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Experimental effect of ozone upon the microbial flora of commercially produced dairy fermented products

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ABSTRACT

Ozone was used to control spoilage microorganisms during the manufacturing of dairy products. Ozone stream was applied onto the surface of freshly filled yoghurt cups just before storage for curd development in order to prevent cross contamination from spoilage airborne microorganisms. Accordingly, brine solution was bubbled with ozone for various periods of time and used for ripening of white (feta type) cheese. Both products were subjected to a continuous monitoring of microbial load and also tested for their sensorial properties. In ozonated yoghurt samples there was a reduction in mould counts of approximately 0.6 Log cfu/g (25.1%) by the end of the monitoring period in relation to the control samples. In white cheese ripened with ozonated brine (1.3 mg/L O_3, NaCl 5%) it seems that ozone treatment during the two months of observation reduced some of the mould load but without offering any advantages over the use of traditional brine (NaCl 7%). However, some sensorial alterations were observed, probably due to the organic load in the brine which deactivates ozone in early stages of application. It is concluded that, if the factors of time and concentration of ozone are configured properly, ozonation could be a promising approach safeguarding the production of some dairy products.

1. Introduction

Dairy products are among the most perishable goods and may present intrinsic hazards in the case of microbiological contamination. Bacteria are the most common cause in food spoilage and besides the pasteurization procedures carried out in dairy industry, preservation in terms of microbial safety of yoghurt and cheese also requires appropriate production, transport and storage conditions (Tamine and Deeth, 1980; Tirloni et al., 2015). To ensure the most prolonged shelf life possible and also to protect the consumers, various methods have been proposed which include addition of CO_2 (*i.e.* for carbonated yoghurt), preservatives and post incubation heat treatment (Karagul-Yuceer et al., 2001; Soukoulis et al., 2007). However, the production of dairy products with high quality begins with good hygienic practices.

Ozone application is generally recognized as safe (GRAS) by FDA for treatment of bottled water and its use was extended to the food processing and preservation which include fruit and vegetable processing and storage, seafood processing, ice-making, and also in cereals and confectionery products (Naito and Takahara, 2006; Cullen et al., 2010). Ozone (O_3) is a gas formed from oxygen under high voltage electric discharge. With an oxidation-reduction potential of 2.07 V, ozone is eligible as one of the strongest and most reactive known sanitizers. This value makes ozone 1.5 times stronger than chlorine against several microorganisms, forming no harmful by-products (Rice, 1999). In the gaseous or aqueous phases ozone is effective against the majority of microorganisms tested (Alexopoulos et al., 2013; Bezirtzoglou and Alexopoulos, 2008; Bezirtzoglou et al., 2008; Kim et al., 1999; Restaino et al., 1995) and has been used in dairy industry as a line sanitizer (Guzel-Seydim et al., 2000), in the treatment of mastitis in cows (Ogata and Nagahata, 2000) and also to control moulds in the cheese ripening room (Serra et al., 2003). The use of ozone in Clean-In-Place (CIP) systems offers many advantages to the industry sanitation. Specifically, ozonized water is a perfect decontaminant for CIP pipework systems in food processing plants (Sopher et al., 2009). Ozonation can be applied in the sanitation of milk storage canisters, containers and tankers (Pascual et al., 2007). Ozone can be added at very low doses to the air in the farm granary with great outcomes, as it could deplete the manure smell in the space and reduce number of flies. Simultaneously, it would act upon *Escherichia coli, Salmonella* sp. populations and many other microorganisms (Whistler and Sheldon, 1989; Varga and Szigeti, 2016).

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The disinfection properties of ozone involve oxidation and destruction mechanisms thus in bacteria, it oxidizes and destroys the cell wall and cytoplasmic membrane. Primarily, ozone assails glycoproteins and glycolipids of the bacterial cellular membrane, as well as certain amino acids, such as tryptophan. It also reacts with the sulfhydryl groups of some enzymes leading to the impairment of cellular activity. Therefore, bacterial death occurs by shifting in the cell permeability causing cellular lysis and reducing bacterial numbers (Dosti et al., 2005). Consequently, differences in the membrane structure of microorganisms could be the cause of their intrinsic sensitivity, given that bacteria are more sensitive than yeasts and fungi and Gram-positive bacteria are more sensitive compared to the Gram-negative (Cullen et al., 2010). Advantageously, the bacterial DNA is the final target of ozone and in this vein bacterium cells are unable to develop ozone resistance (Cullen et al., 2010; Naito and Takahara, 2006). Moreover, reacts with Volatile Organic Compounds (VOC) if it comes in contact and it turns into a harmless non-odorous essence. In this way, ozone continuously reduces until all the ozone has declined to bi-atomic oxygen, which is considered safe and environmentally friendly without leaving any chemical residues (Ölmez and Kretzschmar, 2009). Ozonation is considered a safe method because it does not cause the formation of carcinogenic trihalomethanes (Fawell, 2000) and seems to have no effect on the sensorial properties of the products (Ölmez and Akbas, 2009). In the present study, taking under consideration the bactericidal properties of ozone and in an attempt to conserve the highest keeping quality of the product, the effect of ozone on the microbial ecology of yoghurts and feta cheese was investigated. The aim of this work was to study the potential of ozone as a preservation aid and its effect upon the microflora and the physicochemical characteristics of the selected commercialized dairy products.

