

# **PAPER**

# Detection of improper sealing and quality deterioration of modified-atmosphere-packed pizza by a colour indicator

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The effects of leaking on the quality of modified-atmosphere-packed chilled minced meat pizza were studied. Capillary-like leaks of various sizes were made experimentally in the sealing area with tungsten threads of diameters 50 and 100 µm. Test packages were stored for 5 weeks at 5°C (in darkness or under illumination) or at 10°C in darkness and the microbial and sensory deterioration of pizzas packed in leaking packages was compared to that occurring in intact packages. The diameter of the capillary leakage was found to influence markedly the rate of deterioration of pizzas stored under all conditions. The higher storage temperature accelerated the deterioration of the quality of pizzas in all packages, but illumination increased the rate of deterioration especially in leaking packages. The colour indicator tested reacted to different leak sizes as anticipated and was shown to be reliable in leak detection. © 1997 Elsevier Science Ltd.

Keywords: ready-to-eat pizza; modified-atmosphere packaging; leakage indicator

# INTRODUCTION

Various cooked meat and ready-to-eat products, such as refrigerated minced meat steaks and pizzas, have an important role in the Finnish food market. Although the worldwide market share of refrigerated pre-packed pizza is only 5% of the total pizza market (Brody, 1993), it is a very popular way of purchasing pizza in Finland. The shelf-life of ready-to-eat foods depends greatly on the process hygiene, packaging technique and storage conditions. For example, the shelf-life of refrigerated pizza packed in air is about 1-3 weeks, but can be extended to 4-5 weeks by packing in a modified atmosphere consisting of 20-60% carbon dioxide (CO<sub>2</sub>) balanced with nitrogen (N<sub>2</sub>) (Ahvenainen *et al.*, 1990). The longer shelf-life achieved by gas packaging is of course possible only if

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the package is hermetically heat-sealed and remains intact all the way from production to the consumer.

In contrast to vacuum packaging, it is difficult for consumers to visually detect leaking of modifiedatmosphere packages. Non-destructive testing of modified-atmosphere packages is one way to assure their integrity. A number of colour indicators have been developed in order to indicate tampering with or damage to a package or decreased functionality of oxygen absorbers (Krumhar and Karel, 1992). Factors used to cause such colour changes have included moisture, loss of headspace gas and the presence of atmospheric oxygen. Of these factors, only oxygen appears to be relatively constant as it is present at about 21% in the atmosphere. Patents for colour indicators based on redox dyes have, for example, been published by Yoshikawa et al. (1979), Perlman and Linschitz (1985) and Krumhar and Karel (1992) and at VTT by Mattila-Sandholm et al. (1995). Eilamo et al. (1995) tested the function of one of these for the detection of leakages in modified-atmosphere packages of minced meat steaks, and reported it to be useful and reliable in monitoring for leaking packages.

The present study was carried out in order to determine the sensitivity of chilled modified-atmosphere packed minced meat pizza to oxygen. Pizzas were stored in modified-atmosphere packages with microholes of various sizes in order to determine the maximum acceptable leakage of the package and to test the reliability of the patented colour indicator as a leak detector.

# MATERIALS AND METHODS

### **Packaging treatments**

Commercially produced minced meat pizzas, each weighing 200 g, were used as a test food. The mean nutritional content of the pizzas was: protein 98 g/kg, fat 98 g/kg, ash 20 g/kg, carbohydrates 275 g/kg, energy 1005 kJ/100 g, salt 11 g/kg and moisture 510 g/kg.

The pizzas were packed in nylon-polyethylene pouches, frozen  $(-30^{\circ}\text{C})$  immediately after preparing and chilling in the factory and were transported frozen to VTT. The next day some of the samples were thawed and retail-packed in a Europack HA-01 vacuum chamber packaging machine. The packaging film used was a nylon-polyethylene laminate with a thickness of 90 µm and oxygen permeability of 48 cm<sup>3</sup>/(m<sup>2</sup> d) at 101.3 kPa, 50-75% RH and 25°C. A gas mixture of 70% N<sub>2</sub>+30% CO<sub>2</sub> was used and the residual oxygen (O2) concentration in the headspace of the gas packages was between 0.4 and 0.7%. One-half of the pizza was packed per package. The volume of the package was 255 cm<sup>3</sup> and the headspace volume was 134 cm<sup>3</sup>/100 g corresponding to 53% of the total volume of the package.

# Leakages

During sealing leaks of various sizes were prepared in the seal area using tungsten threads with diameters of 50 and 100 μm. Leaks were made by placing one, three or five threads in the seal area before sealing and drawing them carefully out from the cooled seal after sealing. The leaks were like capillaries, with a length the same as the width of the seal, i.e. 3000 µm. The true diameters of the leaks were measured at Packforsk in Sweden with a microhole-tester (Axelson et al., 1989). Nominal leak sizes and the true diameters of the leaks are presented in *Table 1*.

