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Substitution Effect of the Mesophilic Lactic Starter by the Thermophilic Ones on The Contamination of Standard Soft Cheese Camembert by *Mucor sp*

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Abstract: Cheeses of the Camembert type were ripened at 12°C while varying the mesophilic lactic starter by the thermophilic ones and the relative humidity (85 and 95%) during ripening. The effect of these variations on the evolution of the *Mucor sp* flora, pH and dry extract were studied. The ripened cheeses at 12°C/95 RH% were contaminated definitely by *Mucor* and the contents of dry extract were much weaker than those at 12°C/85RH%. Highly significant effects ($p<0.01$) of the substitution of the starter and relative humidity were observed on the evolution of parameters; pH, dry extract and flora of *Mucor*. A negative but significant correlation ($p<0.05$) between the evolution of the dry extract and the *Mucor* for the samples ripened to 12°C/85 RH% were also noted.

Key words: Camembert cheese · *Mucor sp* · Mesophilic lactic starter · Thermophilic lactic starter · Ripening

INTRODUCTION

During the manufacturing process, the cheese undergoes a great number of handling during which it is prone to various contaminations but generally it is the accident says "hair of cat" caused by various species of *Mucor* (*Mucor hiemalis, Mucor plumbeus, Mucor racemosus*), the principal agent of deterioration in the Algerian cheese dairies, or the qualitative and quantitative losses generating important economic damages for the units [1]. This situation led us to develop a strategy of fight against the contamination by *Mucor sp* based only on the control of the technological process. In this context, it is useful to mention that a harmonious development of the flora of ripening and the good unfolding of this one mainly depend on the former stages more particularly coagulation, draining and the salting and whose interdependence is very narrow so that they are directly responsible for the parameters evolution or ecological extrinsic factors like pH, activity of water (a_w) and the rate of sodium chloride [2]. Also, the quality of draining depends on initial milk (composition, undergone treatments) but also on the lactic acidification at the time of coagulation [3] and also of temperature [4]. Draining fixes the physical (pH, a_w) and chemical characteristics of cheese which partly will control the kinetics of micro-organisms growth and thus of ripening [5]. In addition salting acts on the drainage of the free aqueous phase of curd, by direct action or the activity of water on the development later of the micro-organisms during ripening [6, 7]. The presence of *Mucor sp* would be with the persistence of its disseminated spores in all the workshop of dairy cheese (milk at reception, air and wet vapour, materials, personnel, ground and walls) such as that shown in a preceding study on the determination of the critical points for the control of *Mucor sp* [8] and that in addition, the principal stages of manufacture (coagulation, draining and salting) did not eliminate it. It is the reason for which improvement of the control of the technological parameters of manufacture is essential by applying critical control point in term of analysis of the risks in HACCP system (Hazard Analysis Critical Control

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Point) [9, 10]. Accordingly this present experimental study consisted in a substitution of the mesophilic lactic starter *Lactococcus lactis subsp lactis* and *Lactococcus lactis subsp cremoris* by the thermophilic ones represented by *Streptococcus salivarus subsp thermophilus* at the inoculating stage, regarded as a critical point to control *Mucor* contamination in Camembert cheese.

MATERIALS AND METHODS

Experimental Devices: The experimental cheeses intended for ripening were prepared with pasteurized cow's milk and standardized out of fat content. Maturation was used to improve milk as a culture medium for the lactic acid bacteria and to bring milk to its optimum pH of addition of rennet (fungic enzymes). Milk was heated to 30 and 40°C which corresponds respectively to the optimal temperatures of growth of mesophilic and thermophilic lactic starter. Also during this stage calcium chloride ($CaCl_2$ at 0.05g/l), *Penicillium camemberti* and *Geotrichum candidum* (strains of cover and ripening) were added. Pre-coagulated milk requires a time of one hour maturation to one hour and half and reached an acidity which varies from 22 to 24°D (Dornic). Rennet of fungi origin at a rate of 25ml for 100 liters of milk was added, while maintaining the temperature of the room of manufacture between 26 - 28 °C. The mixed curd obtained was cut out, homogenized then put out of moulds. Salting was done by immersion in sodium chloride brine: NaCl (26%). The time of salting is a function of the Dornic acidity reached at the release from the mould. The fresh cheeses were dried then transferred in room from ripening until the moment of packing (12 days). The conditions of environment, temperature and relative humidity (RH)% were respectively of the order 12 °C and 85 RH%. In order to estimate the effect of the relative humidity on the evolution of the measured parameters, other tests were carried out in an atmosphere of 95 RH%, the temperature being maintained at 12°C. Cheeses were turned over once every 48 hours accompanied by a new pulverization on the surface by moulds. The experimental soft pastes to which our analyses related were divided into two groups of which each one contained 06 Camembert cheese parts (Group I: Mesophilic starter, Group II: Thermophilic starter).

