LWT - Food Science and Technology 66 (2016) 348–354

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

The effect of milk fat substitution with palm fat on lactic acid bacteria counts in cheese-like products

Marek Aljewicz [a, *], Grażyna Cichosz [a], Beata Nalepa [b], Marika Bielecka [a]

[a] Department of Dairy Science and Quality Management, Faculty of Food Science, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland
[b] Department of Industrial and Food Microbiology, Faculty of Food Sciences, University of Warmia and Mazury, Plac Cieszyński 1, 10-726 Olsztyn, Poland

ARTICLE INFO

Article history:
Received 31 May 2015
Received in revised form
6 October 2015
Accepted 18 October 2015
Available online 23 October 2015

Keywords:
Cheese-like products
Gouda
Probiotic
Lactobacillus
Viability
Fingerprinting

ABSTRACT

This study evaluated the influence of complete substitution of milk fat with palm fat on the growth of lactic acid bacteria in Gouda-type cheese-like products, as compared with Gouda cheeses of the same type. Cheese-like products and ripened cheeses were produced in industrial plants with or without the addition of probiotic cultures. The analysed products were evaluated during ripening and storage based on the counts of starter lactic acid bacteria (SLAB), non-starter lactic acid bacteria and the viability of *Lactobacillus acidophilus* NCFM and *Lactobacillus paracasei* LPC-37 probiotic cultures.

The highest SLAB counts were determined in control and experimental Gouda cheeses. SLAB were significantly less abundant in cheese-like products. During ripening, a greater reduction in SLAB counts was observed in control ripened cheeses than in control cheese-like products. *Lactococcus* sp. counts were lower in experimental products containing *L. acidophilus* NCFM than in products with *L. paracasei* LPC-37.

The viability of *L. acidophilus* NCFM was significantly correlated with product type and the time of ripening and storage. The counts of *L. acidophilus* NCFM were higher in ripened cheeses than in cheese-like products. *L. paracasei* LPC-37 cultures were characterised by higher viability rates in products than *L. acidophilus* NCFM.

1. Introduction

Growing awareness about the significance of diet and nutrition for health has increased consumer interest in functional foods. A wide assortment of functional foods are available on the dairy market. Yoghurt is a major functional dairy product because its chemical composition can be easily modified, and it can be enhanced with probiotic cultures. Most of the probiotic cultures were isolated from human or animal digestive tract, but one culture used in the experiment - *Lactobacillus paracasei* LPC-37 was isolated from dairy products.

Cheese constitutes environment more suitable for long-term viability of lactic acid bacteria and probiotic bacteria, than yogurts due to higher buffering capacity, lower titratable acidity, higher pH value, lower oxygen content, higher fat content and higher nutrient availability. One of main differences among probiotic dairy products and probiotic cheeses is that in the first products probiotic bacteria should maintain their viability during relatively long ripening-storage period (Madureira et al., 2008; Karimi, Mortazavian, & Cruz, 2011).

When consumed on a regular basis, probiotic bacteria regulate the quantitative and qualitative composition of the gut microflora and/or modify the immune system to deliver health benefits for both humans and animals (Lahtinen et al., 2012; Vasiljevic & Shah, 2008; Wang et al., 2012). Yet for probiotic bacteria to modulate health, their minimum content in food products should be 6–7 log CFU g^{-1}, and daily yoghurt consumption should not be less than 100 g. Ripened cheeses are consumed in smaller daily amounts than fermented milks due to their higher fat content and relatively high price. For this reason, cheeses should contain minimum 8–9 log CFU g^{-1} probiotic bacteria to deliver health benefits. Due to significantly higher counts of probiotic bacteria in the product, health-promoting effects can be achieved already when ripened cheese is consumed in the daily amount of 20 g.

The survival of probiotic cultures in fresh cheese (Masuda, Yamanari, & Itoh, 2005), fresh cheese with inulin (Buriti, Cardarelli, Filisetti, & Saad, 2007), soft ripened cheese (Coeuret, Gueguen, & Vernoux, 2004) and hard cheese (Bergamini, Hynes,

* Corresponding author. Tel.: +48 89 524 5179; fax: +48 89 523 3402.
E-mail address: marek.aljewicz@uwm.edu.pl (M. Aljewicz).

http://dx.doi.org/10.1016/j.lwt.2015.10.042
0023-6438/

Palma, Sabbag, & Zalazar, 2009; Sharp, McMahon, & Roadbent, 2008) has been investigated by numerous authors. Cheese like-products, due to lower prices than regular cheeses are an integral part of the dairy market in Poland and Eastern European countries. In contrast to original dairy products, very little is known about the viability of probiotic bacteria in Gouda-type cheese-like products where milk fat was completely substituted with palm fat. To compensate for this knowledge gap, the aim of this study was to evaluate the effect of milk fat substitution with palm fat on the viability of starter bacteria (SLAB), non-starter bacteria (NSLAB) and *Lactobacillus acidophilus* NCFM and *L. paracasei* LPC-37 probiotic cultures in Gouda-type cheese-like products and Gouda cheeses during ripening and storage.

