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An improved method for microbiological testing of paper-based laminates used in food packaging

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ABSTRACT

Food packaging materials fundamentally contribute to food quality and safety, as they protect the packaged food against external influences. In this context, the determination of the hygiene status of the packaging material is of great importance. However, European legislation neither sets any microbiological criteria nor provides any approved standard for the microbiological testing of food packaging materials. Nevertheless, reliable routine control is essential for guaranteeing high hygienic quality of packagings.

With the aim to achieve a maximum recovery rate at low contamination levels, an improved experimental design was developed for the enumeration of the total colony count, yeasts and molds and *Enterobacteriaceae* on the surface of roll stock packaging materials. For this purpose, two different types of paper laminates were selected and exemplarily used as objects of investigation. Moreover, the performance of different growth media was compared for each microbiological parameter. This approach was followed by method validation using a selection of quantitative reference materials of representative microorganisms, including resistant forms of microbes such as bacterial endospores and fungal spores.

1. Introduction

Packaging materials play a major role in ensuring microbiological food safety as they pose a physical barrier against various environmental contaminants. In addition, they also fulfill an important function in protecting the packaged food from light, oxygen and humidity, thus contributing to prolonged shelf-life (Marsh & Bugusu, 2007). However, a high hygienic quality of the packaging materials is required for excluding them as a potential source of microbiological contamination for the filling goods.

Today, there exists neither a European regulation setting microbiological threshold levels for food contact materials, nor any internationally approved standard method for the microbiological control of these materials. Although the microbial load of most packaging materials is usually low, the predominance of resistant forms of microbes on packaging materials (Pirttijärvi, Andersson, & Salkinoja-Salonen, 2000; Suihko & Stackebrandt, 2003; Väisänen, Mentu, & Salkinoja-Salonen, 1991), such as bacterial endospores or fungal spores at very low contamination levels, is very challenging for the analytical surveillance. Therefore, under practical conditions, an important focus should be set on the prevention of microbial entrance portals along the entire supply chain to avoid even low microbiological contamination levels of the packaging materials. Thus it is of importance to strictly comply with good manufacturing practices (GMP) (Raaska, 2005).

As far as packaging materials containing paper are considered, such as paper-polyethylene terephthalate (PET) and paper-aluminum laminates, the quality of the commodity paper is decisive for the microbiological quality of the final product (Cerny & Betz, 1999), since paper usually contains a certain microbial load that is not completely eliminated during the production process. Compared to other materials such as metals, glass or plastics, rather low temperature treatment is applied to those laminates (Bergmair, Washüttl, & Wepner, 2010). Experience has shown that undesired exposure of the paper-layer, e.g. by faulty lamination, fosters the release of embedded microbes and thus may pose a hazard for spoilage of the packaged food. Moreover, bacterial and fungal

Abbreviations: TSA, Tryptone Soya Agar; VRB, Violet Red Bile Agar; PCA, Plate Count Agar; DRBC, Dichloran Rose Bengal Chloramphenicol Agar; SAB, modified Sabouraud 1% Glucose 1% Maltose Agar; RM, reference material; FCS, food contact side.

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spores, originating from the surrounding air or from cutting procedures of the laminates, may adhere to the packaging material surfaces (Pirttijärvi, Graeffe, & Salkinoja-Salonen, 1996). Hence not only quality losses of the product may result, but also some theoretical health risk to the consumer.

However, it should not be forgotten that usually process hygiene criteria of food production possess some higher relevance in terms of food safety than the contamination risk by paper-based packaging materials (Ekman et al., 2009).

Microbial growth in foods strongly depends on the food ingredients and on environmental factors. Especially food products with a high water activity (a_w-value) and a high density of nutrients like, e.g., yoghurts and dairy desserts, are prone to microbial spoilage. Even if spores find suited environmental conditions, they are able to germinate and to regrow upon getting contact with food of sufficient a_w-value. In contrast, dry foods with a low a_w-value, such as chocolate with an average a_w of 0.4 (Copetti, Iamanaka, Frisvad, Pereira, & Taniwaki, 2011), possess a significantly reduced risk to undergo microbial spoilage. Most gram-positive microorganisms require a minimum a_w-value of around 0.90 to grow, while gram-negative bacteria prefer higher humidity (a_w 0.97). Mold growth, however, is already possible at an a_w-value of 0.80 (Adams & Moss, 2008).

For the microbiological assessment, only a few official methods exist. For example, DIN 10 050-3:1999 (Deutsches Institut für Normung, 1999) describes the determination of the microbial count of butter wrappers, and DIN 54 378:1993 (Deutsches Institut für Normung, 1993) deals with the determination of the surface colony count (yeasts and molds) of paper and board. The latter method is also part of a recommended procedure outlined by the German Industry Association for Food Technology and Packaging for the microbiological testing of non-absorbent material surfaces (Arbeitsgruppe "Lebensmittelerhaltung und Mikrobiologie," 1974). This method has been widely applied for routine purposes among many packaging material producers.

