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## Mycoflora of milk after several types of pasteurization

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### Summary

A study of the mycoflora in 143 samples of milk, following a serie of laboratory pasteurization treatments (63 °C/30 min, 72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec) and industrial pasteurization was made. The most frequently isolated molds belonged to the *Penicillium*, *Cladosporium* and *Aspergillus* genera and to the Mycelia sterilia group. The greatest number of molds was isolated from milk pasteurized at low temperature and for long periods of time (63 °C/30 min), with an appreciable drop following high-temperature short-time (H.T.S.T.) treatments. Yeasts were isolated only from milk pasteurized at 63 °C/30 min, and then in very low percentages.

*Key words* : Milk - Mycoflora - Pasteurization - Fungi.

### Résumé

#### *Mycoflore du lait après divers traitements de pasteurisation*

Une étude de l'effet produit par divers traitements de pasteurisation sur la mycoflore de 143 échantillons de lait a été réalisée. 103 des échantillons, prélevés du 26 octobre 1981 au 10 janvier 1983, furent soumis à un traitement de pasteurisation à basse température (63 °C/30 mn), tandis que les 40 échantillons restants prélevés du 15 avril au 6 juillet 1983, furent soumis à un traitement de pasteurisation industrielle et à une pasteurisation à haute température (72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec).

Les milieux de culture employés ont été la gélose à l'extrait de malt et la gélose glucosée selon Sabouraud, additionnées de 5 ppm d'oxytétracycline.

Les moisissures les plus fréquemment isolées appartiennent aux genres *Penicillium*, *Cladosporium* et *Aspergillus*, ainsi que le groupe Mycelia sterilia.

Le plus grand nombre d'isollements fut obtenu à partir des échantillons pasteurisés à basse température (63 °C/30 mn), ce nombre diminuant considérablement après les traitements à haute température (H.T.S.T.).

Les levures furent isolées seulement à partir de lait pasteurisé à 63 °C/30 mn et présentèrent un faible pourcentage d'apparition (5,8 %) sur la gélose glucosée et 4,8 % sur l'extrait de malt.

*Mots clés* : Lait - Mycoflore - Pasteurisation - Moisissures.

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## I. Introduction

The majority of researchers point out that yeasts and molds do not constitute a techno-sanitary problem in milk, affirming that the heat resistance of these microorganisms is of the same order as that of non-sporulated bacteria, so that they are totally destroyed by standard pasteurization treatments (ALAIS, 1981 ; COOKE et BRAZIS, 1968 ; DEMETER et ELBERTZHAGEN, 1971 ; NELSON, 1981 ; VEISSEYRE, 1980). Due to this circumstance, studies on fungal heat resistance and, in particular, on possible surviving mycoflora in milk following pasteurization treatment have been scarce. However, other researchers (EECKHOUTTE, 1979 ; JARVIS, 1972 ; SPENCER, 1967 ; WILLIAMS *et al.*, 1941) suggest that some molds demonstrate particular resistance to heat, which has given rise to a conflicting view as to the possible existence of fungi in milk following pasteurization.

This controversy, together with the high level of fungal contamination of fluid milk in the central areas of Spain (VADILLO *et al.*, 1986) and the absence of studies of the mycoflora in pasteurized milk in this country justify our interest in this work on heat resistance of fungi present in fluid milk following different pasteurization processes.

## II. Materials and methods

### A. Sampling

The study of pasteurized milk mycoflora was carried out on a total of 143 samples, proceeding from the bulk tank of a milking cooperative of Madrid (Spain). Of these, 103 were examined between October 26th 1981 and January 10th 1983 after having been subjected to a low pasteurization process (63°/30 min). A study was undertaken with the remaining 40 samples, taken from April 15th until July 6th 1983, to determine the surviving mycoflora following laboratory pasteurization of the milk by using varying high heat treatments (72 °C/15 sec, 76 °C/15 sec, 80°C/15 sec, and industrial treatment).

### B. Culture

The media used were 3 % malt extract agar and Sabouraud's glucosed agar. Oxytetracycline (5 ppm) was added to both in order to inhibit the bacterial flora in milk. Plating was done by spreading 1 ml of milk over the surface of each culture medium, in duplicate.

Incubation was done at 22 °C for 8-10 days.

### C. Isolation

Once the strains had grown in these media, isolation was carried out in 3 % malt extract agar.

### D. Identification

Identification of each of the genera found was mainly carried out according to the guidelines recommended by VON ARX, 1981, although some other standard books on mycology were also consulted for the determination of some genera and species. LODDER's criteria, 1974, were followed for the identification of yeasts.

