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Decontamination of food-packing material using moist heat

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Depending on the microbiological quality, it may be necessary to reduce the number of microorganisms on the packing material before use with food products. Treatment with hydrogen peroxide at elevated temperatures is most commonly used. Residues of hydrogen peroxide, however, are undesirable. Systems not using chemicals obviously would be preferable. For products which do not allow the growth of bacteria, such as many acid products and products with a low water activity, often it is sufficient to inactivate moulds and yeasts. Moulds and yeasts can be inactivated by temperatures below 100°C provided the water activity is high enough. At low humidities at the same temperatures hardly any reduction in viable count is obtained. A prototype machine was built to investigate the inactivation of microorganisms on the surface of packing material, using moist heat for a short time, similar to the time needed for decontamination by peroxide. The number of viable dry spores of *Penicillium roqueforti* can be reduced by a factor of >1000 within 3 s at 90°C and 100% humidity. Moist heat decontamination is a promising method which could help manufacturers pack food in a microbiologically safe manner, without the use of chemicals. Further work is needed, however, to determine the inactivation of other relevant microorganisms.

Keywords: decontamination of packing material; packing machines; mould spores; *Penicillium*; humidity

Introduction

Consumers are more and more demanding with respect to the quality of food products. Therefore, products are often preserved by in-line short-time high-temperature treatments. To prevent re-infection, the product then is packed aseptically.

The microbial load of the packing material must be low enough to assure that the acceptable rate of contamination is not exceeded [1]. Depending on the microbiological quality, it may be necessary to reduce the number of microorganisms on the packing material before use. This may be achieved using hydrogen peroxide, heat, ultraviolet light, or other treatments, either individually or in combination. Treatment with hydrogen peroxide at elevated temperatures is by far the most common technology used. Residues of hydrogen peroxide, however, are undesirable; the acceptable residual concentration in the product packed is very low (<5 mg kg^{-1} in most countries). The use of hydrogen peroxide also requires safety measures to protect personnel from exposure to the solution and to the vapour generated during use. Systems not using chemicals would obviously be preferable.

For products that do not allow the growth of bacteria, such as many acid products and products with a low water activity, it is often sufficient to inactivate moulds and yeasts. Moulds and yeasts, however, can be inactivated by temperatures below 100°C provided the water activity is high enough.

The objective of the work reported here was to determine whether the use of heat in combination with enhanced humidity would be an option for the decontamination of packing materials where the main reason for concern is the presence of moulds.

Spores of *Penicillium* species are frequently found on packing materials even after thermoforming. For this reason and because of availability and ease of cultivation, spores of *Penicillium roquefortii* were used.

Materials and methods

Materials

The packing material comprised rectangular discs 35×45 mm made from a sheet of 550-μm thickness (55 μm of polyester (PETG) and 495 μm of polystyrene) from Cobelplast, Lokeren, Belgium. The fungal spores were *Penicillium roqueforti* type CB2, obtained from Wiesby (Niebüll, Germany), lot 735263. The talc was hydrous magnesium silicate, obtained from Lamers & Pleuger, 's Hertogenbosch, The Netherlands. The mixer was a magnetic stirrer Heidolph type MR 2002; stirring bar diameter 8 mm, length 50 mm.

Media

The diluted malt extract agar contained agar (Difco) 20 g, malt extract (Oxoid CM 57) 20 g, distilled water 1000 ml. The medium was autoclaved at 115°C for 15 min. The peptone salt solution contained peptone salt (Oxoid CM 733) 9.5 g, of which peptone made up 1.0 g and NaCl made up 8.5 g, distilled water 1000 ml. The solution was autoclaved at 120°C for 20 min. The malt extract broth contained malt extract (Oxoid CM 57) 20.0 g, of which malt extract made up 17 g and mycological peptone made up 3.0 g, distilled water 1000 ml. The broth was autoclaved at 115° C for 10 min.

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Packing machine

A prototype machine was constructed in which sheets of packing material could be treated with both moist and dry heat. The design was based on the assumption that this way of decontamination—if successful—would be applied on 'form-fill-seal' machines. The machine therefore was equipped with two heating plates. The machine is represented schematically in Figure 1.

