

## Microbial contaminants cause swelling and inward collapse of yoghurt packs

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**Summary** — A study was carried out on 10 retail samples of yoghurt, belonging to 3 different brands (A, B and C), showing a rarely described alteration consisting of inward collapse of the packs. Microbiological analyses revealed the presence of high counts of different microbial contaminants: in A samples *Acetobacter aceti* was found at  $10^7$  cfu/g, while in B and C samples the main contaminants were *Mucor hiemalis*, *Mucor racemosus* and *Penicillium verrucosum* var *cyclopium* varying between  $10^2$  and  $10^5$  cfu/g. The responsibility of isolated strains for alteration was confirmed by inoculating cellular suspensions in non-contaminated yoghurt packs kept at 5°C and 25°C: samples inoculated with *A. aceti* showed an inward collapse after 20 d at 25°C; packs inoculated with *M. hiemalis*, *M. racemosus* and *P. verrucosum* var *cyclopium* kept at 25°C showed swelling after 5-7 d followed by inward collapse after  $\approx$  20-35 d. In samples kept at 5°C the defect did not appear even extending the incubation time to 60 d. The explanation for the evolution of this defect is the following one: contaminating microorganisms consume oxygen contained in head-space of pack and produce CO<sub>2</sub> whose re-absorption into the product causes a partial vacuum involving collapse of the pack.

**yoghurt contamination / yoghurt defect / *Acetobacter* / *Mucor* / *Penicillium***

**Résumé** — Contaminants microbiens entraînant le gonflement et le pliage des emballages de yaourts. Dix échantillons de yaourts, appartenant à 3 marques différentes (A, B et C), ont présenté un type d'accident rarement décrit consistant en un gonflement ou un pliage des emballages. Les analyses de ces yaourts ont mis en évidence une concentration élevée de divers contaminants microbiens: dans les échantillons A on a trouvé environ  $10^7$  ufc/g d'*Acetobacter aceti* tandis que les échantillons B et C recélaient entre  $10^2$  et  $10^5$  propagules/g de *Mucor hiemalis*, *Mucor racemosus* et *Penicillium verrucosum* var *cyclopium*. La responsabilité des souches isolées dans l'apparition des déformations des emballages a été confirmée par inoculation de suspensions cellulaires dans des pots de yaourts et incubation à 5°C et 25°C. Les échantillons contaminés avec *A. aceti* ont présenté un pliage des emballages après 20 j à 25°C. Ceux inoculés avec *M. hiemalis*, *M. racemosus* et *P. verrucosum* var *cyclopium*, stockés à 25°C ont d'abord gonflé en 5 à 7 j, puis se sont pliés en 20 à 35 j. De telles déformations ne sont pas apparues dans les échantillons maintenus à 5°C, même au cours d'une incubation prolongée de 60 j. L'évolution des déformations constatée pourrait être due

*au métabolisme des micro-organismes: ils consomment l'oxygène présent dans la partie supérieure des pots et produisent du CO<sub>2</sub> dont la réabsorption provoque un vide partiel ayant pour conséquence un pliage des emballages.*

**contamination yaourt / défaut du yaourt / Acetobacter / Mucor / Penicillium**

## INTRODUCTION

Yoghurt defects due to microbial contamination are widely reported in literature; the most frequent contaminants are yeasts and moulds (Suriyarachchi and Fleet, 1981; Garcia and Fernandez, 1984; Fleet and Mian, 1987; Spolaor *et al*, 1988; Foschino and Ottogalli, 1988) usually causing the swelling of packs, the presence of superficial coloured spots, abnormal tastes (Rasic and Kurmann, 1978; Ottogalli, 1991).

In the present paper we describe a kind of rarely observed alteration (Sutherland *et al*, 1986) consisting of an inward collapse of yoghurt packs.

The study was focused on the identification of the microorganisms responsible for the spoilage and on the reproduction of the phenomenon in laboratory conditions.

## MATERIALS AND METHODS

### Samples

Analyses were performed on 10 retail samples of yoghurt in 125-g packs (natural and flavoured, whole and skim products), sold in Italy, belonging to 3 different brands (A, B and C) utilising different packaging systems (table I).

All the samples examined showed inward collapse within the expiry date; in some samples of brand B the swelling of the packs had preceded the inward collapse.

Non-contaminated samples of plain and flavoured yoghurt free from any microbial contamination were utilized in laboratory tests for controlled inoculations.

### Microbiological analysis on retail samples

The following microbial groups were investigated using the FIL/IDF standard methods: lactic acid bacteria (FIL/IDF, 1983); bacterial contaminants (FIL/IDF, 1971); yeasts and moulds (FIL/IDF, 1985).