### E. Pasteurization

#### 1. Laboratory pasteurization

— Low temperature pasteurization.

The method used was based on the technique described by EGDELL *et al.*, 1950, for the study of thermophilic microorganisms in milk.

Prior to pasteurization at low temperature, the sample was homogenized, after which 10 ml of milk were placed in a sterile test tube which was submerged in a water bath, with agitation, at  $62.8 \pm 0.5$  °C for 30 minutes. Following the heating time, the milk was immediately cooled in chilled water.

— Pasteurization at high temperature and short time (H.T.S.T.).

The capillary heat treatment described by STERN and PROCTOR modified by PALACIOS ASENJO, 1984, was followed.

For this procedure, the capillary tubes, once sterilized with dry-heat, were filled with a microsyringe (Angla), fitted with a micrometric device and fine needle 7 cm length, in order to deposit aliquots of 20 ml of milk in each. Once the capillaries were filled, both ends were closed and they were placed in a water bath with agitation at the required temperatures and times (72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec). Once this heat treatment was completed, the capillaries were immediately cooled in a container with chilled water alongside the bath. Then the capillaries were broken at both ends and the milk was extracted with the aid of a syringe containing exactly 10 ml of 1/4 Ringer solution.

#### 2. Industrial pasteurization

The method used in the Milking Cooperative was H.T.S.T. pasteurization, in a Stork plate-type pasteurizer with a working capacity of 12,000 l/h.

Once the milk had been pasteurized and the fat homogenized, it was cooled to refrigeration temperature (3 °C) and transferred to a holding tank which kept it at this temperature. It was later packed under strictly hygienic conditions.

The range of pasteurization temperature varied by less than 1 °C, the limit indicated by the current Spanish legislation (78 °C/15 sec) (DECRETO 2478/1976).

### III. Results

Figure 1 shows the frequency of appearance of the genera isolated from pasteurized milk (63 °C/30 mn) in Sabouraud's glucosed agar and malt extract agar media.

The most commonly isolated genera in the former were : *Penicillium*, *Cladosporium* and *Aspergillus*. All had a frequency of occurrence of over 10 %. *Alternaria*, *Geotrichum*, *Phoma*, *Scopulariopsis*, *Trichosporon*, *Epicoccum*,

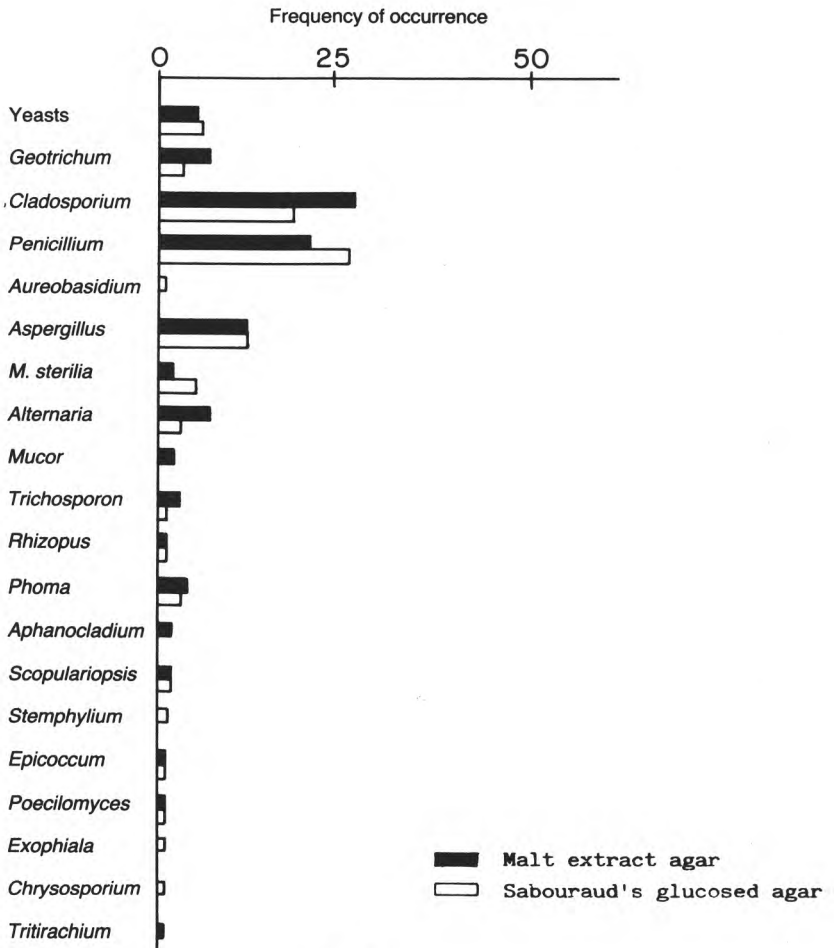


Fig. 1

Frequency of occurrence of the genera isolated from pasteurized milk in Sabouraud's glucosed agar and malt extract agar.