# Storage conditions and sampling

The sample packages were stored under the following conditions: (1) 5°C in darkness, (2) 5°C under illumination (1016±81 lux) and (3) 10°C in darkness. After specified intervals (seven sampling points in all during 5 weeks of the storage test), five replicate

Table 1 The leak sizes, the diameter of tungsten thread used in the preparation of leaks, the number of threads used, and the diameters of the leaks measured with a microhole tester

Leak size (real diameter µm)	Thread diameter (µm)	No. of threads used	Sum of the measured used diameters <sup>h</sup> (µm)
Intact	0	0	8+25
55	50	1	$55\pm 49$
101	100	1	$101 \pm 14$
176	100	3	176±25
248	100	5	$248 \pm 29$

<sup>\*</sup>The microhole tester counts all separate leakages as one

sample units from each leakage size (Table 1) and from each storage condition were tested for gas composition and the colour of the indicator was measured. Two sample units were then assayed for microbial content and three units for sensory quality.

# **Detection of leakage**

The colour indicator tested for leak detection was based on the recipe and formulation presented in the Finnish Patent no. 94802 (Mattila-Sandholm et al., 1995). When the indicator is in a reduced state its colour is white, whereas in its oxygenated state it is

An indicator was attached to an inside corner of each package with a piece of tape immediately before sealing. The changes in the darkness of the indicator colour were measured by a Minolta Chroma Meter CR-200 through the package film before opening the packages. The darkness indices were recorded as the L-values of the analyser. The L-value was 70-80 when the colour was white, about 65 when it was obviously blue and about 55 when the colour was deep blue. At the packaging stage all the indicators were in reduced form and white in colour. Each indicator was measured twice and the L-indices presented in the results are the means of five samples (i.e. ten measurements in all).

#### Gas analysis

On each analysis day, a septum was glued onto the surface of the packages in order to draw gas samples from the headspace with a gas-tight syringe. The oxygen concentration in the headspace of each package was measured using a portable Servomex® oxygen analyser (Model 570A) and the carbon dioxide concentration with a portable Servomex® CO<sub>2</sub> analyser (Model PA404).

# Sensory evaluation

Sensory evaluation was carried out in two parts. The appearance of unopened packages was evaluated first

The means ± standard deviation of measurements from 47 packages.

by three to five selected trained judges. Thereafter, the packages were opened and the odour of pizza in the package was evaluated. The quality of the gas-packed samples was compared with that of a sample which had been stored at  $-30^{\circ}$ C packed in nvlon-polyethylene pouches in normal air (control sample) and thawed at 5°C overnight. The pizzas were then placed on stainless steel trays, on which their appearance, odour and overall quality were evaluated. After the evaluation of cold pizza, the pizzas were heated at 150°C for 10 min in an electric oven and their appearance, odour, taste, consistency and overall quality were evaluated by nine to ten selected trained judges using scores from 0-5 for each property, with descriptions characterizing product defects. The frozen-stored control sample was also included in this second part of the evaluation as a hidden sample. A score of 2 or 1.75 points on an individual score sheet meant that the evaluator considered the commercial quality of the sample to be noticeably decreased. A score of 1.5 points or less meant that the evaluator considered the sample to be unfit for human consumption.

# Microbiological methods

Two duplicate samples were taken from each sample and cut up in Petri dishes. Cut samples (10 g) were mixed with 90 ml autoclaved peptone-saline diluent (Maximal Recovery Diluent by Lab M, Amersham) in a Stomacher-pouch. Further serial dilutions were made in tubes before plating on agar. The media and incubation conditions were: for aerobic plate count Bacto Plate Count agar (Difco), 30°C, 3 days; for yeasts and moulds YGC agar (Difco), 25°C, 5 days; for lactic acid bacteria MRS agar (Oxoid), 25°C, 5 days, 5% CO<sub>2</sub>. Three dilutions were chosen from each sample for cultivation and two duplicate plates were cultivated from each dilution. The number of colonies was counted from plates with between 30 and 300 colonies.

# **Texture measurement**

In addition to the sensory evaluation, the texture of the pizzas during storage was measured by Instron TM-MA 0636 equipment (Instron Ltd, GB). Hardness (maximum power) and elasticity were determined.

# Statistical analysis

Arithmetic means and standard deviations of scores given by each judge for sensory quality were calculated. Analysis of variance using Fisher's least significant difference procedure was carried out to discriminate the means between the samples from every sensory evaluation (Statgraphics Plus 2.1, Manugistic).

