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Surface conditioning of stainless steel coupons with skim milk, buttermilk, and butter serum solutions and its effect on bacterial adherence

Nguyen Manh Dat [a,*], Le Duc Manh [a], Daisuke Hamanaka [b], Duong Van Hung [b], Fumihiko Tanaka [b], Toshitaka Uchino [b]

[a] Food Industries Research Institute, 301 Nguyentrai, Thanhxuan, Hanoi, Viet Nam
[b] Laboratory of Post-harvest Science, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 8128581, Japan

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ABSTRACT

In the dairy industry, dairy by-products such as skim milk, buttermilk and butter serum which possess different specific compositions, could contact with processing surfaces to form conditioning layers and subsequently alter bacterial attachment behavior of the surfaces. In order to simulate and elucidate this process, stainless steel coupons were conditioned with skim milk, buttermilk and butter serum solutions. Formed conditioning layers were examined under a confocal laser scanning microscope (CLSM) and the influence of surface conditioning on bacterial adherence was investigated. The results showed that different conditioning layers were formed by different dairy by-products. The layer formed by skim milk, buttermilk and butter serum was the thinnest, medium and the thickest, respectively. The treatment of stainless steel surfaces with skim milk, buttermilk and butter serum could reduce the adherence of dairy-related bacteria (*Lactococcus lactis* subsp. *lactis* NBRC 100933, *Leuconostoc mesenteroides* subsp. *cremoris* NBRC 107766 and *Lactobacillus casei* FIRI 108) at different levels. In the majority of cases, the adherence-reducing ability of buttermilk and butter serum was found better than skim milk. While skim milk could reduce bacterial adherence during shorter exposure time (almost of 30 min), buttermilk and butter serum could act during the longer period (up to 720 min). The result suggested that, bacterial adherence-reducing effect of buttermilk and butter serum may correlate to their substances associated with milk fat globule membrane. In order to decrease bacterial adherence, surface conditioning with skim milk, buttermilk and butter serum is recommended. Surface conditioning with skim milk is suitable for short bacterial exposure time (30 min), for a longer period of time (more than 180 min), only surface conditioning with buttermilk and with butter serum is advisable.

1. Introduction

It is widely accepted that most surfaces, either in nature or in industry, are colonized by bacterial biofilms. Biofilms residing on food contact surfaces are potential contamination sources which can cause serious problems such as introducing continuous infections (Costerton, Stewart, & Greenberg, 1999), transferring disease-causing organisms (Hall-Stoodley, Costerton, & Stoodley, 2004) and creating mechanical blockages and bio-corrosions (Zottola & Sasahara, 1994), etc. Dairy products, with their high nutrition, are ideally suitable for microorganisms to develop and become a reservoir of contaminants like biofilms. In the dairy industry, processing surfaces could contact with not only dairy products but also a wide range of their by-products such as buttermilk and butter serum. Buttermilk is the term used to refer to the liquid phase released during churning (destabilization) of cream in the butter making process. Buttermilk has been considered as the invaluable by-product of the milk fat industry. However, recently by containing a considerable amount of milk fat globule membrane (MFGM), a new-known fraction but having nutritional benefits and functionalities, buttermilk has been receiving more and more attention from researchers (Corredig, Roesch, & Dalgleish, 2003; Rombaut, Camp, & Dewettinck, 2006; Sodini, Morin, Olabi, & Jiménez-Flores, 2006). Another by-product in the production of anhydrous milk fat (AMF) from butter, butter serum, is also known to have higher MFGM content than buttermilk

* Corresponding author. Tel.: +84 94 6863679; fax: +84 43 8584554.
E-mail addresses: datnm@firi.vn, datnx@yahoo.com (N.M. Dat).

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(Rombaut et al., 2006). MFGM mostly contains polar lipids which act as membrane receptors of various pathogens, involved in pathogen-induced signaling, etc. In addition, polar lipids' amphiphilic property (due to their hydrophobic tail and hydrophilic head groups) and negative charge would affect the attachment ability of processing surfaces to bacteria. Indeed, the consumption of buttermilk was proved to reduce biofilm formation on prosthesis over a period of at least 8 days. It might be due to the total effects of all properties of buttermilk (Busscher et al., 1998).

Despite efforts to maintain the cleanliness of processing surfaces, a very short contacting time is enough for bacteria to attach and form biofilms. Therefore, control of this initial bacterial adhesion step plays an important role in controlling biofilm formation procedure. Of all controlling methods, surface conditioning is seen as the most popular one. Studies on surface conditioning with milk (Speers & Gilmour, 1985), milk at different pH values (Dat, Hamanaka, Tanaka, & Uchino, 2010), individual milk proteins (Barnes, Lo, Adams, & Chamberlain, 1999; Fletcher, 1976), and their effect on bacterial adhesion have been conducted. In the dairy industry, the utilization of dairy by-products for surface conditioning could not only exploit these low-valuable materials for the new application but also decrease the risk of chemical pollution to main products from conditioning materials (because dairy by-products have almost similar composition to dairy products). However, research on surface conditioning with dairy by-products such as buttermilk and butter serum has insufficiently been reported. In the present research, the effect of dairy by-products on properties of stainless steel surface and, consequently, on bacterial adherence were elucidated.

