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# Microbiological assessment of tubular cellulose filters used for liquid foods cold pasteurization

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ABSTRACT

One of the major concerns in food industry is food preservation and microbial spoilage. The most commonly used technique for this purpose is pasteurization, a heat-transferring process. Pasteurization, however, alters the organoleptic characteristics, specifically in heat-sensitive foods. In this study, a novel cold pasteurization technique is proposed as an alternative to thermal methods. This technology concerns the removal of microbial cells from liquid foods at ambient and low temperatures through filtration. The liquid food is continuously pumped through a filter of tubular cellulose (TC), a cellulosic porous material produced after delignification of wood sawdust. The filter can be easily regenerated by washing with hot water. The aim of this study was to assess the ability of the TC filters to entrap and remove microorganisms during cold pasteurization of contaminated wine, beer, olive and pepper brines in both laboratory (2 L) and pilot plant scale (10 L). The microbiological analysis of filter parts showed that TC filter is highly effective for both bacterial and yeast removal, with microbial removal yield higher than 90%. Additionally, the filter presents satisfactory operational stability for long period of time (up to 100 consecutive days), while regenerations of the filter with hot water maintain filter's stability.



## 1. Introduction

Research on bioprocess development has been extensive during the last three decades. However, many of these technologies were not adopted due to shortcomings regarding productivity, ease of application, reduction of capacity and production cost. Pasteurization, one of the most widely employed processes in food production, involves thermal treatment at relatively high temperatures. Therefore this technique has high cost, causes air pollution and also degrades the quality of food.

In various food bioprocesses, such as wine making, membranes (Bartowsky, 2008; Girard and Fukumoto, 2000) are used to remove bacteria such as *Leuconostoc oenos* to avoid malolactic fermentation, but their high cost is a limiting factor to industrial applications. There are still opportunities, however, to use abundant, low cost materials with specific chemical or nano-mechanical properties to create multiple new, effective bioprocessing systems. Natural organic materials such as tubular cellulose (TC), which is an abundant natural material, could have wide applications in food industries. TC has been successfully used as a support for cell immobilization suitable for low temperature alcoholic fermentations leading to increased productivities and improved quality of bio-processed products (Koutinas et al., 2012). The promotion of alcoholic fermentation is due to the catalytic effect achieved by yeast immobilized on the material, which increases the productivity of alcohol production and allows extremely low temperature processing. This last property was found to improve the quality and nutritional value of alcoholic products.

Additionally, TC can be used to remove microbial cells from liquid foods through cell entrapment and/or immobilization at ambient and low temperatures (Gialleli, Kallis, Bekatorou, Kanellaki, & Koutinas, 2015). Nano/micro-tubular cellulose can be employed to produce new composites with different materials having different properties (McClory, Chin, & McNally, 2009; Parker & Townley, 2007), by introducing inorganic or organic nanoparticles in its nano-tubes, or it can be used to strengthen other nano-composites (Beecer, 2007). Starch gel/TC composites have served as rennin enzyme storehouses protecting and maintaining

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the enzymic activity (Barouni, Petsi, Kanellaki, Bekatorou, & Koutinas, 2015) and as bilayer biocatalyst for simultaneous alcoholic and malolactic fermentation (Servetas et al., 2013). Furthermore, alginate/polylactic acid/TC composites exhibited promotional effect to lactic acid fermentation (Kumar et al., 2014). Finally, industrial ethanol production technologies can be more cost effective if their by-products are converted to products of added value e.g. other biofuels.

The main aim of the proposed work is to assess the suitability of nano/micro-tubular cellulose as filter for cold pasteurization of various microbially contaminated liquid foods (wine, beer, olive, pepper brine). Tubular cellulose shows great potential as a model material for the development of a new series of polymeric materials, natural or synthetic, suitable for promoting bioprocessing and contributing to nano-biotechnology development as a scientific field.

## 2. Materials and methods

### 2.1. Support preparation

Delignified pine sawdust (TC) was used as filter for pasteurization of liquid foods at low temperatures. The delignification of sawdust was carried out by treatment of 300 g of sawdust in 3 L of 1% (w/v) NaOH solution for 3 h, while the volume of the mixture was kept constant by addition of distilled water. The final mixture was filtered and the residue was washed several times with hot distilled water (80 °C). Thus, a wet TC material was prepared which contained 65% water and had a degree of delignification higher than 95% (Papafotopoulou-Patrinou et al., 2015). Before any use, the above TC was autoclaved at 120 °C for 15 min.

### 2.2. Filter preparation

The filter used consisted of tubular cellulose. TC was wrapped in a thin, perforated nylon fabric and was placed in either a (i) 2 L glass packed bioreactor or (ii) a 10 L stainless steel packed bioreactor.

### 2.3. Raw materials

Commercial white wine and red wine were employed for cold pasteurization. Wines were supplied by the Greek winery CAVINO S.A. (Achaia, Greece). Commercial pasteurized, hopped lager beer (5% v/v) was supplied by the ATHENIAN BREWERY S.A (Achaia, Greece). Lastly, 10% (w/v) olive brine containing 0.17% (v/v) L-lactic acid, 15% pepper pickle brine with 4.17% (w/v) citric acid, contaminated 7% (w/v) olive brine, "Kalamata" olives and pepperoncini peppers were kindly offered by OLYMPION OLIVES AND PICKLES S.A. (Xavari, Ileia, Greece).