# RESULTS

#### Oxygen and carbon dioxide concentrations

The O<sub>2</sub> concentration increased in all the pizza packages tested at the beginning of storage, regardless of the leakage rate (Table 2). The reason for the slight increase of O<sub>2</sub> concentration in the intact packages was obviously the release of O<sub>2</sub> from the pores of the pizza base. However, the increase in intact packages stopped after 1 day, whereas in leaking packages the

Table 2 The effect of leakage and storage time on the oxygen concentration (ml/l) of the headspace of pizza packages under three different storage conditions: (a) 5°C, dark, (b) 5°C, illumination and (c) 10°C, dark

(a)	Sto	rage tir	ne (d)/(	O <sub>2</sub> con	centrat	ion (m	ıl/l)
Leak size (diameter)	1	6	12	16	21	28	35
Intact							
$\bar{x}$	30 16	14 6	7 6	8 3	4 1	11 6	4
s <sub>x</sub> 55 μm	10	U	O	3	1	U	1
$\tilde{x}$	17	64	53	7	5	7	4
S <sub>x</sub>	12	32	36	1	0	3	0
$\frac{101 \ \mu m}{\bar{x}}$	60	43	36	25	6	9	6
$S_{\mathbf{x}}$	17	17	24	11	1	5	3
176 μm <i>x</i> ̄	31	43	66	55	38	20	7
$S_x$	6	3	17	3	20	6	Ó
248 μm	12	70	05	72	40	20	10
$ar{x}$ $S_{x}$	43 8	79 10	95 19	73 13	49 23	20 12	12 7
(b)	Sto	rage ti	me (d)/	O <sub>2</sub> con	centra	tion (n	1l/l)
Leak size (diameter)	1	7	11	15	19	26	34
Intact						_	
$ar{ar{x}}$	8	6 2	3 0	4 1	8	5 1	27 8
s <sub>x</sub> 55 μm	3	2	U	1	3	1	0
$\dot{\bar{x}}$	11	14	4	16	5	7	24
s <sub>x</sub> 101 μm	5	7	1	12	1	2	2
$\tilde{X}$	15	18	13	20	12	11	34
S <sub>x</sub>	8	6	5	4	6	2	4
176 μm <i>x</i> ̄	32	39	47	53	56	34	42
S <sub>x</sub>	15	11	14	18	10	14	10
248 μm	70	02	102	70	70	00	<b>5</b> 1
$ar{X}$ $S_{\mathbf{x}}$	70 18	92 8	102 9	79 12	78 19	88 21	51 21
(c)	Sto	rage ti	me (d)/	O <sub>2</sub> con	centra	tion (n	nl/l) 
Leak size (diameter)	1	6	11	15	20	27	34
Intact							
$\bar{x}$	3	3 1	6 0	6	7 1	6 2	6
s <sub>x</sub> 55 μm	U	1	U	1	1	2	1
X.	3	3	6	5	5	4	5
S <sub>x</sub>	0	0	0	1	1	0	2
101 μm - x̄	5	23	11	9	5	5	_
$S_{\mathbf{x}}$	_	2	21	1	5 3	5 2	2
176 μm - <del>x</del>	9	28	26	11	8	9	
x S <sub>x</sub>	5	16	10	9	3	7	_
248 μm				10		~	
$ar{x}$ $s_x$	20 14	66 27	38 8	10 3	9 4	7 2	_
	- '						

O<sub>2</sub> concentration increased further, in accordance with the size of the leakage. After 11-12 days of storage, the O<sub>2</sub> concentration started to decrease in the most heavily leaking packages. At a storage temperature of 10°C, the O2 concentration began to decrease after only 1 week.

The CO<sub>2</sub> concentration decreased at the beginning of the storage in all test packages regardless of the leakage rate (Table 3). The decrease in intact packages was caused mainly by the absorption of CO<sub>2</sub> into food and, therefore, slowed clearly after 1 day. The decrease in CO<sub>2</sub> concentration in leaking packages was directly dependent on the leakage rate, and in the most heavily leaking pizza packages (leakages with a total sum of hole diameters of 176 and 248 μm), the CO<sub>2</sub> concentration decreased to almost 0% (Table 3). However, during the latter part of storage, the CO<sub>2</sub> concentration started to increase even in the most heavily leaking packages. In some cases, e.g. pizza packages with microholes of 248 µm total diameter, the concentration reached almost the same level as in intact packages, being clearly higher than in the packages with a total microhole diameter of 55 µm. This increase in CO<sub>2</sub> concentration was caused by microbial growth.

