

Peracetic acid and atmospheric plasma as alternatives for packaging disinfection in the dairy industry

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Date submitted: 28/12/2015

Date accepted: 04/02/2016

Volume/Page(s): 69/2-6

Abstract

In the dairy industry disinfection of packaging material is an established process step. Nowadays the mostly applied disinfection method of packaging materials and machinery is hydrogen peroxide disinfection. Disinfection with atmospheric plasma and peracetic acid are alternatives. In this study the two hydrogen peroxide disinfection alternatives were evaluated for their antimicrobial effect and their interaction with packaging material and packaged good.

To analyze the efficiency of alternative disinfection methods and interactions between packaged goods and packaging materials, films of common coffee cream portion cups were used. The microbial reduction rate was investigated by the wet/dry swap technique (DIN 10113-1: 1997-07) using *Bacillus subtilis* and *Aspergillus niger*. Interactions between disinfection method and packaging material were measured by seal strength (DIN 55529:2005-09) and contact angle measurement (DIN 55660-2). Sensory influences on the packaged goods were detected by using the triangle test (DIN EN ISO 4120). Microbial test showed that the microbial reduction rate of atmospheric plasma was lower than the microbial reduction rate of hydrogen peroxide due to the chosen parameters for atmospheric plasma whereas the microbial reduction of peracetic acid was as effective as of hydrogen peroxide. The sensory test showed that the different disinfection methods had no effect on the flavour of packaged goods. Atmospheric plasma caused a considerably smaller contact angle than both chemical disinfection methods. The seal strength test showed no significant difference ($P > 0.05$) neither between hydrogen peroxide and peracetic acid nor between untreated sample and atmospheric plasma but a significant difference ($P < 0.05$) between these two groups. In the sealing range of 190 - 240 °C the seal strengths were around 8 N/15mm. The results of atmospheric plasma disinfection showed the need for additional tests with different plasma doses in order to generate a higher microbial reduction rate. Experiments with higher plasma doses also require more tests with regard to the

packaging material. Consequently, both disinfection methods could be further investigated as alternatives for disinfection with hydrogen peroxide.

Key words: *Bacillus subtilis*, *Aspergillus niger*, hydrogen peroxide, packaging material disinfection, germ reduction, seal strength, contact angle measurement, sensory test

Introduction

To increase food safety and shelf life of products in dairy industry, packaging materials get disinfected in order to avoid recontamination of the product after the filling process. The standard method of microbial reduction in dairy industry is disinfection by hydrogen peroxide [1]. In view of the continuous improvement processes and the efforts to reduce production costs, an interest in alternative microbial reduction systems is given. Another well-known method for microbial reduction is disinfection with peracetic acid [2]. Disinfection with peracetic acid had a high microbial reduction rate (MRR) at room temperature and a lower application rate. Possible disadvantages of this method are an unpleasant smell of acid and ozone which arises during the application and its corrosive effect at high concentrations [3].

A new innovative technique is microbial reduction with atmospheric plasma. The process is a dry physical method which does not change the technological characteristics of thermolabile packaging material after treatment [4]. Organic residues could remain on the packaging material and the treatment of complex geometries could be difficult [5]. The disinfection of packaging material was carried out in laboratory scale referring to industrial process.

The object of this research was to evaluate the influence of hydrogen peroxide, peracetic acid and atmospheric plasma disinfection on packaging materials and packaged goods. To protect packaged goods against spoilage a high microbial reduction is sought. Therefore a

microbial test was performed to analyse the MRR. To examine possible influences on packaging materials, seal strength test and contact angle measurement test were done. To detect if one of the disinfection methods had an influence on the taste of the packaged good a sensory test was done.

Material and Methods

In order to analyze the different disinfection methods this study used the filling and sealing process of a common coffee cream portion cup which was representative for similar packaging material as applied in dairy industry. Therefore the unformed bottom films, the lidding films and already formed portion cups were used. Portion cups consisted of a polystyrene film, containing high impact and general purpose polystyrene, with a polypropylene inside layer and a total film thickness of 680 μm (bottom film) and an aluminum lidding film with a styrene copolymer as sealing coating with a thickness of 40 μm . These films were used as reference material for the analysis of seal strength, contact angle measurement and MRR. For the sensory test, coffee cream with a fat content of 12 % was filled in portion cups made of the above-mentioned film and sealed with the lidding film. This product was chosen as a representative for many other products filled into thermoformed cups. Due to the amount of water and fat and its low intrinsic flavour, coffee cream is a sensory sensitive product.

