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Effects of encapsulated starter cultures on microbial and physicochemical properties of traditionally produced and heat treated sausages (sucuks)

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ABSTRACT

Starter cultures *(Lactobacillus plantarum* and *Staphylococcus xylosus)* were encapsulated by emulsion method using alginate-starch mixture to increase the live cell number when they used in the heat treated (at ~70 °C for 20 min) and in fermented sucuks. Effects of encapsulated bacteria on microbial and physicochemical properties of heat treated sucuk were investigated. Freeze dried micro particles were characterized in terms of survival rate surface morphology and release profile. The highest survival rate was observed for *L. plantarum* and *S. xylosus* at 2% and 0.5% concentrations, respectively. Release test was completed for 14 days with the release rates of 70.21% and 70.34% for *S. xylosus and L. plantarum*, respectively. The starter cultures were protected by encapsulation against the harsh conditions during fermentation and heat treatment with lower reduction rates suggesting that the microencapsulation of starter cultures is a robust option for the use in the production of heat treated meat products.

1. Introduction

Fermented foods constitute an important part in human diet due to their health-promoting benefits apart from their traditional nutritional values. Within these well-established groups of foods, fermented meat products have been traditionally produced in the Middle Eastern countries and central Europe. The most common type of fermented meat products in Turkey is sucuk, a dry fermented sausage, which is generally manufactured by mixing beef, fat, curing agents, seasonings and starter cultures, stuffing into the casings followed by fermentation and ripening. Properties of sucuk depend on the ingredients in the formulation, fermentation conditions and starter culture use (Hugas & Monfart, 1997; Vural, 1998).

In sucuk production, traditional process by natural flora has some drawbacks that can be eliminated by using the starter cultures, namely, fermentation can be controlled, technological and nutritional quality is ensured, organoleptic properties can be improved and safer product can be obtained by preventing pathogenic and spoilage bacteria (Leroy, Verluyten, & De Vuyst, 2006; Stajić, Perunović, Staniśić, Žujović, & Živković, 2013; Vural, 1998). Lactic acid bacteria (LAB) are preferably used as starter culture in sucuk production. Depending on lactic acid accumulation by metabolic activities of the LAB, pH of the sucuk dough decreases during fermentation. The reduction in the pH is responsible for the development of the typical characteristics of the sucuk such as microbiological safety, formation of desired texture and color (Kaban, 2013; Lücke, 2000; Dalmis & Soyer, 2008). The level of starter culture inoculated into sucuk dough ranges between 10^6 and 10^8 colony forming unit (CFU)/g (Leroy, Verluyten, & De Vuyst, 2006). LAB and staphylococci are generally used as the starter cultures when nitrite is used as a curing agent (Hugas & Monfart, 1997). *Lactobacillus sakei*, *Lactobacillus curvatus*, *Lactobacillus plantarum*, *Lactobacillus pentosus*, *Lactobacillus casei*, *Pediococcus pentosaceus and Pediococcus acidilactici*, are the most widely used LAB in sucuk production (Ammor & Mayo, 2007) while the most commonly used types of Micrococceae family as starter cultures are *Staphylococcus carnosus*, *Staphylococcus xylosus* and *Micrococcus varians* (Guo, Chen, & Liu, 2000).

In recent years, a heat treatment has been added to production steps due to the commercial reasons such as to extend shelf life, to shorten production time and to reduce production cost, etc. The main target in this application is elimination of the foodborne pathogen bacteria at approximately 68–70 °C for 15–30 min

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(Ercoskun, Tagi, & Ertas, 2010). However, LAB and Micrococcus-Staphylococcus (M-S) are also destroyed at the same time. Thus, the lack of sufficient acidification during the fermentation stage causes some quality and safety problems. In addition, extreme fermentation conditions (anaerobic environment, high salt concentration, temperature, low pH, etc.), production steps (heat treatment, crushing, drying, etc.) and unsuitable storage conditions have negative influence on viability and growth rate of starter cultures (Ammor & Mayo, 2007; Kołożyn-Krajewska & Dolatowski, 2012). The level of starter culture increases after an adaptation period during sucuk production, however, significant reductions in the number of viable cells take place in ripening and storage stages. To overcome aforementioned problems, the encapsulation of starter cultures may provide significant advantages.

With the recent developments in new technologies and consumer awareness, utilization of innovative and quality improving techniques and applications in food sector has become widely adopted. Among these technologies, encapsulation is known as a protection method for a core material against severe environmental factors which also provides controlled release of the material (Corbo et al., 2016; De Prisco & Mauriello, 2016; Kailasapathy, 2002). Microencapsulation has been suggested to be one of the most efficient methods for maintaining viability and stability of bacteria during food production process, storage and consumption (De Prisco & Mauriello, 2016; Martín, Lara-Villoslada, Ruiz, & Morales, 2015). Microencapsulation of bacteria is accepted as an emerging technique for extending their storage life and converting them into a powder form for ease of their use (Martín et al., 2015), and even though still underexploited, it could be also considered a promising strategy for the inclusion of starter cultures in meat products (De Prisco & Mauriello, 2016) since the survival rate of microorganisms has been extended to 80–95% when they are encapsulated (Krasaekoopt, Bhandari, & Deeth, 2003).

