GERM LOAD ON PACKAGING PAPER AND BOARD: TRANSFER OF MICROORGANISMS

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STUDY EVALUATES SAFETY OF PAPER

THIS PAPER PROVIDES A SUMMARY OF TEST RESULTS addressing questions of whether a germ transfer takes place in direct contact between a packaging paper and a foodstuff. Also, are there relevant differences between packaging materials made of recycled fibers and packaging materials made of virgin pulp? Transfer tests were carried out with lasagne plates and various paper specimens. In those tests with a dry, non-greasy foodstuff neither a significant germ transfer nor differences between packaging materials containing recycled fibres and packaging materials containing virgin pulp were detected.

The same questions were addressed under comparable test conditions with a moist, greasy foodstuff. The test material was fresh pizza with a greasy topping, a product usually transported in paperaboard boxes. .

TEST STRUCTURE

One specimen was made of virgin pulp and several specimens were made of recycled fibers. One specimen was an original pizza paperboard box sampled at a commercial pizza restaurant. All paper specimens were characterised by the total germ count (DIN 54379), the surface germ count (DIN 54378, bacteria: Merck Plate Count Agar 5463), and the surface germ count (DIN 54378, moulds: Merck Sabouraud Agar 5438).

The virgin pulp specimen and the pizza paperboard box were used in every germ transfer test. Of the specimens containing recycled fibers, the sample with the highest surface germ count was selected for testing. The three specimens were distributed to three laboratories for testing.

TEST PROCEDURE

From each of the three specimens—virgin pulp, pizza paperboard box, and recycled fibers—10 pieces with an area of 50 cm^2 were cut with a sterile circular cutter. Then the 30 pieces were placed in sterile Petri dishes.

Both pizzas were taken out of the paperboard box and wrapped in sterile aluminum foil. One pizza was initially left in the original condition and the other was autoclaved at 120°C for 15 minutes. After the toppings were completely removed from both pizzas, 16 squares, each 5 cm^2 were cut from each pizza. These squares were placed with their bottoms facing the specimens in the prepared Petri dishes.

For each of the three specimens the total germ count was determined according to DIN 54379 and the surface germ count was determined according to DIN 54378, both for bacteria (Merck Plate Count Agar 5463) and moulds (Merck Sabouraud 5438).

After the toppings were removed, the total germ counts for the autoclaved pizza and the non-autoclaved pizza were determined according to DIN 54379 for bacteria and molds and the surface germ count by print-on-plate method (incubation at 30°C for 48 h).

For 30 minutes the Petri dishes were agitated at 50 revolutions/inclinations per minute on a shaker or a gel destainer to ensure sufficient contact between the foodstuff and the paper surface. Then the specimens were removed and placed on a sterile nutrient medium (Merck Plate Count Agar 5463). To ensure controlled contact, each specimen was pressed under a sterile 100-mL beaker containing 50 mL of sterile water for 30 seconds onto the nutrient medium and then discarded. The number of colony-forming units was determined according to DIN 54378. Each determination was made five times **(Table I)**.

Germ identification

If a transfer of germs took place in the transfer test and germs were detectable on the plate count plates, then an identification of possibly present pathogenic germs was made using the stamp procedure.

In this procedure a sterile stamp covered with velvet or cotton and of a slightly smaller diameter than the Petri dish is pressed on the agar surface on which the colonies have grown so that the textile fibers are loaded with the inoculum. When the stamp is then pressed on a non-inoculated agar plate, the entire colony pattern is transferred. One stamp print is transferred from the original plate consecutively to selective nutrient media 1-6 **(Table II)**.

The purpose of the transfer test was to determine the degree to which a transfer of germs from packaging material to foodstuff can take place and relate the outcome with the previously conducted initial germ count.