Fréquence d'apparition des genres isolés à partir de lait pasteurisé sur la gélose glucosée selon Sabouraud et l'extrait de malt.



The seasonal variations for the genera isolated from low temperature pasteurized milk (63 °C/30 min) in Sabouraud's glucosed agar and malt extract agar during 1982 are given in table 1.

In Sabouraud's glucosed agar, only *Penicillium* and *Cladosporium* appeared in the four seasons, although the second occurred in very low percentages in summer (5.8 %) and in fall (9.0 %).

The *Aspergillus* genus and the molds included in the Mycelia sterilia group did not appear in winter and, in the other seasons, the rates of occurrence were very low, with the exception of the *Aspergillus* genus which, in summer, was detected in 35.2 % of the samples. Surprisingly, this percentage is also the highest obtained in pasteurized milk.

In malt extract agar, the mold genera which were most frequently recovered during the four seasons were *Penicillium*, *Cladosporium*, *Geotrichum*, *Aspergillus* and *Phoma*. The last two were isolated in percentages well below those for others.

The *Alternaria* genus, which was detected in summer in very low percentages, nevertheless appeared in fall in 22.7 % of the samples.

In table 2 details of the frequency of occurrence of the genera isolated from milks pasteurized at 72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec, and industrially pasteurized milk, in Sabouraud's glucosed agar and malt extract agar are given.

TABLE II

Frequency of occurrence of the genera isolated from pasteurized milk (72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec and IPM) in Sabouraud's glucosed agar (S.B.) and malt extract agar (M.E.A.)

Fréquence d'apparition des genres isolés à partir de lait pasteurisé (72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec et IPM) sur la gélose glucosée selon Sabouraud (S.B.) et la gélose à l'extrait de malt (M.E.A.)

PASTEURIZATION GENERA	72°C./15" %		76°C./15" %		80°C./15" %		IPM %	
	S.B.	M.E.A.	S.B.	M.E.A.	S.B.	M.E.A.	S.B.	M.E.A.
<i>Penicillium</i>	10,0	2,5	7,5	2,5	20,0	12,5	5,0	12,5
<i>Cladosporium</i>		5,0	7,5	5,0		5,0	5,0	7,5
<i>Aspergillus</i>	2,5		2,5			2,5		2,5
<i>M. sterilia</i>				2,5	2,5	2,5		
<i>Botrytis</i>	2,5							
<i>Harziella</i>							2,5	
<i>Alternaria</i>					2,5			
<i>Scopulariopsis</i>							2,5	
<i>Phoma</i>							2,5	

Abbreviation : IMP = Industrially Pasteurized Milk.



Following pasteurization at 72 °C/15 sec, only the *Penicillium*, *Aspergillus* and *Botrytis* genera were isolated in Sabouraud's glucosed agar. However, in malt extract agar, *Aspergillus* and *Botrytis* were not detected. *Cladosporium* genus was recovered in malt extract agar but not in Sabouraud's glucosed agar.

In milk pasteurized at 76 °C/15 sec, *Penicillium* and *Cladosporium* were isolated from both media. In addition, the *Aspergillus* genus was isolated from Sabouraud's glucosed agar and the Mycelia sterilia group from malt extract agar.

Following heating at 80 °C/15 sec, the following mold genera were isolated from malt extract agar : *Penicillium*, *Cladosporium*, *Aspergillus* and the Mycelia sterilia group. However, in Sabouraud's glucosed agar *Cladosporium* and *Aspergillus* were not detected, but the *Alternaria* genus which was not recovered in malt extract agar, was isolated.

With Sabouraud's glucosed agar, the *Penicillium*, *Cladosporium*, *Harziella*, *Scopulariopsis* and *Phoma* genera were detected in industrially pasteurized milk. From malt extract agar, *Harziella*, *Scopulariopsis* and *Phoma* were not isolated, but the *Aspergillus* genus was found, not having been observed in the Sabouraud's glucosed agar culture medium.

The species identified from each of the genera isolated from these types of milk are shown in table 3.