Disinfection: For each analysis, the samples were treated with the three different disinfection methods. Hydrogen peroxide and peracetic acid disinfection of packaging material were performed in laboratory scale referring to industrial process. Atmospheric plasma is an innovation for packaging disinfection process so that there is no standard use in industry. The samples used in hydrogen peroxide (30 %, Roth, Germany) disinfection were submerged into a liquid solution with a concentration of 30 % at a temperature of 50 °C for 2 s. Then the residual liquid was removed with sterile compressed air. The compressor pressure was 5 bar and the distance between compressed air gun (nozzle diameter of 1.5 mm) and sample was 5 cm. For peracetic acid (15 %, AppliChem, Germany), the samples were submerged into a peracetic acid solution with a concentration of 0.3 % at room temperature for 2 s. Then the samples were dunked in sterile water for 2 s, as it is usual for peracetic acid in industrial processes. The residual liquids were removed with sterile compressed air as for disinfection with hydrogen peroxide. Plasma applications were carried out with atmospheric plasma (MEF 1K, Tigres GmbH, Germany). For plasma disinfection, samples were processed with pulsed atmospheric plasma (200 W) for 0.2 s. The distance between nozzle and samples was 1 cm. Each method was performed under sterile conditions.

Microbiology: To detect microbial reduction with hydrogen peroxide, peracetic acid and atmospheric plasma, samples of the unformed bottom film and the lidding film were contaminated in a circle area with a diameter of 0.8 cm with 20 μl of germ suspension consisting of 1.9×10^8 CFU/ml of *Bacillus subtilis* or 2×10^8 CFU/ml of *Aspergillus niger* (DSM 2143). For the different disinfection methods, microbial reduction was investigated by the wet/dry swab technique DIN 10113-1: 1997-07 using 2 ml of Ringer's solution (1/4 strength, Merck, Germany) per tube. Therefore the first swab was wetted with Ringer's solution and then wiped off the defined area. After that, the area was wiped off with the second dry swab. Both swabs were put into one tube and afterwards the solution was homogenised. After that, swabs were removed and the solution out of one tube was divided up equally between two agar plates. A tenfold determination was performed for each combination of method, film and germ. *Bacillus subtilis* was

detected with plate count skimmed milk agar (Merck, Germany) incubated for 72 h at 30 °C and *Aspergillus niger* with yeast extract glucose chloramphenicol agar (Merck, Germany) incubated for 96 h at 25 °C. Subsequently colonies were counted and sorted in groups of MRRs.

Sensory analysis: To detect possible sensory differences between disinfection methods a triangle test was applied according to DIN EN ISO 4120. This test is useful to determine whether a sensory difference exists between two products. In addition, the test can be used in situations where treatment effects may have produced changes that cannot be characterized easily [6]. Therefore coffee cream (12 % fat) was packed in portion cups which were beforehand treated with one of the three different disinfection methods. As a reference for each disinfection method, untreated coffee cream was used. Overall three triangle tests, one for each different disinfection method, were carried out. Each triangle test consisted of three cups, from which two cups were treated with the same disinfection method and the third cup as the reference (or conversely). For the sensory tests 24 untrained panellists of the University of Applied Science and Arts Hannover (Faculty 2 - Department of Bioprocess Engineering, Germany) were used. Each panellist received these three triangle tests. The triads were presented in plastic cups coded with three digital random numbers.

Seal strength: In addition to the disinfection effect, the processability in the sealing process has to be given which can be tested with the seal strength. The seal strength tests were based on DIN 55529:2005-09 but with a peeling angle of 180 °. This angle was chosen due to the pull mechanism of the testing machine which did not allow a peeling angle of 90°. For the measurement the lidding film was sealed on the unformed bottom film with a seal strength of 500 N for 0.5 s. At several temperatures a fivefold determination was carried out for each of the treated samples by the three different disinfection methods and for the untreated film.

Contact angle measurement: The contact angle was determined by putting drops of ethylene glycol on the sample surfaces. Afterwards the angles of the drops were measured by an image processing program of the contact angle measuring system (OCA 15EC/B by Fa. Dataphysics). The implementation conformed to DIN 55660-2 and a fivefold determination was performed.

Statistical methods: All statistical analyses were performed with the WinSTAT Software (version 2009.1, R. Fitch Software, Germany) with a chosen statistical significance ($P \leq 0.05$).