# Microbiological quality

The yeast and mould count of pizzas was directly dependent on the leakage rate of packages and on the storage temperature (Figure 1) whereas the aerobic bacterial count was dependent on the storage temperature but not on the leakage rate (data not shown). The amounts and growth rates of lactobacilli were equal to those of aerobic bacteria. At the storage temperature of 10°C, pizzas reached the highest aerobic plate count,  $10^7$  cfu/g, in the most heavily leaking packages (total sum of measured hole diameters 176 and 248 µm) after 2 weeks of storage and in other packages (measured diameter of holes 8 (= intact), 55 and 101  $\mu$ m) after 3 weeks. At a storage temperature of 5°C, the aerobic plate count reached this level after only 5 weeks of storage, regardless of the leakage rate. Initial aerobic bacterial counts were 10<sup>2</sup>-10<sup>3</sup> cfu/g. At a storage temperature of 5°C, there was a clear difference between the yeast and mould counts of pizzas with different packaging treatments (leakage rates) after 11-12 days of storage. At 10°C, the leakage already affected the yeast and mould counts of pizzas during the first 6 days. The growth of yeast and moulds increased with increasing leakage. In the most heavily leaking packages (total measured microhole diameters of 176 and 248 µm), visible mould growth could be detected after 21 days when the packages were stored at 5°C without illumination, and after 26 days of storage at 5°C under illumination. At 10°C, moulds were detected after only 11 days. At this temperature, the pizzas in packages with a microhole of only 101 μm true diameter also had visible mould growth after 11 days. The lag-phase of microbial growth was about 2 weeks at 5°C, but at 10°C microbial growth started immediately.

#### Sensory quality

The shelf-life of the sensory quality of pizzas was 10-16 days shorter at 10°C than at 5°C regardless of the leakage rate (Tables 4-7). Furthermore, at 10°C the sensory quality of pizzas in leaking packages already differed quite clearly from that of pizzas in

Table 3 The effect of leakage and storage time on the carbon dioxide concentration (ml/l) of the headspace of pizza packages under three different storage conditions: (a) 5°C, dark, (b) 5°C, illumination and (c) 10°C, dark

(a)	Stor	age tin	ne (d)/	CO <sub>2</sub> co	ncentr	ation (	ml/l)
Leak size diameter	1	6	12	16	21	28	34
Intact							
x	200	170	190	140	150	120	120
S <sub>x</sub>	30	20	20	20	20	20	10
55 μm - x	230	50	40	30	70	90	10
S <sub>x</sub>	20	40	20	20	40	30	10
101 μm							
<del>x</del>	150	40	20	0	0	0	0
s <sub>x</sub> 176 μm	20	10	10	0	0	0	0
Σ	190	90	40	10	10	10	50
S <sub>x</sub>	10	10	10	10	10	10	10
248 μm	100	40	10		40		
χ.	180 20	40	10 0	0	10	50	90
S <sub>x</sub>		10	U	0	0	10	10
<b>(b)</b>	Stor	age tin	ne (d)/	CO <sub>2</sub> co	ncentr	ation (	ml/l)
Leak size (diameter)	1	7	11	15	19	26	34
Intact							
x	220	230	180	180	160	130	90
<i>S</i> <sub>x</sub>	40	0	40	10	30	30	30
55 μm x̄	200	120	140	130	110	80	100
S <sub>x</sub>	60	30	20	10	30	40	20
101 μm			_				
$ ilde{x}$	200	100	80	60	50	20	30
S <sub>x</sub>	20	60	50	20	50	20	20
176 μm <i>x</i> ̄	190	60	30	20	10	0	30
S <sub>x</sub>	20	10	10	10	0	10	10
248 μm							
â.	150	30	10	10	0	0	70
	20	10	10	0	0	0	2
(c)	Stor	age tin	1c (d)/	CO <sub>2</sub> co	ncentr	ation (	ml/l)
Leak size (diameter)	1	6	11	15	20	27	34
Intact							
ž.	240	160	150	170	120	150	120
S <sub>x</sub>	20	30	10	10	20	50	20
55 μm	220	150	140	120	90	70	60
X S <sub>x</sub>	10	10	20	30	10	10	10
101 μm							
x	230	120	100	80	80	90	
s <sub>x</sub> 176 μm	10	10	10	40	20	0	_
170 μm - X	200	100	50	50	100	150	
<i>S</i> <sub>x</sub>	30	40	30	30	50	50	_
248 μm	150	20	10	70	120	160	
X .	170 30	30 20	10 10	70 20	120 30	160 40	_
	50	20	10	20			

