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Effect of cream fermentation on microbiological, physicochemical and rheological properties of *L. helveticus*-butter

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ABSTRACT

The primary objective of this study was to evaluate the physicochemical and rheological properties of butter produced by *Lactobacillus helveticus* fermented cream. The incorporation of putative probiotic – the *L. helveticus*, to ferment cream prior to butter production was anticipated to alter the nutritional composition of butter. Changes in crude macronutrients and the resultant modification relating to textural properties of butter induced upon metabolic activities of *L. helveticus* in cream were focused in this research. Fermented butter (LH-butter) was produced by churning the cream that was fermented by lactobacilli at 37 °C for 24 h. Physicochemical analysis, proximate analysis and rheology properties of LH-butter were compared with butter produced using unfermented cream (control). LH-butter showed a significantly ($P < 0.05$) higher fat content and acid value; lower moisture and ash; and was softer than the control. Cream fermentation modified nutritional and textural properties of butter in which LH-butter contained higher health beneficial unsaturated fatty acids than the control and thus rendered the product softer. Its enrichment with probiotics could thus further enhance its functional property.

1. Introduction

Fermentation of food is one of the earliest food processing methods known by man. It is a metabolic process by yeast or bacteria that converts sugar into organic acid, alcohol and carbon dioxide under anaerobic condition. The resulting metabolites produced during fermentation can then enhance a food's flavour profile, nutritional value, as well as serving the preservation purpose (Caplice & Fitzgerald, 1999). In addition to these valuable characteristics, fermentation by microorganisms could enrich the products further, providing excellent source of health beneficial bioactive compounds (Ewe, Wan-Abdullah, Karim, Bhat, & Liong, 2010).

Butter should be a solid product made from milk or cream, according to Food Regulation 1985 (Malaysia). It contains 80% fat, 16% water and 4% solids non-fat (SNF), such as carbohydrate and protein (Frede, 2002). Industrial production of butter involves the usage of cream as the raw material (Nollet & Toldra, 2010) and is converted from oil-in-water emulsions to water-in-oil emulsions by high-speed churning (Samet-Bali, Ayadi, & Attia, 2009).

Cultured butter is a condiment that is produced under an uncontrolled fermentation manner/natural fermentation manner, such as Tunisian butter (Samet-Bali et al., 2009). The taste and aroma of cultured butter highly depends on the microorganisms which reside and ferment the cream. However, it is more aromatic and tangy, as well as having longer shelf life and better texture than normal non-fermented butter (Caplice & Fitzgerald, 1999). Lactic acid bacteria (LAB) have long been used as a starter culture for the production of various dairy products, such as yogurt, lassi and cheese, which could also be incorporated into cream for the production of butter with enhanced bioactivity and flavour development. By possessing lipase and esterase, LAB is able to utilise fat in the cream and convert it to phospholipids, fatty acids, mono- and diglycerides (Fenster, Parkin, & Steele, 2000). Protein can be degraded by peptidase into peptides and amino acids while lactose can be hydrolysed into lactic acid by lactase (Minervini, 2011). Being a putative candidate for probiotics, the incorporation of LAB for fermentation has indeed further enriched the nutritional composition of dairy products with health beneficial effects beyond what it could normally confer (Lye, Kuan, Ewe, Fung, & Liong, 2009).

To date, butter produced using fermented cream can scarcely be found in the market. The controlled fermentation of cream with LAB could provide consumers with the beneficial effects of fermented-butter (butter produced from fermented cream) as well as probiotics. Therefore, probiotic-fermented-butter may be an excellent alternative to currently available dairy products on the

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market. In this study, *Lactobacillus helveticus* was used for cream fermentation prior to production of butter. The modification done on cream could affect the fat content of butter which in turn affects the physicochemical properties of the product (Rønholt, Kirkensgaard, Pedersen, Mortensen, & Knudsen, 2012). Interactions between the chemical components in butter that, in turn, affects the physical properties of the product can be observed by studying its physicochemical properties. The microbiological and physicochemical characteristics of fermented-butter were compared to non-fermented butter to study the feasibility of the product. The possible effect of fermentation towards microbiological, physicochemical properties and consistency of the product is discussed.

2. Materials and methods

2.1. Bacterial cultures

L. helveticus MTCC 5463 were obtained from Taylor's University (Selangor, Malaysia) and were used for cream fermentation. The bacterial cultures were propagated in sterile de Mann Rogosa, and Sharpe (MRS) broth three successive times using a 10% (v/v) inoculum, and incubated at 37 °C for 24 h prior to use. Then, the activated strains of lactobacilli were washed trice with sterile 0.9% (w/v) sodium chloride solution before cream fermentation.