Microbiology: Counted colonies for each combination of disinfection method, film and germ were sorted in groups of MRRs with which a descriptive statistical comparison was done. Differences between these three disinfection methods were proven with the Kruskal-Wallis test and with the Post-hoc-test.

Seal strength: Data were analysed using a 2-way ANOVA on the factors temperature and treatment method with seal strength as measure variable. In case of significant differences the treatment methods were compared using Fishers least significant difference (LSD) test.

Contact angle measurement: The data of the contact angle were statistically tested using ANOVA to determine statistical differences and the LSD test was used for the comparison of the median.

Results

Microbiology: Groups of MRRs are shown in Table 1. Post-hoc-test showed that MRR of atmospheric plasma differed significantly

Table 1: Groups of microbial reduction rate (MRR) with *Aspergillus niger* and *Bacillus subtilis*

groups of MRR (log-range)	<i>Aspergillus niger</i> (CFU)	<i>Bacillus subtilis</i> (CFU)
initial bacterial count per 20 µl	4.000.000	3.800.000
log 2	< 40.000	< 38.000
log 3	< 4.000	< 3.800
log 4	< 400	< 380
log 5	< 40	< 38
log 6	< 4	< 3

from the MRR of peracetic acid and hydrogen peroxide for each combination of film and germ. For disinfection with hydrogen peroxide and peracetic acid there was a *Bacillus subtilis* MRR in median of 3 log and a *Aspergillus niger* MRR of 6 log on both films. *Bacillus subtilis* MRR ranged for both chemical methods from 3 - 4 log and *Aspergillus niger* MRR from 5 - 6 log. Atmospheric plasma disinfection showed a *Bacillus subtilis* MRR of 2 log for both films in median and ranged from 2 - 3 log. *Aspergillus niger* MRR ranged from 3 - 4 log and was 4 log in median for bottom films and about 3 log for lidding films. The MRRs with *Bacillus subtilis* and *Aspergillus niger* for bottom films and lidding films with the different treatments were shown in Table 2.

Sensory analysis: The triangle test showed that nine persons out of 24 detected flavour deviations for samples treated with hydrogen peroxide, seven for peracetic acid and also seven for atmospheric plasma. At least 14 persons out of 24 should have detected flavour

Table 2: Microbial reduction rates (MRRs) with *Bacillus subtilis* and *Aspergillus niger* for treatment methods

	log-range					
	hydrogen peroxide		peracetic acid		atmospheric plasma	
	<i>B. subtilis</i>	<i>A. niger</i>	<i>B. subtilis</i>	<i>A. niger</i>	<i>B. subtilis</i>	<i>A. niger</i>
bottom film	3	6	3	6	2	4
lidding film	3	6	3	6	2	3

deviations to obtain a significant difference at a 5 % level. Consequently there was no significant difference in the sensory analysis carried out according to DIN EN ISO 4120 annex A.1. Furthermore none of the panellists reported an acidic flavour although the peracetic acid has a characteristic acrid odour [7].

Seal strength: As shown in Figure 1 sealing started at 140 °C. The curve ended at 240 °C because the polystyrene film melts at higher temperatures. The sealing temperatures were described with the median. Between temperatures from 140 °C - 180 °C the seal strength showed a higher variance between the various samples within the three different disinfection methods. In the sealing range, the strength and the temperature were high enough to get a consistent seal strength, so there was a lower variance within the different treated samples. The sealing range reaches from 190 - 240 °C. These temperatures allowed a practical processability in the sealing process due to the low variation in seal strength. Within the sealing range, temperature and treatment