2. Materials and methods

2.1. Ozone sanitation trials

2.1.1. Yoghurt samples

Freshly filled yoghurt cups (240 g) were withdrawn from the production line of a middle-sized production company and were exposed to a sterilized air/ozone stream of a relatively stable concentration (2.5–3 ppm) for 0, 10, 30 and 60 s. After ozonation the samples were sealed and placed in the maturation chamber for curd development (24 h). The ozonated air stream was generated from a portable device (corona discharged), filtered for sterilization (0.45 μm pore diameter) and directed on the surface of the yoghurts. This configuration targeted the possible cross-contamination with airborne microflora during the experiment. After curd development, the cups were kept in a refrigerator (5 °C) and their microbial population was monitored for the subsequent 90 days and specifically at 0, 1, 2, 3, 5, 10, 20, 50, 70 and 90 days although nominal shelf-life of the Greek type yoghurt is appx. 50 days. Additionally, all samples were inspected daily for any signs of mould development. Classical microbiological techniques involving the preparation of decimal dilutions of the samples, were used to cultivate and enumerate total mesophilic count, yeasts and moulds, lactobacilli, coliforms, staphylococci and enterococci.

2.1.2. Feta cheese samples

Twenty samples of 400 g of feta cheese were left for ripening in pre-ozonated brine with a lower (5%) than usual (>7%) salt concentration. Ozone gas was supplied for 0, 10, 20 and 30 min to the brine where "feta" cheese was then left for maturation for a period up to 2 months. Although ozone concentration in bubbled air stream was constant (2.5–3 ppm) the final saturation of the brine to ozone differs due to the different time the gas was supplied. Total mesophilic count, coliforms, moulds/yeasts, staphylococci, enterococci and lactic acid bacteria were assessed during the ripening period at 0, 2, 5, 10, 20 and 60 days using classical microbiological techniques as described below.

2.2. Microbiological determinations

Triplicate samples of yoghurt and cheese were collected aseptically. Amounts of 10 g each were placed into a sterile blender jar. Then, 90 mL of Butterfield's phosphate buffer were added (dilution 1:10) and homogenized for 2 min at high speed (18,000–21,000 rpm). Furthermore, decimal dilutions were performed in 1/4 Ringer's solution and plated (0.1 mL) onto the surface of the following plate media:

Gelatin peptone agar (HiMedia Laboratories Pvt. Ltd, Mumbai – India) for enumeration of total mesophilic count (TMC) excluding lactic acid bacteria after incubation at 30°C for 48 h.

Violet Red Bile agar with 1% lactose (HiMedia) was used to enumerate coliforms. Medium incubation was performed at 44 °C. The pink or pinkish red colonies which appeared within 24 h were considered as presumptive coliforms.

Potato dextrose agar acidified with 10% tartaric acid (pH 3.5) was used to enumerate total moulds and yeasts. Inoculated plates were incubated at 25°C for 5 days.

Mannitol salt agar (HiMedia) was used to detect staphylococci. Plates were incubated at 30 °C for 24 h. Representative colonies with a yellow/white color surrounded by a yellow zone were picked and subjected to Gram-stain, catalase and agglutination tests (HiStaph Latex Test Kit, HiMedia) in the case of pathogenic staphylococci as *Staphylococcus aureus* presence.

Slanetz and Bartley medium (HiMedia) was used for the detection and enumeration of faecal *Streptococcus* spp. (enterococci). For increased sensitivity, incubation was performed at 35 °C for 4 h and then at 44 °C for additional 48 h. Red or maroon colonies were counted as enterococci.

In yoghurt samples, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* were enumerated by using the methodology proposed by ISO/IDF (ISO 7889/IDF 117, 2003). *L. delbrueckii* ssp. *bulgaricus* was enumerated on de Man-Rogosa-Sharpe agar (HiMedia) acidified at pH 5.2 and incubated under anaerobic conditions at 37 °C for 72 h, while *Streptococcus thermophilus* was enumerated onto M17 agar (HiMedia) supplemented with lactose (5 g/L) and incubated under aerobic conditions at 45 °C for 24 h. A representative number of colonies with typical morphology from both media were further examined with Gram staining and catalase reaction according to ISO/IDF guidelines.

In feta cheese, mesophilic lactic acid bacteria (LAB) were enumerated on double layer MRS agar (HiMedia) following a 48 h incubation at 30 °C.

2.3. pH, ozone concentration and syneresis index in yoghurt samples

Ozone concentration in the air stream was measured photometrically, after bubbling on 1 L of distilled water, with the indigo method using a commercially available kit (Hach Co., Loveland Co., USA). pH was measured by means of a portable digital pH meter (WTW pH meter, Weilheim, Germany).

The whey separated from yoghurt samples during storage was removed using a syringe. The amount of whey drained off expressed as milliliters per container (240 g) was calculated as the syneresis index according to Salvador and Fiszman (2004).

2.4. Sensory analysis

Sensory analysis of yoghurt and cheese samples was conducted by 5 trained panelists. The sensory attributes of flavor, texture, color and overall quality acceptance were scored on a 10 point scale where 1 represents "totally unacceptable" and 10 "totally acceptable". Samples of 20 g in duplicates were prepared for sensory analysis and offered separately to the panelists in a clean environment at 20 °C with ambient light. To protect the panelists, the sensorial analysis in yoghurts was