| P-043 | **Wax encapsulation to improve shelf-life and sensory properties of cheese** |  |
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## INTRODUCTION AND OBJECTIVES

In Mexico there are more than thirty types of traditionally-made cheeses. Most of them are artisanally produced, regionally distributed and have strong historical roots. Those cheeses are produced from raw milk. Usually, they have physicochemical and compositional characteristics (low pH, high salt concentration and low water activity) that can be explained by the need to store them in a difficult ecological environment, which encourages spoilage by microbial growth (Villegas, 2004).

Poro cheese is a raw milk handmade product with soft slightly pressed paste; coated with paraffin wax and packed in yellow cellophane; involuntarily matured during its sale period (1 month) (Villegas, 2004).

*Carnobacterium* species constitute a genus of Lactic Acid Bacteria (LAB) present in different ecological niches. This LAB is important in dairy products. Indeed, it synthesizes flavor compounds such as 3-methylbutanal. Furthermore, it can inhibit the growth of food-borne pathogens like, as *Listeria monocytogenes,* due to its ability to produce bacteriocins (Afzal *et al*., 2010).

Bioactive food components are subjected to a rapid inactivation or degradation. Many bioactive food components would therefore benefit from an encapsulation procedure that slows down degradation. Bioactive components encompass lipids, vitamins, peptides, fatty acids, antioxidants, minerals but also living cells (De Vos et al., 2010) v.g. flavoring compounds, 3-methylbutanal and bacteria like *Carnobacterium maltaromaticum.*

Desired properties for food applications microcapsules are water impermeability, process resistance and ambient stability (Vandamme, 2007a). Waxes are suitable for food industry applications thanks to their water insolubility and the minimal effects they produced in food properties (Kamble et al., 2004). Many encapsulation procedures have been proposed but none of them can be considered as universally applicable for bioactive components because bioactive components have their own characteristics (De Vos et al., 2010).

The aim of this work is thus (1) to improve sensorial characteristics and shelf life of cheese by incorporating into the paraffin, microcapsules containing strains of *Carnobacterium maltaromaticum* (a bacteria known for

its production of olfactory notes and bacteriocins) and (2) compare the sensory impact on cheese versus directly encapsulated volatile compounds as 3-methylbutanal. Microcapsules will be made using two different materials selected by their melting point: candelilla wax and carnauba wax.

## MATERIALS AND METHODS

Coating paraffin, carnuba wax and candelilla were purchased from Koster Keunen Holland BK (The Netherlands), plasticizer and emulsifier from Sasol (Germany).

Microbiological enumeration cheese was achieved in samples collected from two different artisanal factories (F1 and F2). Ten grams of each cheese were aseptically taken and putted with 90 mL of sterile 2% sodium citrate solution into a sterile stomacher bag, and homogenized by 2 min; eight decimal dilutions were prepared and used for enumeration on agar plates. Medias and enumeration conditions were: Total count (PCA /30°C/72h/Aerobiosis (A)), Total Coliforms (VRBA /30°C/24h/A), *Pseudomonas spp*. (CFC/30°C/48h/A), *Staphylococcus aureus* (BP/37°C/48h/A), Molds and Yeasts (OGA/25°C /120h/A), *Enterococcus spp.* (BEA /37°C/48h/A), Salt-tolerant flora (BHI + 5% NaCl /25°C/48h/A), *Streptococcus mesophilus* (M17/30°C/48h /A), *Streptococcus thermophilus* (M17/ 44°C/48h/A), *Lactococcus mesophiles* (M17/25°C/48h/Anaerobiosis), *Lactobacillus mesophilus* (MRS pH 5,7/30°C/72h/A) and *Lactobacillus thermophilus* (MRS pH 5,7/42°C/48h/A). After incubation, microorganisms were counted and means and standard deviations were calculated.

The ability of *Carnobacterium maltaromaticume* to produce 3-methylbutanal was tested by Head-space GC, in a chromatograph (PR 2100, Périchrom, Zac-du-Moulin, 91160 Saulx-lés-Carthusian, France) equipped with an head space injector (Headspace HT 300A Périchrom) and a detector flame ionization (FID, Périchrom). Volatile compounds were speared using a column of 30m x 0.25 mm x 0.2 µn (integrator WINILAB III, Périchrom). Carrier gas was nitrogen under a constant flow of 2.0 $mLmin^{-1}$, with 190°C as detection temperature. Solutions of 3-methylbutanal varying between 1 and 5000 µM concentration were used for the standard curves.