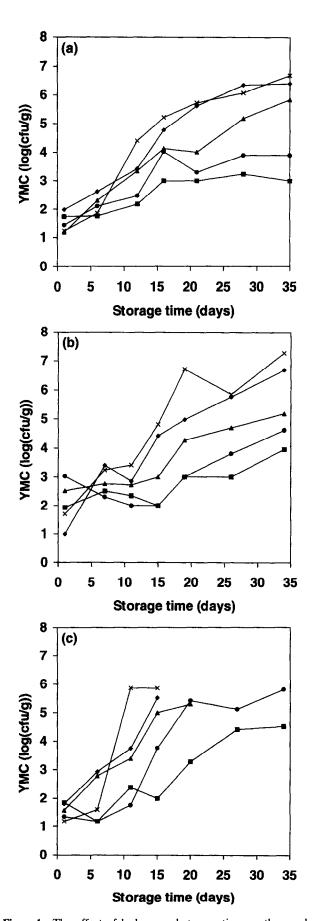


Figure 1 The effect of leakage and storage time on the number of yeast and moulds (YMC) in pizza under three different storage conditions: (a) 5°C, dark, (b) 5°C, illumination and (c) 10°C, dark. Leak sizes are presented as total measured diameters ( $\mu$ m) of the microholes and coded as follows: -m- intact, -•- 55, - $\Delta$ - 101, -•- 176 and -×- 248

Table 4 The effect of leakage and storage time on the sensory quality of pizzas stored at 5°C in darkness: (a) appearance of pizza in unopened packages, (b) odour of cold pizza after transfer to stainless steel trays and (c) flavour of heated pizza\*

(a)	Storage time (d)/score						
Leak size (diameter)	1	6	12	16	21	28	35
Intact	5.0°°	4.9"	4.6°	4.2°	3.6 <sup>d</sup>	2.8d	2.7
55 μm	$5.0^{\rm ns}$	4.9°	4.5"	3.9ª	$3.2^{\circ}$	$2.6^{a}$	2.5°
101 μm	$5.0^{\circ}$	4.9"	4.6"	4.1"	3.1°	2.0	0.8
176 µm	5.0 <sup>···</sup>	4.9"	4.6"	4.1"	2.86	1.0⁵	0.2
248 μm	$5.0^{\rm ns}$	4.9	4.6°	4.1"	$1.0^{a}$	$0.3^{\circ}$	$0.1^{\circ}$
Control	5.0°s	5.0°	5.0 <sup>6</sup>	5.0°	5.0°	5.0	5.0°
(b)	Storage time (d)/score						
Leak size (diameter)	1	6	12	16	21	28	35
Intact	4.7"	4.3	3.8ª	3.7 <sup>d</sup>	3.6°	2.6°	2.4
55 μm	$4.8^{ab}$	4.3ª	3.8°	3.4 <sup>ed</sup>	2.8 <sup>b</sup>	2.4°	1.8
101 μm	$4.9^{ab}$	4.3°	3.7ª	3.2th	2.7 <sup>b</sup>	1.4 <sup>r</sup>	1.2
176 µm	4.8ab	4.2°	3.7°	$2.8^{ab}$	2°	$0.9^{a}$	$0.2^{\circ}$
248 μm	4.7°	4.1ª	3.4°	$2.6^{a}$	1.6	$0.6^{a}$	$0.0^{\circ}$
Control	5.0 <sup>b</sup>	4.9°	5.0 <sup>b</sup>	5.0°	5.0 <sup>d</sup>	5.0⁰	5.0
(c)		S	torage	time (d	i)/score	;	
Leak size (diameter)	1	6	12	16	21	28	35
Intact	4.4"	3.6"	3.6ª	3.3h	3.0ab	3.2"	2.9
55 μm	4.6 <sup>h</sup>	3.9ab	3.7ab	3.21x	2.7	3.1ª	_
101 μm	4.5ab	4.0™	3.5ª	3.5°	2.8 <sup>a</sup>		_
176 μm	4.5ab	3.8ab	$3.3^{a}$	2.9°			_
248 μm	4.5ah	4.0bc	$3.3^{a}$	$2.1^{a}$			
Control	4.6 <sup>b</sup>	4.2°	4.1 <sup>b</sup>	4.34	3.7⁵	4.1 <sup>b</sup>	3.7

<sup>\*</sup>The same letter in each column after the mean indicates no significant difference between the samples (P < 0.05).

intact packages after 6 days and very clearly after 11 days regardless of the leakage rate. When pizzas were stored at 5°C without illumination, the sensory quality of pizzas in leaking packages differed from that of the pizzas in intact packages after 16 days. When they were stored at 5°C under illumination, a significant difference between pizzas in intact and leaking packages was already evident after 7 days. The reasons for the unacceptability of pizzas stored under illumination were mainly defects in odour, whereas without illumination the main reason was defects in appearance (mould growth). At a storage temperature of 5°C, pizzas in the most heavily leaking packages were judged unfit for retailing after 12 days when stored under illumination and after 18 days when stored without illumination (Table 5). However, when pizzas were packed in intact packages, illumination had no effect on the sensory quality retention. At a storage temperature of 10°C, pizzas in the most leaking packages were unfit for retailing after 8 days.