### Identification of microorganisms

The microbial strains were isolated in specific media and were subsequently identified according to the classical methods referred in literature for bacteria (De Ley *et al*, 1986), yeasts (Barnett *et al*, 1983) and moulds (Smith, 1969; Samson *et al*, 1976).

### Reproduction of alteration

The alterative phenomenon was reproduced inoculating cellular suspensions of pure strains at concentration of  $\approx 10^7$  colony forming units per gram (cfu/g) in non-contaminated controlled samples of plain and flavoured yoghurt packaged in 125 g polystyrene cups. The packs were then incubated at 5°C and at 25°C for a maximum period of 60 d.

Apart from the visual observation of the defect, the following determinations were performed: bacterial contaminants (FIL/IDF, 1971); yeasts and moulds (FIL/IDF, 1985); D-lactic acid, L-lactic acid, acetic acid and ethanol contents, by spectrophotometric methods utilising enzymatic kits (Boehringer, 1984); percent composition of head-space atmosphere by gas chromatographic method (Gas chromatograph Perkin-Elmer Mod 8500; thermoconductivity detector HWD; column CTR1, 2 m, Alltech Italia).

## RESULTS

### Microbiological determinations on retail samples and identification of contaminant microorganisms

The alteration in all samples consisted of inward collapse observed as a bending of the packs and lids.

The analyses showed the presence of high microbial counts of different types of contaminants in samples from brands A, B and C; in brand A samples the bacterial counts reached  $10^7$  cfu/g, while in brand B samples the main contaminants were moulds ( $10^2$ – $10^5$  cfu/g) with a lesser content of bacteria; in brand C sample yeasts and moulds reached  $10^6$  and  $10^5$  cfu/g, respectively (table I).

The typical lactic microflora of yoghurt was not influenced by this kind of contaminants.

The bacterial strains isolated from yoghurts of brand A were classified as *Acetobacter aceti*.

In samples from brand B, moulds were ascribed to species *Mucor hiemalis* Wehmer, *Mucor racemosus* Fres and *Penicillium verrucosum* Dierckx var *cyclopium* (Westling) Samson *et al*, and bacteria to genus *Bacillus*.

In samples from brand C, moulds were ascribed to species *M hiemalis* and *M racemosus* and yeasts to species *Debaromyces hansenii* (Zopf) Lodder and Kregger-van Rij.

### Reproduction of alteration

Samples inoculated with *A aceti* showed an inward collapse of the packs after 20 d at 25°C, with organoleptic modifications such as browning, sour taste and 'vinegar odour': this was due to high production of acetic acid, while D-lactic acid and L-lactic acid did not show important variations (table II); the samples kept at 5°C did not show any alternative process for 60 d.

In samples inoculated with *M hiemalis*, *M racemosus* and *P verrucosum* var *cyclo-*

**Table I.** Microbiological analyses of retail samples of yoghurt packs showing inward collapse within the expiry date. Standard deviations are indicated. *Analyses microbiologiques des échantillons de yaourt du marché qui montraient le pliage des emballages. Les écarts types sont indiqués.*

Brand	No samples	Pack	Flavour	Type	Lactic flora (Str + Lact) cfu/g	Contaminants cfu/g		
						bacteria	moulds	yeasts
A	4	PS°	bilberry	whole	$5.4 \times 10^8 \pm 1.5$	$2.5 \times 10^7 \pm 2.0$	$< 10^2$	$< 10^2$
B	5	EPS§	coconut	whole	$2.1 \times 10^8 \pm 0.8$	$3.0 \times 10^2 \pm 3.9$	$4.7 \times 10^4 \pm 5.0$	$< 10^2$
C	1	PS°	plain	skim	$3.0 \times 10^6$	$< 10^2$	$6 \times 10^5$	$2 \times 10^6$

° Polystyrene cup; § Expanded polystyrene box.

° Pot en polystyrène; § boîte en polystyrène expansé.

**Table II.** Behaviour of yoghurt samples, inoculated with isolated strains, kept at different temperatures. Standard deviations or variation range are indicated.