Physicochemical Analyses: The physicochemical analyses were related primarily to the measurement of the variation of the pH during all the stages of the production line as well as the measurement of the total dry extract during the period of ripening.

The pH: The measurement of pH was carried out with a pH-meter (WTW, pH340, +-0.01) and an electrode of contact (WTW sentix, pH 2 to 13), calibrated using buffer solutions with pH 4 -7 and 10 [11].

Dry Extract: The measurement of the rate of dry extract was applied according to standard AFNOR NF V 04-282 [11]. It requires a balance of precision (OHAUS, Analytical plus AP 310, 0-310g±0,0007 G) and a drying oven (Memmert, ULP 600, 20-300°C). The principle of this measurement rests on a difference in mass between the initial sample and the same sample dried during 24 to 48h with 102°C±2°C. The difference in mass represents the quantity of evaporated water. Mass obtained after drying brought back to the initial mass, % of matter represents dries.

Research and Enumeration of Mucor sp: The analysis of *Mucor sp* was carried out during the phase of refining. After taking away, each sample was put in a sterile test tube containing 9 ml of physiological water.1 ml of each sample was inoculated onto the selective medium for *Mucor* and whose composition in grams per liter was as follows: Malt extract: 20; yeast extract: 2; Chloramphenicol: 0,5 ; Ketoconazole: 0,05; Agar-agar: 15; pH = 5,4 - 5,8. The inoculated plates were incubated for 05 days at 20°C [12].

Statistical Analysis: The variance analysis adopted was the mono factorial in total randomization for treatment of pH results during the production line of Camembert cheese until the salting stage. During ripening, a tri factorial in total randomization was applied for the treatment of pH, dry extract and *Mucor sp* flora parameters. Studied factors were the substitution of starters Mesophilic/Thermophilic, hygrometry (85 and 95 RH %) and time (ripening).The averages of the three parameters results were compared according to Newman-Keuls test [13].

RESULTS AND DISCUSSION

Evolution of the *Mucor* flora: The *Mucor sp* evolution during ripening was characterized by a progressive and rather important fall for 3rd day until the 12th day in the samples inoculated by mesophilic starter and ripened at 12°C/85 RH%. However a total absence of *Mucor sp* as from the 6th day of ripening was noted in camembert cheeses inoculated exclusively by thermophilic starter ($p<0.01$). According to Le Bars-Baily *et al*, [14], *Mucor sp* appeared at the end of 2 to 3 days of ripening, i.e. 4 to 5

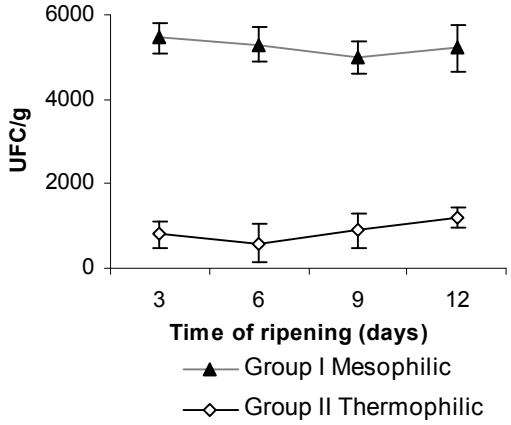


Fig. 1a: Evolution of *Mucor sp* flora during ripening (12°C/95 RH%)

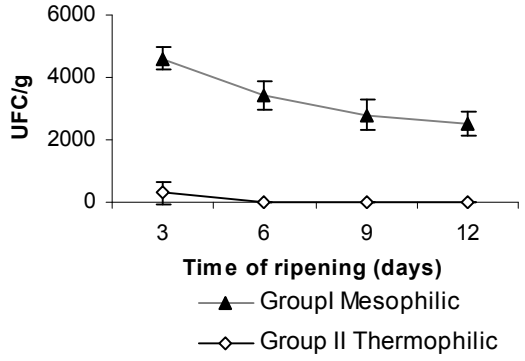


Fig. 1b: Evolution of *Mucor sp* flora during ripening (12°C/85 RH%)