Within the *Penicillium* genus, eight different species were identified from milk pasteurized at 80 °C/15 sec, five in industrially pasteurized milk, four in milk pasteurized at 76 °C/15 sec and three in milk pasteurized at 72 °C/15 sec : The species *P. sartoryi* Thom and *P. yarmokense* Baghdadi were extracted from the four types of milk. *P. commune* Thom was not isolated, only from milk pasteurized at 72 °C/15 sec, and *P. chrysogenum* Thom was not isolated from milk pasteurized above 76 °C/15 sec.

In the case of *Cladosporium* genus, *Cl. macrocarpum* Preuss was found in the four types of milk, *Cl. herbarum* Link ex Fr. was isolated only from milk pasteurized at 76 °C/15 sec and from industrially pasteurized milk.

As to the *Aspergillus* genus, *A. amstelodami* Margin Thom & Church occurred in milk which had been pasteurized at 80 °C/15 sec and in industrially pasteurized milk, *A. unilateralis* Thrower in milk pasteurized at 76 °C/15 sec and *A. niger* van Tieghem in milk pasteurized at 72 °C/15 sec.

The *Botrytis cinerea* Pers. ex Nocca & Balbis species was isolated only from milk which had been pasteurized at 72 °C/15 sec, the species *Harziella capitata* Cost. & Matr. and *Phoma herbarum* Westend from industrially pasteurized milk, and *Alternaria tenuisima* (Fries) Wiltshire from milk pasteurized at 80 °C/15 sec.



TABLE III

*Species isolated from pasteurized milk (72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec and IPM)*  
*Espèces isolées à partir de lait pasteurisé (72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec et IPM)*

PASTEURIZATION GENERA	72 °C. /15 "	76 °C. /15 "	80 °C. /15 "	IPM
<i>Penicillium</i>	<i>P. chrysogenum</i> <i>P. sartoryi</i> <i>P. yarmokense</i>	<i>P. commune</i> <i>P. cyclopium</i> <i>P. sartoryi</i> <i>P. yarmokense</i>	<i>P. chrysogenum</i> <i>P. commune</i> <i>P. cyclopium</i> <i>P. decumbens</i> <i>P. lanosum</i> <i>P. sartoryi</i> <i>P. trzbinskianum</i> <i>P. yarmokense</i>	<i>P. chrysogenum</i> <i>P. commune</i> <i>P. decumbens</i> <i>P. sartoryi</i> <i>P. yarmokense</i>
<i>Cladosporium</i>	<i>C. macrocarpum</i>	<i>C. macrocarpum</i> <i>C. herbarum</i>	<i>C. macrocarpum</i>	<i>C. macrocarpum</i> <i>C. herbarum</i>
<i>Aspergillus</i>	<i>A. niger</i>	<i>A. unilateralis</i>	<i>A. amstelodami</i>	<i>A. amstelodami</i>
<i>Botrytis</i>	<i>B. cinerea</i>			
<i>Harziella</i>				<i>H. capitata</i>
<i>Alternaria</i>			<i>A. tenuisima</i>	
<i>Phoma</i>				<i>Ph. herbarum</i>

#### IV. Discussion

In our study, the most common genus in all the pasteurized milk types (63 °C/30 min, 72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec, and industrially pasteurized milk) was *Penicillium*. This result may, to some extent, corroborate the statements of JARVIS, 1972, EECKHOUTTE, 1979, and SPENCER, 1967, as well as the experimental results in respect of heat resistance obtained by WILLIAMS *et al.*, 1941.

The *Aspergillus* genus was isolated from pasteurized milk at 63 °C/30 min in high numbers, which may confirm the findings of JARVIS, 1972, and EECKHOUTTE, 1979. In milk pasteurized at 72 °C/15 sec, 76 °C/15 sec, 80 °C/15 sec and industrially pasteurised milk, the genus was also found, although in smaller percentages, probably as a result of the greater sensitivity to these temperatures and pasteurization times.

Other fungal genera were also found in pasteurized milk, however, a discussion on them is difficult due to the lack of any information on their heat resistance.

Fungi isolated from pasteurized milk are of the greatest interest given the harmful effect which they may have on the organism of human beings and animals consuming this type of food. On the other hand, in view of the short shelf-life of pasteurized milk (72 hours) and to the temperature at which it is kept (4-6 °C), it is difficult that this microorganism may give rise to important alterations in milk. However, in dairy products of long shelf-life produced with pasteurized milk fungi could cause notorious alterations either in the manufacture process or in the finished product.

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