In this document (German designation: "Merkblatt 21") a cultural technique for the determination of the total aerobic colony count, of yeasts and molds and of *Enterobacteriaceae* is described. According to this guideline, packaging materials first are cut into a format that fits into standard petridishes (diameter 94 mm) by using round templates. The petridishes are filled with a thin layer of culture medium, which varies according to the microbiological parameter. For the total colony count, Nutrient agar is used, while a modified Sabouraud medium containing 1% Glucose and 1% Maltose agar is used for yeasts and molds, and Violet Red Bile agar for *Enterobacteriaceae*. After solidification of the medium, the cut sample is put onto the agar and a second layer of medium is poured over the sample.

This method shows some general as well as some specific deficiencies regarding paper-based laminates: the procedure is time consuming, and the assessment of the recommended sample area of 100 cm^2 cannot be achieved when using standard petridishes with a diameter of 94 mm. Additionally, undesired growth of microbes originating from the cutting edges may hinder the unambiguous enumeration of the colonies. Also, the overlaying process sometimes causes some curling of the laminate sample and hence additional equipment is necessary to fix the sample into a correct position. This again increases the risk of re-contamination and may thus lead to false-positive results.

In order to overcome the above mentioned problems, this study was undertaken to improve the cultural method for the microbiological assessment of laminated packaging materials. The particular challenge was to simplify the working procedure and to enhance method robustness considerably with respect to the observed deficiencies of the currently used routine method, especially when considering roll stocks of paper-based laminates.

2. Materials and methods

2.1. Materials

2.1.1. Quantitative reference materials

A selection of BioBall MultiShot-550 products (BTF, Sidney, Australia), which are accredited reference materials (RM) under ISO Guide 34 standard, was used for the microbiological spiking experiments. According to the corresponding Certificates of Analysis (BTF, n.d.), these quantitative reference materials contain a precise number of colony-forming particles of a specified type culture strain taken from the ATCC reference culture collection (ATCC, American type culture collection), ranging between 500 and 600 cfu per BioBall. For method validation, the following microorganisms were selected: *Bacillus subtilis*, ATCC 6633 (Lot no.: B1978 containing a stated reference value per BioBall of 568.7 ± 46.1 cfu examined on Nutrient agar after 24 h aerobic incubation at 37 °C); *Escherichia coli*, ATCC 8739 (Lot no.: B1961 with a reference value of 588.5 ± 33.6 cfu on Nutrient agar after 24 h aerobic incubation at 37 °C); *Aspergillus brasiliensis*, ATCC 16404 (Lot no.: B1982 having a reference value of 548.5 ± 43.8 cfu on Dichloran Rose Bengal Chloramphenicol (DRBC) agar after 48 h aerobic incubation at 37 °C). A strict protocol (BTF, 2014) was followed for each BioBall rehydration procedure to ensure a homogenous suspension for providing aliquots with a defined composition for each experiment.

2.1.2. Packaging material samples

Two different types of paper laminates, a paper-PET laminate and a paper-aluminum laminate, served as sample material and were examined for their microbial contamination prior to and after spiking. The paper-PET laminate (termed as *PaP* in the following) uses a metalized PET layer as food contact side (FCS) and is usually intended for pot sealing of yoghurts and chilled dairy desserts. It is delivered as large reels to the customers – usually food producers or bottlers – who cut the material right after sealing it to the plastic cups containing the food. The paper-aluminum laminate (termed as *PaA*), which is used for wrapping chocolate bars, uses the paper layer as FCS. Considering the risk of microbial growth, *PaP* is of highest priority regarding food safety and was therefore selected for the method development and validation with quantitative reference materials.

2.2. Methods

2.2.1. Sampling

PaP samples were drawn right after the final cutting process in the manufacturing site. Several layers of *PaP* were cut from the large roll that is delivered to the customers, immediately wrapped in polyethylene foil and transferred to the laboratory.

2.2.2. Method according to "Document 21"

The traditional method according to the guideline given in "Document 21" is illustrated in Fig. 1 (on the left). For the determination of the total aerobic colony count, Nutrient agar was replaced by Plate Count agar (PCA; Merck, Ref. 1.05463.0500) since this culture medium has been recommended in several ISO standards, e.g., ISO 4833-1:2013 (International Organization for Standardization, 2013). For the enumeration of yeasts and molds, modified Sabouraud 1% Glucose 1% Maltose agar (SAB; Dinkelberg, Ref. DB15970.0500) and for *Enterobacteriaceae*, Violet Red Bile agar (VRB; Merck, Ref. 1.01406.0500) were used according to the given guideline. In order to avoid a contamination of the samples, the following steps were carried out in a laminar flow work bench.

Round samples of 40 cm^2 – suitable to fit into a conventional petridish – were cut using sterile scissors. To weigh down this