours for bacteria and at 30 ± 1 °C for 5 days for mould and yeast. The grown colonies were counted and the number of bacteria and fungi per 1 g of dry paper mass was calculated (u/g) [14].

Examination of the total number of microorganisms in paper materials by agar flooding method

20 mm × 20 mm sized samples were prepared in a total amount of 1 g. Two-layer dishes were used in the examination with top and bottom layer of agar nutrient medium between which the tested sample was placed. The tested surface was directed to the bottom of the Petri dishes. Incubation was done at 37 °C for 48 ± 2 hours for the microorganisms

on the PM2 substrate (agar with casein and soy hydrolyzate), and at 28 ± 1 °C for 5 days for fungi on the PM3 substrate (agar Sabouraud with benzylopicillin Fluka). After the incubation had finished, the amount of grown colonies of bacteria (PM2) and fungi (PM3) was counted for each of the sample. The number of the microorganisms was calculated per 1 g of tested sample, per dry mass of the paper, and per 100 cm² of the paper [15].

Examination of the total number of microorganisms in paper materials by smear method

Since no standard is available for the microbiological testing on the paper surface by smear method, the standard for the sampling from flat surfaces was applied (PN-ISO 18593 'Microbiology of food and fodder. A horizontal method of sampling from the surface by using contact dishes and smears') as well as the standard for the estimation of total number of bacteria and fungi in paper products. The microorganisms were washed out from a paper surface limited by a template pattern of 25 cm² by means of a swab wetted in Ringer fluid. The swab was then put to a flask holding 20 ml of Ringer fluid and the stick was cut off. The flask was shaken for 30 seconds. 4 Petri dishes were inoculated with 1 ml of the fluid, of which 2 dishes were flooded with PDA medium (tripton glucose extract agar) (BTL) and 2 with glucose-starch agar (TEAG). For much contaminated samples incubation was made from a series of dilutions from 10⁻¹ - 10⁻³ in Ringer fluid. Incubation of the Petri dishes with the TEAG medium was made at 37 °C for 48 to 72 hours and at 30 ± 1 °C for 5 days for mould and yeast with medium PDA. The number of microorganisms per 1 cm² of the tested paper or board surface was calculated [13, 16].

Results

The total number of bacteria and fungi estimated by three methods is shown in *Tables 1* and *2* (see page 188); it was on a low level, and depended upon the applied method.

The number of bacteria by the defibering method was in the range of 10² - 10³ u/g. Highest bacteria number of 1.2 × 10³ was

Table 1. Total number of bacteria in the tested paper samples.

Kind of sample	Dry mass, %	Method to estimate the total bacteria number			
		Defibering method	Flooding method		Smear method
		Bacteria number, u/g	Bacteria number, u/g	Bacteria number, u/100 cm ²	Bacteria number, u/1 cm ²
Paper wrapping for soft candies	96.0	1.0×10 ²	4.0×10 ¹	3.0×10 ¹	<1.0
Pad for box of confectionary	93.6	5.4×10 ²	uc.	uc.	<1.0
Paper bag for dry material, sugar	96.0	1.2×10 ³	uc.	uc.	<1.0

Table 2. Total number of fungi in the tested paper samples

Kind of sample	Dry mass, %	Method to estimate the total fungi number			
		Fibrion method Defibering Smear method	Flooding method		Smear method
		Bacteria number, u/g	Bacteria number, u/g	Bacteria number, u/100 cm ²	Bacteria number, u/1 cm ²
Paper wrapping for soft candies	96.0	<1.0×10 ¹	5.7×10 ⁰	4.3×10 ⁰	<1.0
Pad for box of confectionary	93.6	<1.1×10 ¹	0	0	<1.0
Paper bag for dry material sugar	96.0	2.0×10 ¹	1.0×10 ¹	7.1×10 ⁰	<1.0

Table 3. Total number of bacteria in tested paperboard samples; „-“ - not estimated.

Kind of ample	Dry mass. %	Method to estimate the total bacteria number			
		Defibering method	Flooding method		Smear method
		Bacteria number. u/g	Bacteria number. u/g	Bacteria number. u/100 cm ²	Bacteria number. u/1 cm ²
Board box for confectionary (1)	92.8	7.4×10 ⁵	uc.	uc.	-
Board box for confectionary (2)	92.6	5.3×10 ⁵	uc.	uc.	<1.0
packaging paperboard (1)	93.6	4.4×10 ⁶	1.8×10 ²	2.8×10 ²	-
packaging paperboard (2)	93.6	4.4×10 ⁶	uc.	uc.	-
Paperboard packaging for pizza	91.9	6.5×10 ⁶	1.1×10 ²	3.9×10 ²	-

Table 4. Total fungi number in tested paperboard samples.

Kind of sample	Dry mass, %	Method to estimate the total fungi number			
		Defibering method	Flooding method		Smear method
		Bacteria number, u/g	Bacteria number, u/g	Bacteria number, u/100 cm ²	Bacteria number, u/1 cm ²
Board box for confectionary (1)	92.8	<1.1×10 ¹	0	0	-
Board box for confectionary (2)	92.6	<1.1×10 ¹	2.0×10 ⁰	5.0×10 ⁰	<1.0
packaging paperboard (1)	93.6	2.6×10 ³	8.0×10 ¹	1.3×10 ²	-
packaging paperboard (2)	93.6	4.3×10 ¹	uc.	uc.	-
Paperboard packaging for pizza	91.9	4.4×10 ²	uc.	uc.	-

found in sugar bags and the lowest - 1.0×10² in paper for soft confectionary. The method of agar flooding enabled to estimate the number of bacteria only for the least contaminated sample (paper for

soft confectionary) and the result was half of that from the defibering method. Reading the result was difficult due to high error. With numerous samples the counting of colonies was deceiving due

to fast growth of the bacteria making the computing of the single colonies impossible. They were marked as uncountable (uc). In the flooding method in case of high contamination, the reading of the result is impossible (*Figure 1.a*).