#### Texture

Pizzas became harder and lost their elasticity immediately at the beginning of the storage, regardless of the storage or packaging conditions (data not shown). There was no clear difference in the textures of pizzas with different packaging (intact and leaking packages). However, pizzas became harder at a slightly slower rate at 10°C than at 5°C. This was as

Table 5 The effect of leakage and storage time on the sensory quality of pizzas stored at 5°C under illumination: (a) appearance of pizza in unopened packages, (b) odour of cold pizza after transfer to stainless steel trays and (c) flavour of heated pizza\*

(a)	Storage time (d)/score						
Leak size (diameter)	1	7	11	15	19	26	34
Intact	4.9ª	4.6ª	4.3 <sup>b</sup>	4.1 <sup>h</sup>	3.7°	2.8°	2.8
55 μm	5.0 <sup>h</sup>	4.6a	$4.2^{a}$	3.9 <sup>h</sup>	3.5 <sup>b</sup>	2.6°	2.4
101 μm	5.0 <sup>b</sup>	4.6ª	4.2"	3.4ª	$3.4^{ab}$	2.3 <sup>h</sup>	1.6°
176 μm	5.0	4.6°	4.1°	3.4°	3.3°	1.1"	0.4
248 μm	5.0ab	4.6°	4.1ª	3.4ª	3.3°	1.1ª	0.1
Control sample	5.0 <sup>b</sup>	5.0 <sup>b</sup>	5.0°	5.0°	5.0 <sup>a</sup>	5.0 <sup>d</sup>	4.9 <sup>r</sup>
(b)	Storage time (d)/score						
Leak size (diameter)	1	7	11	15	19	26	34
Intact	4.6ª	3.8 <sup>h</sup>	3.6°	3.5ª	3.34	2.8d	2.6
55 μm	4.6a	3.6 <sup>b</sup>	3.2 <sup>n</sup>	$3.0^{\circ}$	2.8°	2.1°	$1.6^{\circ}$
101 μm	4.6°	3.4 <sup>b</sup>	$3.0^{h}$	2.5	1.9 <sup>b</sup>	1.4 <sup>h</sup>	1.2 <sup>h</sup>
176 μm	4.5a	3.3 <sup>b</sup>	2.6ª	1.7ª	1.4ª	0.9°	0.4
248 μm	4.4*	2.8ª	$2.3^{a}$	1. <b>7</b> °	1.3ª	0.9"	$0.1^{a}$
Control sample	5.0 <sup>b</sup>	5.0°	5.0 <sup>d</sup>	5.0°	5.0°	5.0°	4.9°
(c)		S	torage	time (c	i)/score	;	
Leak size (diameter)	1	7	11	15	19	26	34
Intact	4.1ab	4.0de	3.5°	3.2hc	2.8 <sup>b</sup>	2.8ª	2.7ª
55 μm	4.1ab	3.8 <sup>ed</sup>	3.5°	2.8 <sup>h</sup>	2.4ªh	2.4ª	
101 μm	$4.0^{ab}$	3.5hc	3.2be	1.9 <sup>a</sup>	1.5"		_
176 μm	4.2ab	3.2ab	2.7ab	_	_		_
248 μm	4.0ª	2.9*	2.4ª		_		_
Control sample	4.2 <sup>b</sup>	4.2°	3.8c	3.6°	3.4 <sup>h</sup>	3.9⁵	3.9⁵

<sup>\*</sup>The same letter in each column after the mean indicates no significant difference between the samples (P < 0.05).

anticipated, because the retrogradation of starch is inversely proportional to storage temperature.

#### Function of the leakage indicator

The indicator detected the O<sub>2</sub> entering leaking packages very well under all three storage conditions. In intact packages, the indicator remained white but in leaking packages it changed to blue (Figure 2). When the total sum of measured diameters of microholes in the seal area was at least 101 µm, the indicator changed from white to blue much more quickly than the sensory quality deteriorated. In the packages with a measured total microhole diameter of 55 μm, the colour change was much slower (except at 5°C in the dark) and it followed closely the deterioration of pizzas at 5°C under illumination. However, at 10°C, the colour change in packages with a total microhole diameter of 55 µm was slightly too slow when compared to the spoilage rate of pizzas. This means that the amount of oxygen-absorbing compound ( = oxygen absorbing capacity) in the indicator should have been somewhat lower. On the other hand, even though the indicator tested at 5°C without illumination had the same recipe as the indicators tested under other storage conditions, it was more sensitive to oxygen. At 5°C without illumination, the colour of the indicator changed slightly even in intact packages during the first day. However, it turned back to white after 5 days.