The machine is equipped with means to measure and control the relative humidity (RH) between 0 and 100%. Measurement was done with a Rotronic C-80-F/I-350 humidity sensor/transmitter (Rotronic AG, Zurich, Switzerland). The humidity was controlled by adjustment of the temperature of an electric heater in a water bath and adjustment of the speed of the fan which circulated air in the sterilizing chamber. It took up to 2 h to obtain a constant humidity. Water was added to the waterbath as required. After 2 h the relative humidity varied less than ±1%.

The temperatures of the two heating plates could be controlled independently. The plates could be pressed to the sheet of packing material by two air cylinders. The contact time could be adjusted.

Sheets of packing material could be transported manually through the sterilization chamber of the machine using a guided pulling device. It took less than one second to pull a section of sheet with a length equal to the length of the sterilization chamber into that chamber, between the heating plates. To pull the same section of sheet out again took also less than one second.

Deposition of spores on packing material

Investigation of spores in the flask, as delivered, revealed that the material consisted of flakes containing approximately 10^8 spores per gram. A small quantity of flakes (5 g) was ground in a mortar to obtain a finer powder. This procedure was performed very slowly for about 5 min, in a laminar air flow cabinet. Electron microscopy was used to check whether the grinding resulted in single spores.

Five grams of ground spore material and 0.2 g of talc were transferred to a 100-ml Erlenmeyer flask, using a powder funnel. A magnetic stirring bar was added and the flask was closed with a chromatography spray nozzle (Figure 2). The spray nozzle was connected to a compressed air supply, pressure 1.2 bar, using an electromagnetic shut-off valve. The air was filtered using a sterile air filter (Gelman, Loughborough, Leics, UK, Acro 50, 0.45 μm, PTFE). The contents of the flask were mixed using the magnetic stirrer for 10 min at 750 rpm.

The rectangular discs of packing material were contaminated on the polystyrene side using the spray system shown in Figure 2. To prevent contamination of the environment with spores, this was done in a laminar air flow cabinet. The discs were placed 60 mm from the spray nozzle. Before placing a disc in front of the nozzle, the supply of compressed air to the conical flask was opened twice for 0.5 s. With the disc in front of the sprayer, the supply valve was opened for the time needed to deposit the desired number of spores on the disc. Opening the supply valve for 0.5 s resulted in the deposition of approximately 4000 colony forming units (CFU).

Tests

For each test, three discs of contaminated packing material were used. One (sample *a*) served as the control, to determine the number of CFU per disc before the decontamination treatment. The other two (samples *b* and *c*) were

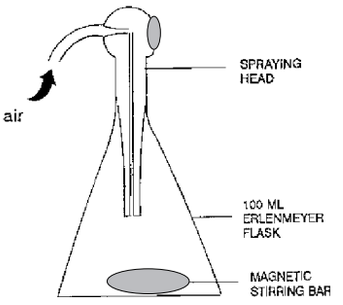
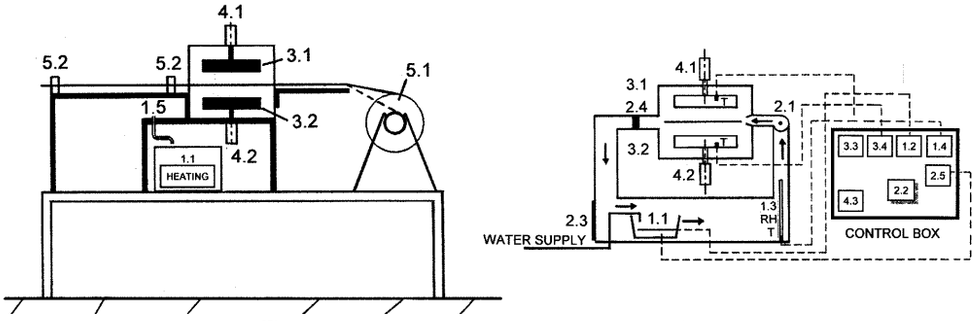


Figure 2 Spray system.