The smear method applied did not reveal the contamination of the paper surface. It could have been caused by the presence of the bacteria in the deeper layer. Even samples with smooth surface imbibe water making the smearing sampling difficult.

The total amount of fungi according to defibering method was 2.0×10¹ u/g in the sugar bag (*Table 2*). In most of the samples tested the number of fungi was undetectable. In the wrapping paper for candies, the method detection limit of 1.0×10¹ units per 1 g did not allow to estimate the number of fungi. The number of fungi in the sample according to the flooding method was 4.3×10⁰ u/100 cm² and calculated on 1 g - 5.7×10⁰ u. Alike in bacteria, the smear method did not yield results in the form of washed out colonies of mould or yeast. In one of the samples (pads for confectionary box) mould was not detected by any of the methods.

The number of estimated microorganisms in paperboard was distinctly higher than in paper (*Tables 3* and *4*). The total amount of bacteria in paperboard by the defibering method was in the range of 10⁵ - 10⁶ u/g. Counting of the bacteria colonies in the flooding method was impossible in most of the samples tested due to the intensive growth. In the board (1) and pizza packaging the number of bacteria was in the range of 1.1 - 1.8 ×10² u/g, much lower than in the defibering method. The counting of the colonies of bacteria and mould was difficult in the agar flooding method due to the character of the growth. Even at a low contamination, the counting was restrained with high measurement error. Bacteria tend to flood along the edge of the sample which practically makes the counting of the singular colonies impossible (*Figure 1.b*). When colonies were growing on the sample surface, the reading was hindered because the colour of the paper and the colonies was the same.

The smear method was applied to only one sample of paperboard with smoothest surface. Presence of bacteria was not detected by the method. A strong swelling of the samples was seen during the

smearing; the samples though smooth on the surface quickly imbibed the Ringer fluid from the smear stick. Growth of neither bacteria nor mould was observed by the method in the sample though it revealed microbiological contamination by the defibering- and agar flooding methods.

The photos given in **Figures 1 & 2** present the growth of bacteria and mould on samples of packaging paperboard.

The total number of fungi in the paperboard estimated by the defibering method was no higher than 2.6×10^3 u/g. The number of fungi by the flooding method was also lower or uncountable alike in the counting of the total bacteria number.

The agar flooding method seems suited to very low microbiological contamination only. At high contamination the test result may only be described in words without a quantitative assessment of the bacteria or fungi number. Photos illustrating the growth of mould on paperboard samples are presented in **Figure 2**.

An intensive growth of the mould proceeds on the samples. The appearance of the colonies is diversified depending on the type of sample confirming the contamination by different kinds of mould.

On the basis of the examinations made, it can be concluded that the smear method is not suited to the testing of microbiological contamination of paper and paperboard. It could only be used if the tested material would contain a water impermeable aluminium foil or plastic coat.

■ Conclusions

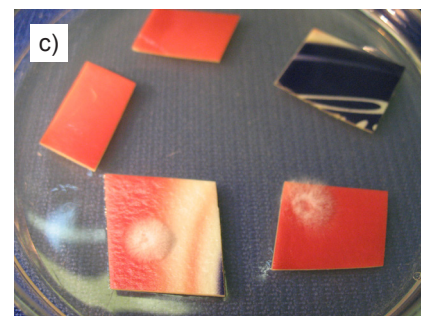
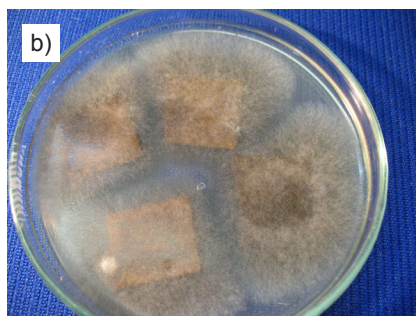


Figure 2. Agar flooding method. Growth of mould on: a) pizza packaging, b) paperboard samples, c) box paperboard.



Figure 1. Method of agar flooding. Growth of: a) bacteria on box pad for confectionary, b) board box.

- Establishing the criteria concerning microbiological purity of packaging materials prepared of paper and paperboard is recommended. The present regulations demand only from the food producers to eliminate all sources of food contamination including those coming from the packaging.
- In the investigation made, assessed was only the total number of microorganisms. It seems, however, that the in standardized criteria, except of the total number of bacteria and fungi, the obligatory absence of some pathogenic bacteria should be included similarly as in demands concerning alimentary products.
- The samples of tested paper revealed a low, however not zero, level of microbiological contamination. The contamination was high in paperboard, which is probably caused by a not compact structure of the fibrous composite.
- Testing made for the same samples according to the agar flooding and smear methods produced results by several orders lower than in the defibering method. Otherwise, the assessment appeared impossible in the two methods due to their specificity.
- Testing by the agar flooding method according to DIN 54 378 standard is not time-consuming, however, it only permits to approximately assess the contamination level due to the mutual over-flooding of the colonies and a difficult reading of results.
- The smear method is not suited to the estimation of bacteria and fungi number in samples of paper and paperboard.
- The defibering method according to standard ISO 8784.1 is best suited to the testing of microbiological purity of paper and paperboard intended for contact with food. The method enables a precise estimation of bacteria and fungi number in both very pure and highly contaminated samples.
- The investigation made was merely of an introductory character. Further work is needed in that direction to provide a statistical base for the selected method. □

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