### 2.4. Microbial strains and culture media

The alcohol-resistant and psychrotolerant yeast strain *Saccharomyces cerevisiae* AXAZ-1 (*13*), the acetic acid bacterium *Acetobacter pasteurianus* DSM 3509 (DSMZ, Germany), the lactic acid bacterium *Lactobacillus brevis* DSM 1268 (DSMZ, Germany), the lactic acid bacterium *Lactobacillus plantarum* DSM 10492 (DSMZ, Germany), a mixed Lactic Acid Bacteria (LAB) culture isolated from pickled peppers and contaminated brine after olive ripening were used in this study.

*S. cerevisiae* was grown in a synthetic medium containing (% w/v) 0.1 $NH_4SO_4$, 0.1 $KH_2PO_4$, 0.5 $MgSO_4$, 4 Glucose and 0.4 yeast extract with aeration (500 $cm^3$/min, 0.007 bar) at 30 °C. *A. pasteurianus* was cultivated at 30 °C under aerobic conditions (500 $cm^3$/min, 0.007 bar) in an YPM medium consisting (% w/v) of 0.5 yeast extract, 0.3 peptone and 2.5 mannitol. *L. brevis* and *L. plantarum* were grown in MRS broth (LabM, United Kingdom) without aeriation at 30 °C and 37 °C respectively. The mixed LAB culture was isolated from the 15th day of fermentation (pH = 4.3 ± 0.14) of uninoculated peppers in 15% (w/v) brine containing 4.17% (w/v) citric acid. Brine samples (1 mL) were aseptically transferred to 9 mL sterile 1/4 Ringer's solution. Decimal dilutions in the same Ringer's solution were prepared and duplicate 1 or 0.1 mL samples of the appropriate dilutions were mixed on MRS agar medium and incubated at 37 °C for 48 h. Morphologically distinct colonies were picked from the petri plates respectively of the highest dilution and isolates were transferred to liquid cultures of the above medium. All media were autoclaved at 120 °C for 15 min prior to use without pH adjustment (Di Gagno et al., 2009).

Lastly, contaminated olive brine after one-month olive ripening was obtained by the "Olympion Olives and Peppers S.A." and used as inlet during the cold pasteurization process of olives and olive brine.

### 2.5. Cold pasteurization of liquid foods

#### 2.5.1. Laboratory cold pasteurization

A cylindrical glass bioreactor of 2 L (60 cm × 7 cm i.d.) packed with the TC filter (1733 $cm^3$) was used in the laboratory scale experiment. White or red wine was contaminated with *S. cerevisiae* AXAZ-1 (70 × $10^3$ CFU/mL) or *A. pasteurianus* (70 × $10^3$ CFU/mL) respectively. Commercial beer was contaminated with a mixture of *L. brevis*/*S. cerevisiae* AXAZ-1 (total microbial load 70 × $10^3$ cfu/mL). The contaminated liquids were continuously pumped into the system (2 L/day for 100 days for wine and 34 days for beer) using a high accuracy peristaltic pump (Masterflex L/S 7016, Cole–Parmer, USA) at 4 °C. Additionally, olive or pepper brine was contaminated with *L. plantarum* (70 × $10^3$ CFU/mL) or mixed LAB cells isolated from pepper pickles (70 × $10^3$ CFU/mL) respectively. The contaminated liquids were continuously pumped into the system (1.5 L/day for 30 days) using a high accuracy peristaltic pump (Masterflex L/S 7016, Cole–Parmer, USA) at room temperature (17–20 °C).

The effectiveness of the pasteurization technique was evaluated by standard plate counting and/or optical density (*A. pasteurianus*-600 nm, *S. cerevisiae* and contaminated olive brine-700 nm, mixed LAB culture isolated from peppers and *L. plantarum*-625 nm) using a Jenway 6300 UV/VIS spectrophotometer (Staffordshire, UK) of the inlet and outlet liquid stream. When the microbial load removal was reduced below 90%, regeneration of the filter with hot water (70–80 °C) was performed.

#### 2.5.2. Scale up of cold pasteurization

Cold pasteurization of contaminated white wine with *S. cerevisiae* AXAZ-1 was studied on a 10 L (50 × 17 cm i.d) packed with tubular cellulose bioreactor with flow rate of 4 L/day for 100 days. Furthermore, cold pasteurization of contaminated olive and pickle brines was conducted on a 10 L packed bioreactor with flow rate of 2.5 L/day for 30 days at room temperature (17–20 °C). For the evaluation of operational stability and filter regeneration the same procedure as the laboratory scale experiments was followed.

### 2.6. Samples

TC filter samples were obtained before each filter's regeneration and at the end of the pasteurization for each liquid food. TC samples were obtained in each case from (a) the upper part of the filter, (b) the central part of the filter and (c) the lower part of the filter so as to determine the removal pattern at each part of the TC filter.