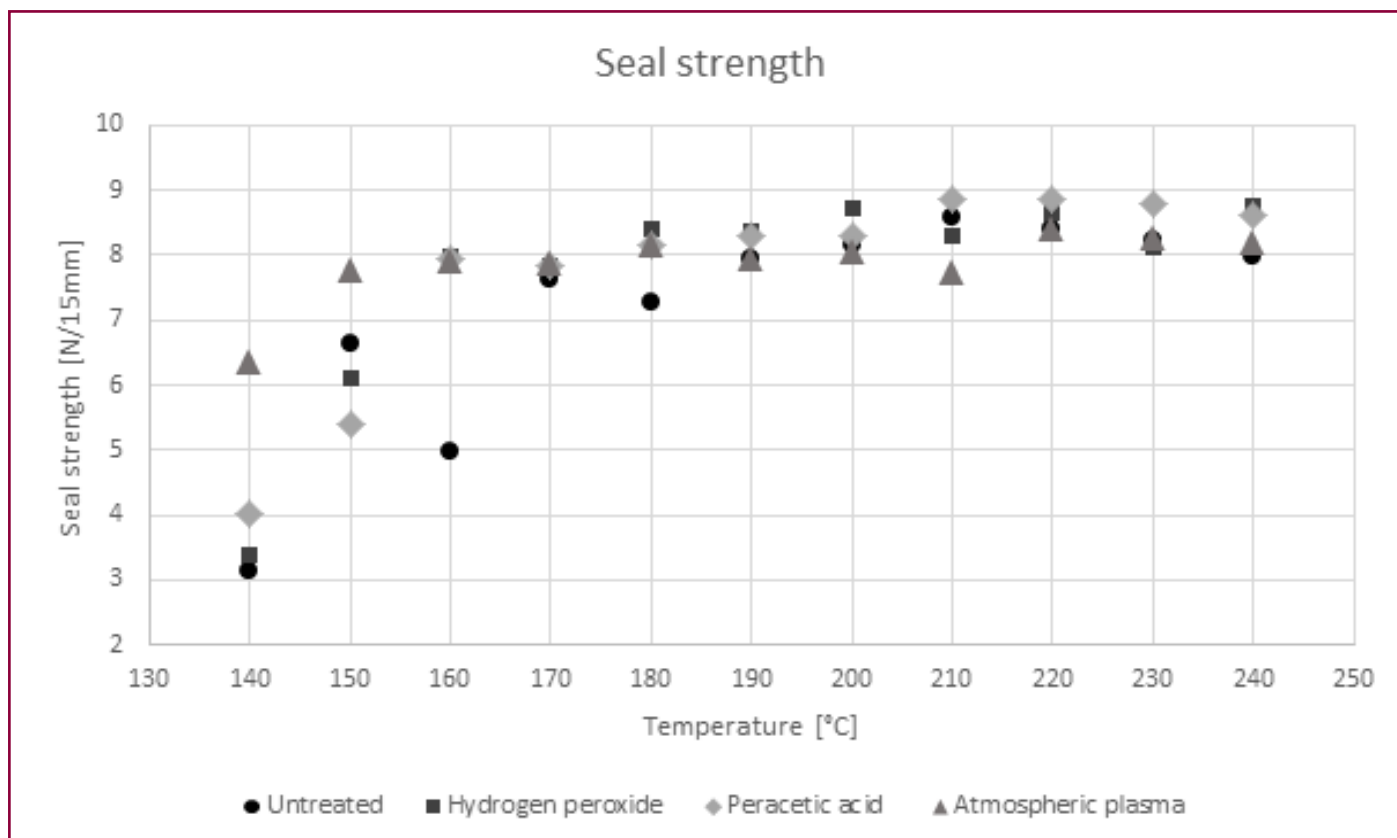


Figure 1: Median values of the seal strength for packaging material with three different treatment methods and the untreated sample. Median values were calculated from five measurements, respectively