Table 6 The effect of leakage and storage time on the sensory quality of pizzas stored at 10°C in complete darkness: (a) appearance of pizza in unopened packages, (b) odour of cold pizza after transfer to stainless steel trays and (c) flavour of heated pizza

(a)	Storage time (d)/score						
Leak size (diameter)	1	6	11	15	20	27	34
Intact	4.9ah	4.1ª	3.3°	3.0°	2.3 <sup>d</sup>	2.2°	2.04
55 μm	4.9a	4.1ª	$3.2^{c}$	$2.8^{\circ}$	1.8°	1.8⁵	$0.7^{\circ}$
101 μm	4.9°	4.1ª	3.1°	1.3 <sup>b</sup>	0.3⁵	$0.3^{a}$	0.3
176 μm	4.9 <sup>nb</sup>	4.1"	1.9 <sup>b</sup>	0.3"	0.0	0.0	$0.0^{\circ}$
248 μm	4.9ab	4.1ª	1.3ª	$0.0^{\circ}$	$0.0^{\circ}$	$0.0^{\circ}$	$0.0^{\circ}$
Control sample	5.0 <sup>h</sup>	5.0 <sup>6</sup>	5.0 <sup>d</sup>	5.0 <sup>d</sup>	5.0°	5.0 <sup>a</sup>	4.9
(b)	Storage time (d)/score						
Leak size (diameter)	1	6	11	15	20	27	34
Intact	4.9ah	4.1°	3.2°	2,7 <sup>d</sup>	2.0 <sup>d</sup>	1.9°	1.8°
55 μm	4.9ab	4.0°c	2.8™	2.1°	1.4°	1.3 <sup>h</sup>	1.0 <sup>h</sup>
101 μm	4.8ª	3.9⁵	2.4 <sup>h</sup>	1.2 <sup>h</sup>	$0.3^{h}$	$0.3^{a}$	0.2
176 μm	4.9ab	3.6ª	1.7°	$0.1^{a}$	$0.0^{\circ}$	$0.0^{\rm a}$	$0.0^{\circ}$
248 μm	4.8ª	3.6*	1.5°	$0.0^{\rm a}$	$0.0^{\rm a}$	$0.0^{\circ}$	$0.0^{\circ}$
Control sample	4.9 <sup>b</sup>	5.0⁴	$5.0^{\rm d}$	4.9°	5.0℃	5.0 <sup>d</sup>	4.9 <sup>d</sup>
(c)		S	torage	time (	d)/scor	e	
Leak size (diameter)	1	6	11	15	20	27	34
Intact	4.4ns	4.0ns	3.3ns	2.9ns	2.7ns	3.1 <sup>ns</sup>	2.5ª
55 μm	4.5 <sup>ns</sup>	3.8ns	3.7 <sup>ns</sup>	3.2ns			_
101 μm	4.5 <sup>ns</sup>	4.1 <sup>ns</sup>	3.5ns	_		_	_
176 µm	4.5 <sup>ns</sup>	3.8ns	_	_	-	_	_
248 μm	4.4 <sup>ns</sup>	4.0 <sup>ns</sup>	_	_			
Control sample	4.4ns	4.1 <sup>ns</sup>	3.8 <sup>ns</sup>	3.5 <sup>ns</sup>	4.2ns	3.8 <sup>ns</sup>	3.9h

<sup>\*</sup>The same letter in each column after the mean indicates no significant difference between the samples (P < 0.05).

# DISCUSSION

Leakage increased the growth of yeasts and moulds more than the growth of aerobic bacteria. The amounts and growth rates of lactobacilli were equal to those of aerobic bacteria. Pizzas were very sensitive to O<sub>2</sub>, but were less sensitive to the effect of storage temperature than minced meat steaks (Eilamo et al., 1995). Minced meat pizzas lost their quality more quickly in leaking packages (even with a total microhole diameter of only 55 µm) than intact packages. When the microholes were bigger than 55 μm in diameter, the pizzas deteriorated very quickly. The main reasons for poor sensory quality were heavy yeast and mould growth, rancid odour

Table 7 The effect of package leakage and storage condition on the commercial shelf-life of packed minced meat pizzas. Commercial shelf-life was determined on the basis of odour scores given for cold pizza samples\*

Leak size (real diameter μm)	Storage conditions/shelf-life (d)							
	5°C, dark	5°C, illumination	10°C, dark					
Intact	35	34	20					
55	28	26	15					
101	21	15	11					
176	21	11	6					
248	16	11	6					

<sup>\*</sup>Scores below 2 were regarded as unacceptable for retailing.