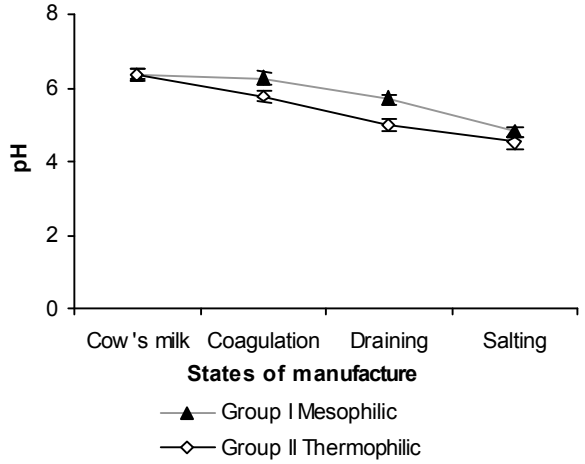
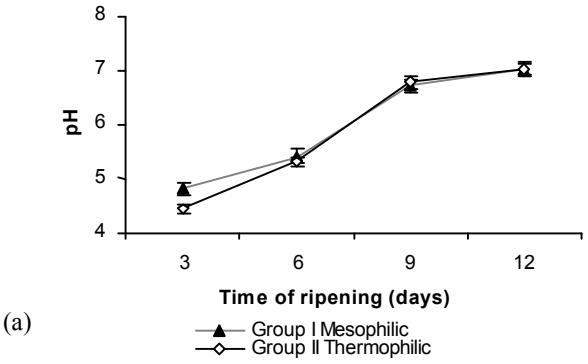


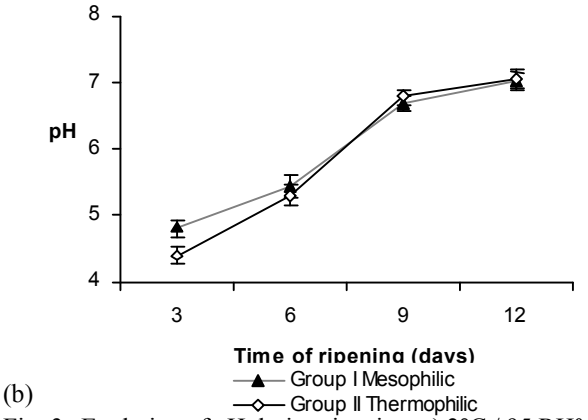
Fig. 2: Evolution of pH during manufacture

days after the addition of rennet (fungic enzymes) and that its development obstructed considerably normal flora of ripening made up mainly of *Penicillium*. It's also noted that the average number of *Mucor sp* reached in ripened cheeses at 12°C/95 RH % was much more important ($p<0.01$) compared to those obtained at 12°C 85 RH%. The difference of was about 45% on average (Fig 1a, 1b). These results partly join those obtained by Bergere and Lenoir, [15] which noted that a too important hygroscopy, higher than 85 RH% during drying surface of cheeses and more than 91% at ripening state constitute favourable conditions to the appearance of this deterioration.

Evolution of the pH and effect on *Mucor* flora: Concerning pH parameter, the noted values followed a decreasing pattern at all experimental sample. It's the consequence of production of lactic acid by lactic starters, but the values of pH were much lower with cheeses obtained by thermophilic lactic bacteria (Fig. 2). These results can probably partly explain the evolution of *Mucor sp* during ripening stage. However, a thermophilic starter had a recognized property dependent on their metabolism which was very active compared to mesophilic starter resulting in an important consumption of lactose with consequence for an accelerated and intense acidification. Thermophilic lactic bacteria would be less sensitive to the low values of pH than mesophilic lactic bacteria [16]. According to Béliard *et al*, [17] the specific inhibiting effect of organic acids is allotted to their not dissociated form which penetrates freely in the cell where it ionizes, causing a lowering of internal pH and blocking some transport mechanisms. Baired-Parker [18], brought back the concentrations of lactic acid not dissociated necessary to cause an inhibition of about 1 mmol (millimol) for yeasts and Enterobacteriaceae against 2 mmol for the moulds. It is thus at the end of coagulation where the recorded pH showed a clear significant difference ($p<0.01$) between experimental samples. According to Le Bars' Bailly *et al*, [14] there is a positive correlation between frequency of contamination by *Mucor sp* and pH after the mould state if it is higher than 4.8. This variability continued during all the stages of manufacture and which reached 0.40 pH units at the first day of ripening (Fig. 3). Increases in pH were very fast between 6th and 9th days or the cheeses passed from an acid pH (pH=5) to a pH close to neutrality (pH included 6.68 and 6.78). This increase of pH was the consequence of the establishment of yeasts and *Geotrichum candidum* [19, 20]. These micro-organisms are very abundant at a beginning of ripening stage consume the lactic acid and the pH of curd gradually rises [2, 21]. Beyond the 5th day, a fine carpet of *Penicillium camemberti* appears and the pH of cheese surface quickly increases, because in addition to consumption of lactate, it has been produced from ammonia by diamination of