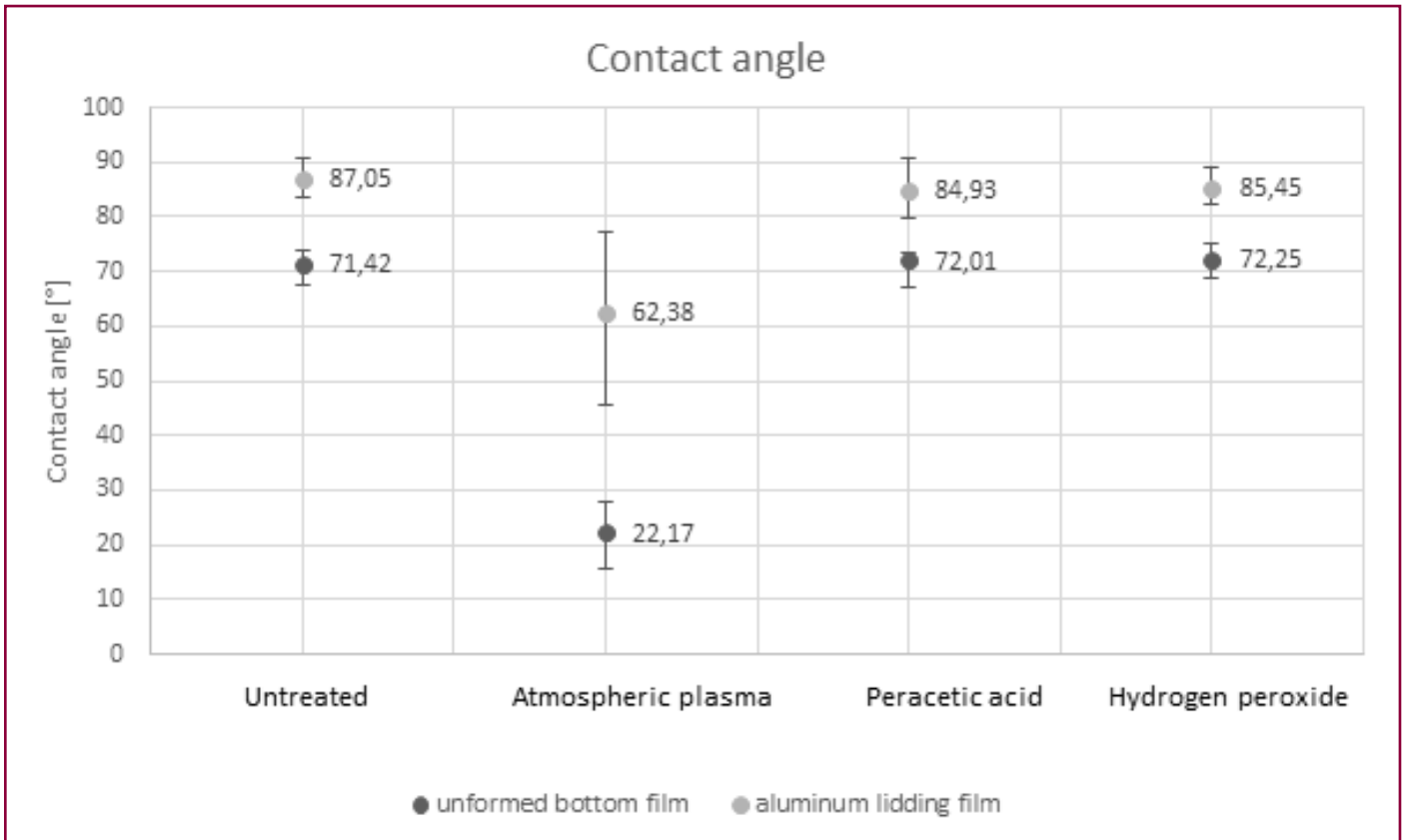


Figure 2: Contact angle measurement of treatment methods for polystyrene film and aluminum lidding film. Values were calculated from five measurements, respectively

method had a significant influence on the detected seal strength (temperature $P = 0.003$, treatment method $P < 0.001$). There was no significant difference in seal strength neither between the untreated and the plasma samples ($P > 0.05$) nor the hydrogen peroxide and peracetic acid samples ($P > 0.05$). There was a significant difference between these two groups ($P < 0.05$).

Contact angle: The contact angle of ethylene glycol in Figure 2 showed no significant differences ($P < 0.05$) between untreated and treated samples for unformed bottom and lidding films. The atmospheric plasma-treated samples showed a considerably smaller contact angle amounting to 22.2° on unformed bottom film and 62.2° on aluminum lidding film. The atmospheric plasma-treated aluminum lidding film showed a wider range in contact angles.

Discussion

Microbiology: Recommended minimum requirement for aseptic packaging machines is a MRR of 4 log on packaging material [8]. This recommendation could not be complied using *Bacillus subtilis* for all disinfection methods and *Aspergillus niger* for disinfection with atmospheric plasma. Under chosen conditions disinfection with atmospheric plasma had a lower MRR than the disinfection methods with hydrogen peroxide and peracetic acid. Disinfection with hydrogen peroxide showed a *Bacillus subtilis* MRR of 3 log and *Aspergillus niger* MRR of 6 log for both films with a disinfection time of 2 s at a concentration of 30 %. Under those conditions the above-mentioned process was more effective than other studies showed [9]. This could be due to the fact that this study approximately reproduced an industrial process. Referring to industry the laboratory test of disinfection used tempered hydrogen peroxide and sterile compressed air to remove residual liquid.

In different sectors of industry, peracetic acid is an established disinfection method [10]. In this study peracetic acid showed a *Bacillus subtilis* MRR of 3 log and *Aspergillus niger* MRR of 6 log for both films with a disinfection time of 2 s at a concentration of 0.3 % at room temperature. As it had a similar MRR as hydrogen peroxide, peracetic acid could be used as an alternative for disinfection with hydrogen peroxide.