*Comportement des échantillons de yaourt, contaminés avec les souches isolées, maintenus à différentes températures. Les écarts types ou les variations des valeurs sont indiqués.*

Inoculated strains	Temp (°C)	Time (days)	Aspect of the pack	Microbial contaminants cfu/g	Lactic acid g/l	Acetic acid g/l	Ethanol g/l	Head-space % composition		
								CO <sub>2</sub>	O <sub>2</sub>	N <sub>2</sub>
<i>Acetobacter aceti</i>	25	0	normal	$1.0 \times 10^5$	8.2–9.6	0.1–0.7		nd	nd	nd
		20	ic	$2.2 \times 10^8 \pm 1.7$	6.7–9.3	20.7–40.4		nd	nd	nd
<i>Mucor hiemalis</i>	25	0	normal	$2.5 \times 10^5 \pm 2.1$	10.7–14.3		< 0.1	1.2	18.5	80.3
		5–7	swollen	$3.5 \times 10^5 \pm 3.5$	12.0–15.0		4.0–5.0	75.4	0.4	24.2
		19–20	ic	$2.5 \times 10^4 \pm 0.7$	10.2–15.0		8.1–23.3	19.6	11.2	69.2
	5	0	normal	$2.0 \times 10^5$	12.0		< 0.1	1.5	18.0	80.5
19		swollen	$5.0 \times 10^3$	12.0		3.0	62.4	0.5	37.1	
<i>Mucor racemosus</i>	25	0	normal	$9.5 \times 10^4 \pm 0.7$	10.7–14.8		< 0.1	1.2	18.7	80.1
		5–7	swollen	$1.0 \times 10^4$	9.7–18.8		2.5–3.5	74.4	0.3	25.3
		20–33	ic	$2.5 \times 10^3 \pm 2.1$	10.2–16.5		7.2–20.0	31.3	4.7	64.0
	5	0	normal	$1.0 \times 10^4$	12.0		< 0.1	1.5	18.0	80.5
		26	swollen	$5.0 \times 10^3$	13.5		2.5	59.8	1.2	39.0
<i>Penicillium verrucosum cyclopium</i>	25	0	normal	$4.0 \times 10^5$	13.0–13.8		< 0.1	1.2	18.8	80.0
		7	swollen	$1.0 \times 10^4$	12.3–12.9		< 0.1	74.5	0.5	25.0
		35	ic	$1.0 \times 10^3$	11.2–12.3		< 0.1	28.5	5.7	65.8

ic: inward collapsed

*pium* kept at 25°C swelling of the packs occurred within 5–7 d followed by inward collapse (fig 1) after ≈ 20–35 d; in samples kept at 5°C the swelling occurred after ≈ 20–30 d (table II) only in packs contaminated with *M hiemalis* and *M racemosus*, but the inward collapse did not appear even when the incubation time was extended to 60 d.

Samples contaminated with *Bacillus* spp and *D hansenii* did not show any modification of packs kept at both temperatures.

During the swelling phase, samples contaminated with moulds showed significant changes of head-space composition which began with a strong increase of CO<sub>2</sub>, followed by a CO<sub>2</sub> drop during the inward collapse phase.

In samples inoculated with strains of moulds belonging to *M hiemalis* and *M racemosus* production of ethanol was observed.

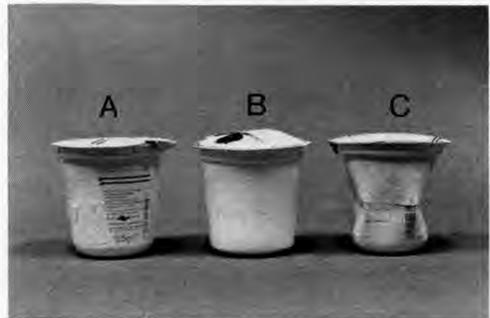
## DISCUSSION

Inward collapse of packs is a rare type of alteration which has practically not been reported in the literature; in this paper we have described some different cases of this defect due to microbial contaminants.

The microorganisms responsible for this alteration can be either bacteria belonging to *Acetobacter*, or moulds belonging to *Mucor* or *Penicillium*; *Bacillus* and *Debaryomyces*, which were also found in spoiled yoghurts, cannot be considered responsible since they were unable to reproduce the phenomenon in our laboratory tests.

The development of such defect can be attributed to:

- microbial contamination of the product by strains able to grow at low pH and provided with an oxidising respiratory metabolism;



**Fig 1.** Non-contaminated pack (A); swollen pack (B); and pack showing inward collapse (C). *Emballage sans défaut (A), emballage gonflé (B) et emballage plié (C).*

- consumption of head-space oxygen by the above-mentioned microorganisms with CO<sub>2</sub> production;

- re-absorption of CO<sub>2</sub> into the product with a subsequent partial vacuum causing collapse of the pack.

Strict observance of good manufacturing practices and correct conditions of refrigeration constitute the best means of avoiding or slowing down this kind of alterations.

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