Foodstuff	Virgin Pulp	Recycled Fibers	Pizza paperboard box	Total
Pizza (original)	5x	5x	5x	15
Pizza (autoclaved)	5x	5x	5x	15

I. Summary of conducted tests: transfer tests

Germ / Germ Group	Selective Nutrient Media	Merck No.
1 *Salmonellae* + enterobacteria	XLD Agar,Merck 5287	
2 *Escherichia coli*	ENDO C Agar,	Merck 4044
3 *Pseudomonas aeroginosa*	Cetrimid Agar,	Merck 5284
4 *Streptococci*	Streptocci Selective Agar,	Merck 5468
5 *Staphylococci*	Baird Parker Agar,	Merck 5406
6 *Clostridia*	RCM Agar	Merck 5410

II. Selective nutrient media used to test for specific organisms

RESULTS

Preliminary Tests, Control Tests

When comparing the results obtained by the various participants in the tests, some differences were noted in the outcome of the total germ count/surface germ count of the starting material. **(Figures III and IV)** This was true especially for paper specimens 1 and 4. Differences by roughly 2 potencies of 10 cannot be avoided altogether with a test material as non-homogeneous as paper. Because microorganisms tend to agglomerate, concentrations at certain points may occur. Such non-homogeneity cannot be excluded in a natural product. These tests have shown that non-homogeneity does not effect the final results.

Transfer Tests

For germ identification: stamping of germ growth from plates after transfer on selective nutrient media (Laboratory W), no final evaluation of the test was possible. Initial results pointed to bacillaceae and some enterobacteria.

Paper specimen	Bacteria/g	Yeasts/g	Molds/g
1 pulp, fresh	16	0	0
2 recycled fiber	18	0	0
3 recycled fiber	4	2	0
4 recycled fiber	20	0	82

III. Determination of surface germ count

Paper specimen	Bacteria/g	Yeasts/g	Molds/g
1 pulp, fresh	1.6×10^{6}	2.8×10^{5}	$<10^{2}$
2 recycled fiber	2.7×10^{6}	7.6×10^{5}	$<10^{2}$
3 recycled fiber	6.4×10^{6}	7.5×10^{5}	$<10^{2}$
4 recycled fiber	8.0×10^{5}	1.0×10^{5}	1.1×10^{2}

IV. Determination of total germ count

Specimen	GERM COUNTS (bacteria/molds) Total CFU/g Lab B	Surface CFU/100cm² Lab B	Total CFU/g Lab T	Surface CFU/100cm² Lab T	Total CFU/g Lab W	Surface CFU/100cm² Lab W
Paper 1	1.6×10^{6}	16	1.6×10^{5}	96	2.7×10^{2}	25
Paper 4	8.0×10^{5}	18	6.7×10^{6}	24	1.1×10^{4}	growth on edge
Pizza paperboard	1.1×10^{5}	0	5.5×10^{4}	68	1.5×10^{4}	growth on edge
Pizza (original)	1.6×10^{3}	0	2.4×10^{4}	0	1.5×10^{2}	8
Pizza (autoclaved)	$<10^{2}$	0	$<10^{2}$	0	$<10^{2}$	0

V. Controls of total germ counts/surface germ counts: bacteria

Specimen	GERM COUNTS (bacteria/molds) Total CFU/g Lab B	Surface CFU/100cm² Lab B	Total CFU/g Lab T	Surface CFU/100cm² Lab T	Total CFU/g Lab W	Surface CFU/100cm² Lab W
Paper 1	2.8×10^{5}H $<10^{2}$S	16	$<10^{2}$	0	1×10^{3}H	19 $<10^{2}$S
Paper 4	1.0×10^{5}H 1.1×10^{3}S	18	$<10^{2}$	0	1×10^{3}H	137 6×10^{1}S
Pizza (paperboard)	<100	0	$<10^{2}$	0	1.8×10^{3}H	24 1×10^{2}S
Pizza (original)	<100	0	$<10^{2}$	0	$<10^{2}$H $<10^{2}$S	0
Pizza (autoclaved)	<100	0	$<10^{2}$	0	2×10^{1}H $<10^{2}$S	0