MRR of disinfection with atmospheric plasma showed significant differences from both chemical methods. There was a *Bacillus subtilis* MRR of 2 log for both films and a *Aspergillus niger* MRR of 4 log for bottom films and about 3 log for lidding films. Plasma is an alternative [11] disinfection method for hydrogen peroxide but there is a need for further studies to get suitable conditions. It is worth to investigate radiation intensity, radiation time and distance between air gun and sample. In detail, the interactions between power increase of atmospheric plasma and effects in relation to microbial reduction should be tested. Further it is necessary to know if pulsed atmospheric plasma is more efficient than non-pulsed atmospheric plasma. Consequently, the interactions of different chosen parameters for a plasma treatment should be analyzed.

Sensory analysis: The different disinfection methods had no effect on the coffee cream's flavour. This conclusion is supported by other studies in which sensory tests with lettuce disinfected with peracetic acid were carried out [12]. In this case, the treatment did not affect the sensory quality of the lettuce, although small color changes were observed after colorimetric measurements. Other studies tested the sensory of tomatoes, sweet pepper, cucumbers or strawberries after peracetic acid disinfection. The panel could not find any significant differences in fruits washed with and without peracetic acid [13, 14]. Referring to the flavour peracetic acid and atmospheric plasma are alternatives for hydrogen peroxide disinfection.

Seal strength: For all three disinfection methods and the untreated reference sample the seal strength was investigated to determine its alteration as shown in Figure 1. The seal strength test was carried out according to DIN 55529:2005-09. The angle of 180 ° used in the test differed from DIN and might have had an influence on the results. Within the sealing range the treatments achieve similar seal strengths around 8 N/15mm. There was no significant difference between hydrogen peroxide and peracetic acid. With chosen parameters for atmospheric plasma an increasing effect on the seal strength was manifested at 140 - 180 °C. A reason could be the increase of surface polarity due to the formation of carbonyl and hydrolytic groups on the surface [15]. It is expected that the seal strength would decrease from a specific point of plasma dosage due to the separation of polymers into oligomers which causes a weak boundary layer [16]. The weak boundary layer was not obtained yet, so there might be a possibility to heighten the plasma dosage to increase its seal strength within the sealing range. Regarding seal strength, peracetic acid and atmospheric plasma could be used as alternatives for hydrogen peroxide.

Contact angle: With the knowledge of the contact angle or the resulting surface energy of the samples a statement can be made about the wetting behavior and the adhesion of printing inks. To examine how the different disinfection methods changed the surface of the unformed bottom film and the aluminum lidding film, the contact angle was determined by putting ethylene glycol drops on the surface. The smaller contact angle of the plasma-treated samples was the result of an increased polarity of the surface. The increased polarity was primary caused by surface oxidation [17]. In Figure 2 the contact angle measurement for treated polystyrene film and aluminum lidding film is shown. The unformed bottom film which consists of polystyrene is more oxidative as the lidding film. This results from the high amount of electrostatic double bonds around the aromatic ring of polystyrene [18]. Therefore the contact angle of the atmospheric plasma-treated lidding film sample has not decreased to such an extent as the unformed bottom film.

Conclusion

Peracetic acid and atmospheric plasma were examined as alternatives for hydrogen peroxide disinfection. Especially peracetic acid had almost the same efficiency as hydrogen peroxide in MRR, seal strength, contact angle and sensory test. Even the expected vinegar taste in the sensory analysis was not detected. In view of these investigations, peracetic acid can be used as an alternative for hydrogen peroxide. Between atmospheric plasma and hydrogen peroxide there were differences in seal strength and MRR for the applied conditions. At sealing temperatures between 140 - 180 °C the seal strength of samples disinfected with atmospheric plasma was higher. With the applied treatments the MRR of atmospheric plasma was lower compared to disinfection with hydrogen peroxide. Further investigations with higher applied plasma dosages or a longer treatment time should be done to examine the full potential of this disinfection method.

Acknowledgements

The authors are grateful to Prof. Dr. med. vet. Volker Krömker and Prof. Dr. rer. nat. Rainer Brandt for their constant and valuable support. The results reported in this paper would not have been possible without the technical assistance of Dipl. Ing. Anke Bormann and Dr. agr. Jan-Hendrik Paduch in the microbiology laboratory.

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