2. Materials and methods

2.1. Bacterial cultures

In this study, *Lactococcus lactis* subsp. *lactis* NBRC 100933 (*Lactococcus lactis*), *Leuconostoc mesenteroides* subsp. *cremoris* NBRC 107766 (*Leuconostoc cremoris*), and *Lactobacillus casei* FIRI 108 (*Lactobacillus casei*) were used to represent lactic acid bacteria which are commonly present in dairy products. The first two strains were obtained from the collection of National Institute of Technology and Evaluation Biological Resource Center–Japan (NBRC). The last strain was isolated from imported cheese in Vietnam. *Lactococcus lactis* was anaerobically grown in Trypticase Soy Yeast Extract Medium (30 g trypticase soy broth (Bacto™ Trypticase Soy Broth, Becton, Dickinson and Company, USA) plus 3 g yeast extract (Bacto™ Yeast extract, Becton, Dickinson and Company, USA) and 15 g agar (Wako Pure Chemical Industries, Ltd., Japan) if needed for 1 L of medium), *Leuconostoc cremoris* was anaerobically grown in de Man, Rogosa and Sharpe medium (MRS agar and broth, Merck, Germany), *Lactobacillus casei* was anaerobically grown in M17 Medium (37.25 g Difco™ M17 Broth or 48.25 g Difco™ M17Agar (Becton, Dickinson and Company, USA) plus 5 g of glucose (Wako Pure Chemical Industries, Ltd., Japan) for 1 L of medium). Bacterial cells of each strain were incubated at 30 °C for 48 h in agar plates. Anaerobic condition was created in a parafilm-sealed plastic box using oxygen-absorbing and carbon dioxide-generating agents (AnaeroPack-MicroAero, Mitsubishi Gas Chemical Co., Inc.). Grown cells were harvested by spreaders and suspended in growth medium. After being reincubated at 30 °C for 48 h, they were removed from growth suspension and water-washed twice by centrifugation (3000 rpm, 10 min, and 4 °C). Bacterial cells were then resuspended in sterile distilled water to obtain the initial concentration of 10^8–10^9 colony-forming unit per milliliter (CFU/ml). This could be done by adjusting the suspension to a previously calculated absorbance at 600 nm (Morimatsu, Eguchi, Hamanaka, Tanaka, & Uchino, 2012). This suspension was stored at 4 °C until the inoculation for the bacterial adhesion test.

2.2. Test surfaces

2.2.1. Preparation of surfaces for testing

Stainless steel coupons (type 304, finish number 4, size 1 × 7 cm) were used as solid surfaces for bacterial adhesion. After being washed with neutral detergent containing sodium alkyl ether sulfate as surfactant (Kao Corp., Tokyo, Japan), and gently rinsed with distilled water, coupons were sonicated for 15 min in distilled water to remove any pre-attached materials using a Bransonic Ultrasonic Cleaner (Model 2510J-MTH, frequency of 42 kHz, Yamato Scientific Co. Ltd., Tokyo, Japan). Before treatment of surfaces, stainless steel coupons were autoclaved at 121 °C for 30 min and dried in clean bench air.

2.2.2. Treatment of surfaces with skim milk, buttermilk, and butter serum solutions

Skim milk (Skim milk powder, Wako Pure Chemical Industries, Ltd., Japan), buttermilk and butter serum solutions were used for conditioning coupon surfaces. Buttermilk and butter serum were prepared from cream (whipping cream, Meiji, Japan) by following the procedure of standard dairy processes (Fredrick, Sichien, & Lewille, 2010), briefly described as below. Buttermilk was produced by churning cream at 10 °C by a hand mixer (Braun Hand Blender, MR5555MCA, Braun Inc., Poland). Churning process damaged the membranes of butterfat in cream, resulting in the production of small butter grains. These butter grains floated in the water-based portion of the cream called buttermilk. The buttermilk was then separated from butter by draining and rinsing the grains with sterile distilled water (if needed). Butter serum was produced from butter by melting at 60 °C for 30 min. After melting, butter was transformed into butter oil and butter serum. The mixture was self-separated, and when cooling to about 10 °C, oil was solidified and butter serum was then withdrawn from the solidified oil. Major components in these products such as proteins, fats, and carbohydrates were analyzed by Kjeldahl (Graef & Vermeule, 2010a), Roese–Gottlieb (Graef & Vermeule, 2010b), and Luff–Schoorl (Sichien & Vermeule, 2010) methods which were modified for dairy products, respectively. The composition of these dairy by-products was presented in Table 1.

In this study, stainless steel coupons were immersed in sterile screw-cap polystyrene test tubes containing the above skim milk, buttermilk and butter serum solutions (all at adjusted concentration of about 10%). After 30 min of conditioning, coupons were then removed, washed 3 times by sterile distilled water and dried in clean bench air. Obtained coupons were used for the bacterial adhesion test.

2.3. Bacterial adhesion

Treated stainless steel coupons were immersed in test tubes containing 10 ml of cell suspension of about 0.5 × 10^8 CFU/ml in sterile physiological solution. Clean stainless steel coupons were

Table 1
Composition of some dairy by-products used as surface conditioning media.

Dairy by-products	Composition, %			
	Dry matter	Protein	Carbohydrate	Fat
Skim milk	10.0	4.6	4.8	0.2
Buttermilk	9.7	4.0	3.8	1.8
Butter serum	11.0	4.2	3.5	3.1