2.2. Butter samples

2.2.1. General

Two types of butter were produced, namely, (1) butter fermented by *L. helveticus* MTCC 5463 (LH butter) and (2) non-fermented butter (control).

2.2.2. Cream fermentation

Commercially purchased UHT-whipping cream (ARLA Foods, Slagelse Dairy, Denmark) was used for fermentation prior to butter production. Fermentation of cream was performed using 10% (v/v) of activated lactobacilli obtained from Section 2.1 at 37 °C for 24 h. Upon fermentation, the fermented cream was refrigerated for 24 h prior to churning.

2.2.3. Butter production

Control and LH butters were produced using a small scale laboratory setup according to the method of Britten, Lamothe, and Robitaille (2008) with slight modification. Firstly, cream was churned with a mixer Professional 600TM Series model (KitchenAid, US) at high speed (speed of 10) until separation of the fat grains from buttermilk was observed. The churning process took 10 min and was maintained at 10–15 °C throughout. Butter grains were then pressed to remove the excess buttermilk. Next, the butter grains were mixed continuously with sterile cold water, four times, to remove excess buttermilk. Lastly, the butter was shaped into the desired shape, stored in plastic containers and kept refrigerated at 4 °C. A similar churning process was performed separately for the production of LH butter using fermented cream.

2.3. Viable counts of L. helveticus

The viable counts of *L. helveticus* in cream, before and after fermentation, and fermented-butter were determined using the pour plate method with MRS agar. Samples were serially diluted with 0.9% (w/v) sterilised NaCl and subsequently plated in duplicate, followed by incubation at 37 °C for 48–72 h.

2.4. pH of cream and fermented cream

The pH of cream, before and after fermentation, was measured using a digital pH meter, Eutech pH 700 (Thermo Scientific, Germany).

2.5. Fatty acid profiles of control and LH-butter

2.5.1. Lipid extraction

Extraction of lipid from butter was performed according to Folch's technique, described by Christie (1989) with slight modification. Briefly, approximately 10 g of butter was mixed with 100 ml of a chloroform–methanol mixture (2:1 v/v). The mixture was homogenized (IKA T25 digital Ultra-Turrax homogenizer, Germany) and then agitated using an orbital shaker (Protech model 719, United State) for 20 min. Next, the mixture was filtered and 25 ml saturated NaCl solution was added to the filtrate; the chloroform phase was subsequently recovered. An adequate amount of anhydrous sodium sulphate (Na_2SO_4) was added to absorb moisture, and the chloroform mixture was dried with rotary evaporator (Eyela, Japan) at 40 °C under vacuum. The extracted oil sample was then subjected for derivatization of fatty acid to fatty acid methyl ester (FAME).

2.5.2. Derivatization of fatty acid (FA) to fatty acid methyl ester (FAME)

Two millilitre of hexane was added to dissolve 20 mg of oil sample that was weighed into a micro reaction vessel. Subsequently, 2 ml of BCl_3-methanol followed by few drops of 2, 2-dimethoxypropane were added. The mixture was heated at 50 °C for 10 min and then cooled to 25 °C under running water. Then, 1 ml of water and 1 ml of hexane were added into the reaction mixture and the reaction vessel was shaken vigorously. Upon the formation of two distinct layers, the upper layer was transferred to a clean vial and adequate amount of anhydrous Na_2SO_4 was added to absorb moisture.

2.5.3. Gas chromatography (GC) condition for FAME determination

FAME was determined using a GC (Perkin Elmer Clarus 600) equipped with a flame ionization detector (FID) and a BPX 70 column (30 m × 0.25 mm i.d., 0.25 μm film; SGE, Austin, TX). The injector and detector were maintained at 220 °C and 350 °C, respectively. The column temperature was programmed as an initial temperature at 60 °C holding for 2 min, ramping to 220 °C at 10 °C/min and 210 °C at 5 °C/min and holding for 7 min. Hydrogen was used as carrier gas with flow rate at 1 ml/min. Identification of peaks was based on comparison of retention time with standard FAME.

2.6. Physico-chemical compositional analyses

2.6.1. Protein, moisture and ash contents

Protein, moisture and ash contents of LH butter and control were determined according to the Official Methods of Analysis of AOAC International. Samples were analysed for protein using the Kjeldahl method (AOAC official method 991.20, AOAC International 2000d) by measuring the nitrogen content and multiplying by a conversion factor of 6.38. Moisture content was determined by the loss of mass of samples in an oven heated at 102 ± 2 °C (AOAC official method 920.116, AOAC International 2000a). Ash content in samples were determined gravimetrically using a dry ashing method, heated at 550 °C in a muffle furnace (AOAC official method 920.117, AOAC International 2000b).