Microorganism delivery systems in meat products were summarized by Cavalheiro et al. (2015). Alginate, wheat grains and Arabic gum were used as the wall materials for encapsulating of common probiotic bacteria in meat products such as dry fermented sausages (Muthukumarasamy & Holley, 2007; Sidira, Karapetsas, Galanis, Kanellaki, & Kourkoutas, 2014), salami (Barbosa, Todorov, Jurkiewicz, & Franco, 2015) and cooked meat batters (Perez-Chabela, Lara-Labastida, & Rodriguez-Huezo, 2013). Four LAB were successfully encapsulated with Acacia gum and inoculated in cooked meat batters. It was stated that the microbial safety as well as nutritional value of cooked meat products could be provided with encapsulated LAB (Perez-Chabela et al., 2013). Alginate beads was used as carrier for LAB in the meat model system that simulated the fermented meat products and then in a commercial preparation of pork meat. Microencapsulated cells exhibited similar acidification activity to the control samples containing the free cells after 7 days (Corbo et al., 2016).

The most common coating materials used in the microencapsulation are polysaccharides (starch, cellulose, alginate, pectin, carrageenan, and chitosan), proteins (soy, whey, casein, gelatin, and β-lactoglobulin), and lipids (waxes). The success of the alginate gel encapsulation technique is due to the gentle environment it provides for the entrapped material, its non-toxic nature, inexpensiveness, simplicity, and its biocompatibility (Krasaekoopt et al., 2003). Alginate is a naturally derived polysaccharide extracted from various species of algae and composed of β-D-mannuronic and α-L-guluronic acids. Sodium alginate is capable of forming hydrogels with the divalent cations such as Ca^{+2}, Ba^{+2} and Sr^{+2}. However, stability of the alginate under acidic medium was found to be poor that limits its usage in fermented foods. Alginate-starch combinations providing increased chemical and mechanical stability have been proposed as the wall material to overcome this problem (Sultana et al., 2000). Strains microencapsulated in alginate have been included in food formulations such as ice cream, cheese, yogurt, mayonnaise and biomass (Corona-Hernandez et al., 2013; Krasaekoopt et al., 2003).

Heat treated sucuk is defined as “sucuk like product” in Turkey. This treatment provides some economical advantages to the producers. However, problems in physical, chemical and sensory quality as well as product safety may arise during production and storage due to the significant reduction of desirable bacteria. Encapsulation thus may protect the viability of starter cultures from heat treatment. Studies using free or encapsulated starter cultures in heat treated sucuks are still scarce. In this context, the purpose of the present study was to evaluate morphological properties, survival rate and release behavior of starter cultures of *L. plantarum* and *S. xylosus*, microencapsulated using an emulsion method with the alginate-starch mixture, and also to monitor the effects of addition of these encapsulated starter cultures on physicochemical and microbiological characteristics of heat treated and fermented sucuks.

2. Materials and methods

2.1. Starter culture preparation

Lactobacillus plantarum (ATCC No: 2331) was activated in MRS broth and maintained on MRS agar. Freeze dried cultures of *Staphylococcus xylosus* (ATCC No: 29971) was activated in BHI broth. Fresh cultures were obtained after activation by three successive transfers in MRS (Merck, Darmstadt, Germany) and BHI broth (Merck), respectively at 37 °C for 48 h. *L. plantarum* and *S. xylosus* cultures in late-log phase (with the cell numbers of 3.2×10^{11} CFU/mL and 8.4×10^{11} CFU/mL, respectively) were harvested by centrifugation at 3000 rpm for 10 min, washed in sterile saline solution (0.8% NaCl) under the same centrifugation conditions, and used for encapsulation. All other chemicals and solvents, unless otherwise stated, were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Microencapsulation

Starter cultures were encapsulated according to the emulsion technique as commonly applied (Sultana et al., 2000). Sodium alginate alone and sodium alginate- maize starch mixtures were used as the wall material in different ratios (Table 1) to determine the proper wall material ratio in terms of highest survival rate described below. Glassware and reagents used in the experiments were sterilized before use. Alginate-starch solution was sterilized at 121 °C for 15 min and cooled to room temperature before encapsulation. The mixture containing 40 mL of alginate-starch and 10 mL of active cell suspension (with 11.5 log CFU/mL and 11.92 log CFU/mL, for *L. plantarum* and *S. xylosus* respectively) were prepared. Concentrations of the bacteria and the alginate-starch suspension to active cell suspension ratio were selected based on our preliminary determination and the values of a previous study (Sheu, Marshall, & Heymann, 1993). The suspension was dropped into 250 mL of sunflower oil containing 0.5% of Tween 80 and stirred at 600 rpm on a magnetic stirrer for 20 min. While stirring sterile 100 mL of $CaCl_2$ (0.1M) was added quickly until the emulsion was broken. After 20 min at room temperature, the beads were removed from the aqueous phase, washed with a solution containing 0.9% salt and 5% glycerol. Microcapsules were harvested by centrifugation at 15,000 rpm for 5 min and freeze dried using a freeze dryer (Armfield, Ringwood, UK). Dry microcapsules were stored in a sterile container at +4 °C until use.