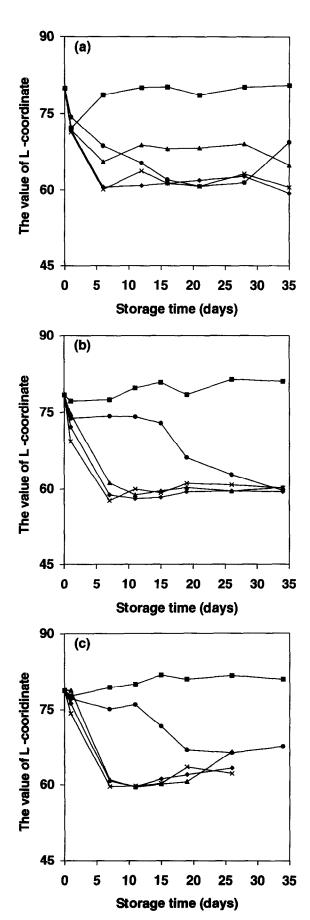


Figure 2 The effect of leakage and storage time on the colour of the indicator (measured as the L-value) in pizza packages stored under three different conditions: (a) 5°C, dark, (b) 5°C, illumination and (c) 10°C, dark. Leak sizes are presented as measured total diameters ( $\mu$ m) of the microholes and coded as follows: -mintact, -•- 55, - $\Delta$ - 101, •- 176 and - ×-248

and taste and also greying under illumination. Bleaching of tomato colour to a more orange colour was not observed. Minced meat steaks even tolerated a leakage corresponding to a leak in the seam area with a diameter of 169 µm (Eilamo et al., 1995). The leak size had some influence on the quality of minced meat steaks stored at 5°C, but no effect was noticed when the steaks were stored at 10°C. For the quality of pizza, the leak size also had an effect when stored at 10°C. It seems that if a leakage limit were to be set for pizzas, a leakage rate higher than that corresponding to a microhole diameter of 55 µm and situated in a seal area of 3 mm in width could not be allowed. This information is also useful for a manufacturer planning to take into use a non-destructive on-line leak tester to automatically test the integrity of packages.

Pizzas are also sensitive to illumination, when packed in leaking packages. In this study, the pizzas were packed in clear materials without printing. In commercial packages the material is printed and, at least in Finland, only a very small area of the pizza can be seen through the package. This is useful for the retention of quality, particularly if a package is leaking. On the other hand, the spoilage of pizza cannot be detected without opening the package. Therefore, a colour indicator for package leakages in pizza packages could help to detect defective packages in shops before the product is spoiled and, the indicator would also serve to improve the reliability of the pizza manufacturer in the eyes of the customer.

Deterioration in the quality of pizza was not indicated by the appearance of unopened packages in all cases. Spoilage was noticed only when an intact package had expanded or when there was mould growth on the pizza stored in a leaking package. The colour changes in pizzas stored in leaking packages under illumination were not noticeable enough to judge them unacceptable, but their odour had to be evaluated before final judgement. The odour of pizza in the freshly opened package did not differ noticeably from that of pizza on a stainless steel tray. The sour odour often associated with gas packed products, caused by CO<sub>2</sub>, was not noticed with gas packed pizza, although it was noticeable in the case of minced meat steaks (Eilamo et al., 1995). This difference may be caused by the fact that the initial pH of pizza is rather low, around 4.5, whereas the pH of minced meat steaks is more neutral, around 6.5. Furthermore, the sour odour of tomato sauce is an essential part of the aroma of pizza.

The colour indicator tested in this study (Mattila-Sandholm et al., 1995) generally detected the O<sub>2</sub> entering packages through leaks very well and the colour change was dependent on the leakage rate. Thus, in the least leaking packages in which the quality deterioration was slower, the colour change matched quality deterioration rather well. This means that the colour indicator was not too sensitive to

leakage. Rather, it seemed that the amount of O<sub>2</sub>-absorbing compound included in the indicator was somewhat too high. However, because the presence of an O<sub>2</sub> absorbent increases the shelf-life of this type of food, as has been observed, for example, by Hurme et al. (1995), no risk is involved if the amount is rather too high. More important is that the indicator should change its colour at the same rate as the spoilage occurs.

The colour of the indicator in intact packages did not change during the storage periods studied, but remained white. The colour change in leaking packages was stable and, therefore, the indicator seems to be reliable. Although the O<sub>2</sub> concentration decreased almost to zero in leaking packages due to microbial growth at the end of storage, the colour stayed dark blue. It must be noted that because microbes consume O<sub>2</sub>, measurement of the O<sub>2</sub> concentration in the headspace of the package cannot be used for the detection of leakage.

Another interesting result concerning the functioning of the indicator was that even though the indicator was placed at the furthest point from the leakage (in the bottom corner of pizza pouches), it detected the presence of O<sub>2</sub> very quickly. It should be noted that in pouch-like pizza packages the headspace (free gas volume) is mainly situated in the corners of the pouch (this can also be seen in commercial packages). The material of pouches is very close to the pizza surface. This means that when O<sub>2</sub> entered the package through the microhole in the seal area, it had to travel by a rather difficult route to the indicator. Thus, the results show that the indicator developed is reliable even if it is not situated near the leakage point.

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