A first encapsulation test was made adapting Gowda *et al.*, 2009 and Malanovic *et al.*, 2011 described methodology. Wax was melted and water was heated (95°C) using a thermo stated water bath. Wax was added to the water phase heated at a temperature by 5°C to 10°C higher than the melting point of wax. Dispersion of melted wax was obtained by 6000 $min^{-1}$ mixing speeds and a 4 min time of mixing, using a mechanical stirrer with tow blade im-

pellers. Solidification of micro droplets was performing by cooling with cold water (2 – 5°C). Microparticles obtained after solidification were collected by filtration under reduced pressure, and dried at 40°C.

Volumetric size distributions of microcapsules were determined using a laser light diffusion granulometer (Mastersizer S, Malverninstruments, UK). Wax thermal behavior was investigated using differential scanning calorimetry (DSC 204 F1, Netzch, Phoenix). Samples (20 mg) were heated in a standard alumina perforated sample pan. Samples were first pleased at 20°C for 5 min, then cooled to -60°C (-10°K/min), kept for 5 min, heated to 120°C (5°C/min), again cooled to -20°C (-20°K/min) and finally maintained at 20°C for 4 min.

## RESULTS AND DISCUSSION

Figure 1 shows the average bacterial population in cheeses from the two factories. It is clear that cheeses from F2 have higher values in every case except *Pseudomonas ssp.* Actually, *Pseudomonas ssp.* was found only in F1 cheeses. This is probably due to higher moisture content of F2 (data not show). Total coliforms were not found in any case.

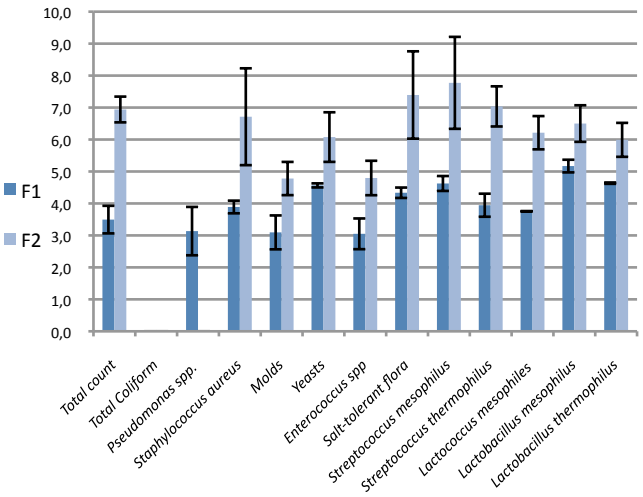


**Figure 1: Average bacterial population in Poro cheese (log UFC/g).**

After bacterial enumeration, principal aromatic compounds produced by *Carnobacterium maltaromaticus* incubated in a medium TSB-YE at 30°C for 16h were studied. Production of 3-methylbutanal was 60 µM, i.e. significantly higher than its production of other compounds such as 2-methylpropanol (5 µM).

As bacteria are living cells, ensuring their survival during the presses is important. That is why the encapsulation method must be carefully chosen in other to not expose bacteria to temperatures above 55°C for prolonged time periods that could kill them. The most commonly applied encapsulation technologies are emulsification, conservation, spray cooling, freeze drying, fluid bed coating and extrusion technologies (De Vos *et al*., 2010). There are two most frequently used techniques for wax capsules preparation. The "solid" technique involves deposition of hot wax with the functional ingredient on a plate whereas "liquid" technique involves an injection of hot wax with a (model) functional ingredient into cold oil, followed by stirring using a high shear mixer. Preliminary tests using another technique called melt dispersion were carried out. This technique is based on the emulsification of the molten mass in the aqueous phase, followed by solidification based on chilling (Milanovic *et al*., 2011). Usually, it is not used in bacterial encapsulation because of the high temperature process. As a consequence, it would be necessary to test several formulations in order to reduce the melting point of wax to temperatures that allow the survival of bacteria. Another interesting technology would also be prilling, which consists in the formation of drops through a needle (Vandamme *et al*., 2007b).

## CONCLUSIONS

Bacterial population of studied cheeses was in the normal range for this kind of products. Moreover, it is important to note that there were not any coliforms in both cheeses. It has been shown that the used *Carnobacterium mataromaticum* strain produces 3-methylbutanal. Bacterial survival to the encapsulation processes is a crucial point. Therefore the most suitable encapsulation methodology should be found.

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