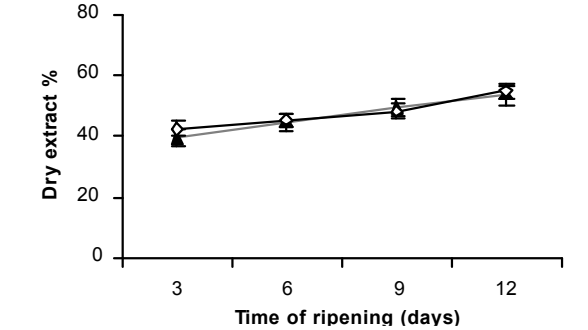


(a)

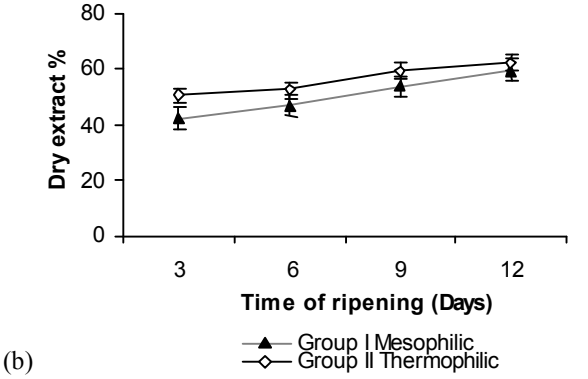


(b)

Fig. 3: Evolution of pH during ripening: a) 2°C / 95 RH%, b) 12°C/ 85 RH%



(a)



(b)

Fig. 4: Evolution of dry extract during ripening a) 12°C/ 95 RH%, b) 12°C/85 RH%

amino acids [22-24]. After 12 days, the average pH of the whole of batches reached a value of about 7.02. However, these values would be rather far away from the optimum pH of *Mucor sp* growth such as that shown in a study on experimental cheeses or the *Penicillium camemberti* could adapt to a broad range of pH ranging between 3.5 and 9, contrary to a strain of *Mucor* of contamination which did not develop in a range of pH much more narrow to 4.9 [25].

Evolution of the Dry Extract and Effect on the *Mucor sp* Flora: Generally, the average values of the rates of dry extract followed an increasing evolution at the two experimental groups from first to the end of ripening period. However, the rates were much more significant ($p<0.01$) in cheeses obtained by thermophilic starters (group II) than those obtained by mesophilic starters (group I) for values about 50.3 and 42.3% respectively. In addition, the effect of relative humidity appeared highly significant ($p<0.01$) as from the 3rd day of ripening. The difference was 6.25% between ripened samples at 12°C/85 RH% and those at 12°C/95 RH% (Fig. 4). One deduced that cheeses which have high dry extract rate will have the losses of more significant weights and conversely, this appears a logical consequence because the lost weight corresponds primarily to a significant water loss per exudation during the draining of curd. This involved a fall of activity of water available for the *Mucor sp* and consequently slowed down its growth. According to Mescle and Zuca, [26], a fall of activity of water involves a fall of the osmotic pressure and various stresses for the micro-organisms. Results obtained agree to those obtained by Agioux, [27] which showed that the experimental ripened cheeses at 96 RH% have very weak dry extract rate, therefore less water lost compared to ripened cheeses at 92 RH%. This increase in the rate of dry extract corresponds to an evaporation of water on cheeses surface. In another study, it was shown that the strains of *Mucor sp* contamination put at the test was very quickly inhibited by a lowering of the activity of water, in lower part of a_w 0.92 no development was noted, even after 14 days of incubation. On the other hand, *Penicillium* strains could still develop with a speed slowed down until towards 0.85. All these quoted factors, the temperature of ripening practised of 12°C is also a parameter contributing in slowing down the development of *Mucor sp* whose optimum of growth is at the approximately about 24°C [28, 29].

A significant correlation ($p<0.05$) was obtained between the evolution of the flora of *Mucor sp* and the rate of dry extract for the samples refined to 12°C/85 RH%.

It can be concluded that the choice of lactic starter during the sowing of the milk presents a risk (critical control point), which will have an influence on the possibility of development and contamination by *Mucor sp*. The use of thermophilic lactic starter brings a guarantee in term of acidification and can help in the improvement of the technological parameters of manufacture by the fast decline of the pH and an important decrease of the activity of water.

This study can be of use to the implementation of the systems HACCP in cheese dairies by the identification of the risks and take measures to avoid these accidents of manufacture

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