1.1 Electrically heated water bath
1.2 Waterbath temperature controller
1.3 Humidity & temperature transmitter
1.4 Humidity indicator
1.5 Pipe to fill water bath

2.1 Fan
2.2 Fan speed controller
2.3 Filter
2.4 Filter
2.5 Air temperature controller

3.1 Upper heating plate
3.2 Lower heating plate
3.3 Upper plate temperature controller
3.4 Lower plate temperature controller

4.1 Upper heating plate air cylinder
4.2 Lower heating plate air cylinder
4.3 Plate closing time controller

5.1 Packing material
5.2 Device for pulling packing material

Figure 1 Prototype machine for the decontamination of sheets of packing material.

Table 1 Inactivation of spores of *P. roquefortii* under various conditions[a]

Treatment				Results			
Temperature inside sterilization chamber (°C)	Relative humidity (%)	Temperature of heating plates (°C)	Time between heating plates (s)	N_o	N	$R = N_o/N$	Log R (mean)
49	100	60	1	1410 52 500	2640 74 600	<1 <1	—
57	100	70	1	1410 52 500	986 42 700	1.4 1.2	0.11
62	100	80	1	1410 52 500	898 13 550	1.6 3.8	0.43
			1	3060 58 700	≤9 1660	≥340 35	2.27
67	100	85	0	3060 58 700	31 3040	99 19	1.77
			1	1400 55 300	≤1 11	≥1400 5028	3.50
73	100	90	0	1400 55 300	<1 19	>1400 2910	3.33
			1	4410 55 000	≤5 ≤3	≥882 ≥18333	3.98
76	100	95	0	4410 55 000	<3 12	>1470 4583	3.48
			1	5660 53 300	<1 ≤5	>5660 ≥10 660	3.91
77	100	88	0	5660 53 300	<1 ≤9	>5660 ≥5922	3.76
	30	100	3	4040	3050	1.3	0.11
	32	120	3	4560 82 600	1830 38 900	2.5 2.1	0.36
Not measured	27	150	3	4040 82 600	300 832	13 99	1.75
	18	180	3	4560 82 600	≤3 ≤4	≥1520 ≥20 650	4.04

[a]In all tests with 100% humidity the total treatment time was slightly less than 3 s. Values of N are averages of CFU counts of three identical experiments, using discs of equal initial contamination. Log R was calculated after averaging the values of R from identical experiments; the '≥' signs have been ignored; thus the Log R values may be minimum values.

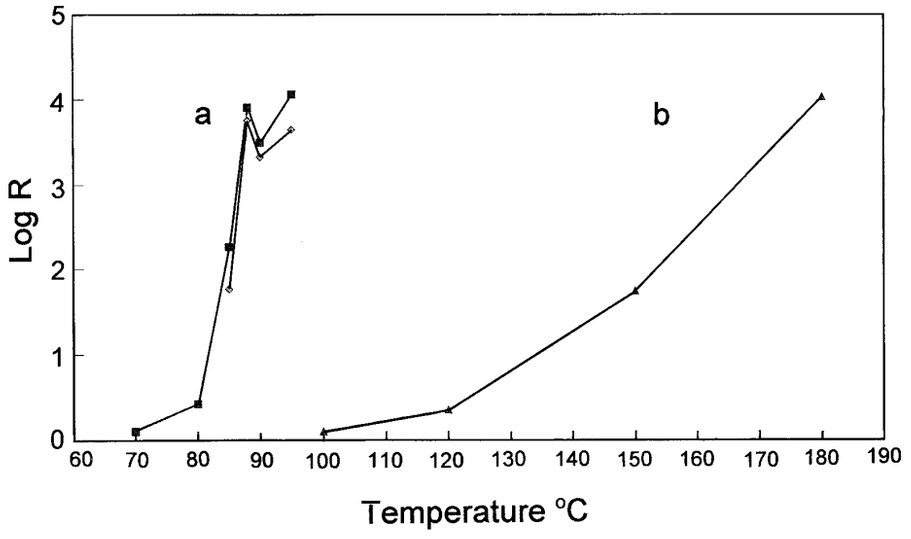


Figure 3 The reduction in number of viable spores of *P. roqueforti* as a function of the temperature at high (a) and low (b) relative humidity. –■– t = 1 s; –◆– t = 0 s; –▲– t = 1 s, RH <40%.

subjected to the decontamination treatment. For that purpose discs *b* and *c* were glued on a larger portion of packing material sheet using double adhesive tape. The discs were positioned on the sheet in such a way that they would be either between or beside the heating plates. The place of the discs thus depended on the conditions to be tested. Disc *a* was used to determine the inoculum level (N_o), discs *b* and *c* were used to determine the number (N) of mould spores surviving the treatment. Each experiment was done in triplicate, with the same treatment conditions and the same inoculation level.