VI. Controls of total germ counts/surface germ counts: yeasts (Hefen [Y]) and molds (Schimmelpilze [M])

Transfer From/to	Determination x 5 (CFU) Lab B					Lab T					Lab W				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Paper 1 / Pizza (original)	0	2	0	0	0	0	0	1	0	0	0	0	1	0	2
Paper 1 / Pizza (autoclaved)	0	1	0	1	1	2	0	1	0	1	0	2	4	1	3
Paper 4 / Pizza (original)	2	1	0	0	0	3	2	0	2	1	4	0	3	0	0
Paper 4 / Pizza (autoclaved)	0	3	1	3	1	3	2	0	0	1	0	1	0	0	0
Pizza (paperboard) / Pizza (original)	0	0	1	1	1	0	1	0	0	0	1	0	12	0	3
Pizza (paperboard) / Pizza (autoclaved)	0	3	1	0	0	1	0	1	0	0	0	0	0	0	0

VII. Transfer test: Surface germ count on pizza (bacteria)

Transfer From/to	Determination x 5 (CFU) Lab B					Lab T					Lab W				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Paper 1 / Pizza (original)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Paper 1 / Pizza (autoclaved)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Paper 4 / Pizza (original)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Paper 4 / Pizza (autoclaved)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pizza (paperboard) / Pizza (original)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Pizza (paperboard) / Pizza (autoclaved)	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0

VIII. Transfer test: Surface germ count on pizza (yeasts and molds)

SUMMARY OF RESULTS

The following summarize the results of the test (See Tables V-X).

No significant differences in the germ load could be detected for paper boards produced on the base of virgin pulp or recycled fibers.

The maximum transfer of germs from paper to the moist, greasy foodstuff was in the worst case (15 tests each, mean value) 1.33 germs from a paper specimen sized 100 cm^2 to a pizza square sized 25 cm^2. This quantity is negligible when compared with the inherent germ load of foodstuffs.

No significant differences were noted in the germ transfer behavior from paper made of virgin pulp, recycled fiber, and the pizza paperboard to the foodstuff. This evaluation demonstrated that the use of recycled fibers as raw material does not increase the risk potential when compared with virgin pulp as raw material.

In the identification of all transferred germs, no pathogenic microorganisms were detected. This was applicable for paperboard made of virgin pulp, in paperboard made of recycled fibers, and in the original pizza paperboard box.

This evaluation demonstrated that no significant germ transfer from paper to foodstuff takes place with either dry or moist and greasy food. **TJ**

Transfer From / to	RCM	Cetrimid	LABORATORY B Baird-Parker	Strepto-cocci	Endo C	XLD Salmonellae	XLD Entero-bacteria
Paper 1/Pizza (original)	0	0	1*	0	0	0	0
Paper 1/Pizza (autoclaved)	0	0	0	0	0	0	0
Paper 4/Pizza (original)	0	0	0	0	0	0	0
Paper 4/Pizza (autoclaved)	0	0	3*	0	0	0	0
Pizza (paperboard)/ Pizza (original)	0	0	1*	0	0	0	0
Pizza (paperboard)/ Pizza (autoclaved)	0	0	0	0	0	0	0

* Coagulase negative, micrococci

In each case one of the colonies grown after the transfer tests "virgin pulp/pizza original" and "virgin pulp/pizza autoclaved" were streptomyceae. All other colonies not grown on selective nutrient media were gram positive, oxidase negative rods, at an advanced stage with sporulation (in all likelihood bacillaceae).

IX. Germ identification: Stamping of germ growth from plates after transfer on selective nutrient media

Transfer From / to	RCM	Cetrimid	LABORATORY T Baird-Parker	Strepto-cocci	Endo C	XLD Salmonellae	XLD Entero-bacteria
Paper 1/Pizza (original)	0	0	1*	1	0	0	0
Paper 1/Pizza (autoclaved)	2	1	2*	0	2**	0	2***
Paper 4/Pizza (original)	2	0	0	0	1**	0	0
Paper 4/Pizza (autoclaved)	3	0	1*	1	2**	0	0
Pizza (paperboard)/ Pizza (original)	0	0	1*	0	0	0	0
Pizza (paperboard)/ Pizza (autoclaved)	1	0	0	0	0	0	0

* opaque-beige small colony
** reddish colony
*** agar yellow, yellow slimy colony

X. Germ identification: Stamping of germ growth from plates after transfer on selective nutrient media.

This work is an ad hoc paper of the TEGEWA technical committee, Microbial Products, Karlstrasse 21, 60329, Frankfurt/Main, Germany; Ute Höütmann, lead author.