Enumeration of colony forming units

Discs *b* and *c* were removed from the sheet with sterile tweezers, immediately after the treatment. Spores were removed from the surface of discs *a* and *b* by submerging the discs in 50 ml peptone salt solution with 0.1% Tween 80 added and shaking them manually for about 30 s, followed by an ultrasonic treatment for 5 min. As this treatment did not remove all spores from the surface, the discs were placed in Petri dishes and covered with malt extract agar. Of the suspension obtained by shaking and ultrasound a tenfold dilution series was made in peptone salt solution. One millilitre of the undiluted suspension, and 1 ml of the dilutions 10^{-1} to 10^{-3}, were mixed into diluted malt extract agar in pour plates. Disc *c* was placed in a Petri dish and covered with diluted malt extract agar.

Plates were incubated at 25°C for 4–5 days. If the number of surviving spores was high, N was determined by counting the plates of discs *b*. If the number of survivors was low, N was determined by counting the CFU on disc *c*. The values of the three experiments were averaged. During analysis of the number of microorganisms, the risk of contamination as a result of handling cannot be totally avoided. With higher numbers, the contribution of handling is insignificant. With very low numbers, the importance of such contamination becomes more apparent. Therefore, counts below ten are preceded by '≤' to indicate that the actual number of relevant microorganisms (ie survivors) may be the value indicated or somewhat lower. Consequently, reduction factors R calculated ($R = N_o/N$) are preceded by '≥' as the actual reduction obtained might have been higher. In the case of no survivors, the number is indicated as <1 and consequently the reduction factor is preceded by a '>' (larger than) sign.

Results

The results of all experiments are presented in Table 1. To obtain a useful reduction in the number of viable spores within 1 s at a low relative humidity, the temperature must be >150°C.

At high humidity, within the same time a reduction of >1000-fold is obtained at plate temperatures of approximately 90°C. The results, however, indicate that it is not so much the temperature of the plates, but rather the temperature in the chamber which is important, at least at 100% RH. The value of R proved to be >1000 when the plates were at 90°C and the temperature inside the machine was ≥73°C, independent of whether or not the samples had been pressed between the heating plates for 1 s.

The reductions obtained are presented graphically in Figure 3. Line (a) shows the results of treatments at 100% humidity. Line (b) shows the average of the results obtained at low humidity (<40%). Clearly, at high humidity the temperature can be significantly lower than at low humidity for the same degree of inactivation of spores of *P. roquefortii*. For a 1000-fold reduction the difference is approximately 90°C.

The high humidity treatments left a film of moisture on the surface of the packing material. Therefore, if moist-heat treatment would be applied in practice, depending on the product to be packed, a drying section may have to be added to the packing machines, situated between the treatment section and the filling unit.

Discussion

To investigate whether, at least for some purposes, the use of chemicals for the decontamination of packing material can be avoided, a prototype machine was built to investigate the inactivation of microorganisms on the surface of packing material, using moist heat for a short time (similar to the time needed for decontamination by peroxide). For spores of *P. roqueforti* a reduction factor of $R > 10^3$ can be obtained within 3 s at 90°C and 100% humidity, while at low humidities at the same temperature hardly any reduction in viable count is obtained.

All experiments were done with dry mould spores. As most vegetative cells are less resistant than mould spores, this method may prove to be suitable to inactivate vegetative cells on packing material. Nevertheless, before using moist heat to decontaminate packing material, the inactivation of other relevant microorganisms, such as other moulds, yeasts and relevant vegetative bacteria, should be proven experimentally.

At 100% relative humidity, water condenses on the packing material, which may be undesirable. Therefore, it would be interesting to know if the relative humidity can be decreased to a point where there is no condensate left on the packing material, but decontamination is still sufficient. Otherwise, it may be necessary to add a drying section to the packing machine.

Moist heat decontamination is a promising method which could help manufacturers pack food in a microbiologically safe manner, without the use of chemicals.

References

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