2. Materials and methods

2.1. Production of ripened cheeses and cheese-like products

The experimental material comprised ripened Gouda-type cheese-like products and Gouda-type cheese manufactured in an industrial plant in Giżycko, Poland. Gouda-type cheese-like products and Gouda-type cheese were manufactured as described by Aljewicz and Cichosz (2015).

2.2. Chemical composition

Samples of representative cheeses were collected as indicated by AOAC 955.30 (AOAC., 2005). Cheeses were grated using a Santos 2 grater (Lyon, France). Grated cheese samples were analysed in triplicate to determine their salt content by the AOAC 975.20 Volhard method (AOAC., 2005), fat content by the Van Gulik method (ISO, 2008) and moisture content by the AOAC 926.08 procedure of oven drying at 102 °C (AOAC., 2005). Total nitrogen and protein content was determined by the Kjeldahl method (ISO, 2011). The pH of cheese slurry, prepared by blending 10 g of grated cheese with 10 mL of deionised H_2O, was measured with a pH meter (Elmetron CP 501, Zabrze, Poland, electrode: Inode, Zabrze, Poland) after calibration with pH 4.0 and pH 7.0 buffers (Merck, Darmstadt, Germany).

2.3. Microbiological analysis

Cheese samples were prepared according to PN-EN ISO (2010). A cheese sample of 10 g was added to a 90 mL of sodium citrate solution (20 g L^{-1}, POCh, Gliwice, Poland) with a temperature of 40 °C. Samples were homogenised in a stomacher (BagMixer 400W, Saint Nom, France) to produce a uniform suspension. *L. acidophilus* NCFM was determined according to ISO (2006). Total *L. paracasei* LPC-37 counts were determined on MRS agar modified through the addition of 30.0 g of sodium chloride (POCh). pH was adjusted to 5.8 ± 0.1. All ingredients were dissolved in distilled water and sterilised by autoclaving at 118 °C for 15 min. After melting and cooling, they were supplemented with filter-sterilised vancomycin (Applichem, Darmstadt, Germany) to final concentration of 30 μg mL^{-1}. Samples were incubated anaerobically at 37 °C for 72 h with the use of the AnaeroGen system (Oxoid, Poznan, Poland). Total non-starter lactobacilli counts were determined in control and experimental cheeses on Rogosa agar (Merck). Samples were incubated anaerobically at 37 °C for 72 h with the use of the AnaeroGen system (Oxoid).

The total counts of starter bacteria were determined in control and experimental cheeses on M17 agar (Merck, Darmstadt, Germany). Samples were incubated aerobically at 30 °C for 48 h (mesophilic cultures).

2.4. Confirmation of strains belonging to the L. acidophilus and L. paracasei DNA isolation

In order to confirm the belonging to the *Lactobacillus* genus, *L. acidophilus* and *L. paracasei* species and *L. acidophilus* NCFM and *L. paracasei* LPC37 strains randomly selected bacterial colonies were checked in the Gram stain test (Olympus BX51, Japan). Then, the material from the collected colonies was grown in MRS broth at 37 °C for 24 h under anaerobic conditions. Next, total genomic DNA was extracted with the Genomic Mini kit (A&A Biotechnology, Poland) according to the manufacturer.

species-specific PCR. Amplification was carried out using a thermal cycler MJmini (Bio-rad, Poland) and two pair species-specific primers: Laci-1 (5′- TGCAAAGTGGTAGCGTAAGC-3′) and 23-10C (5′- CCTTTCCCTCACGGTACTG-3′) for the identification of *L. acidophilus* (Song et al., 2000) or Y2 (5′-CCCACTGCTGCCTCCCG-TAGGAGT-3′) and para (5′-CACCGAGATTCAACATGG-3′) for *L. paracasei* (Ward & Timmins, 1999). The reaction mixture (20 μl) contained 1x*Taq* buffer with 2.0 mM $MgCl_2$, 0.25 mM of species-specific primers, 0.2 mM of each dNTPs, 20–40 ng of bacterial DNA and 1 U of Taq DNA Polymerase (Thermo Scientific, Lithuania). DNA fragments were amplified as follows: *L. acidophilus* – 35 cycles consisting of denaturation at 95 °C for 20 s, annealing at 68 °C for 2 min, extension at 72 °C for 2 min, and a 5 min final extension step at 74 °C; *L. paracasei* – initial denaturation at 94 °C for 3 min, followed by 30 cycles consisting of denaturation at 94 °C for 45 s, annealing at 45 °C for 45 s, extension at 72 °C for 1 min, and a 5 min final extension step at 72 °C.

rep-PCR. Amplifications were performed with 1 U of Dream Taq DNA polymerase (Thermo Scientific), 2.5 mM $MgCl_2$, 0.2 mM of each dNTPs, and 0.1 mM of primer GTG_5 (5′-GTGGTGGTGGTGGTG-3′) (Markiewicz, Biedrzycka, Wasilewska, & Bielecka, 2010). An initial denaturation at 94 °C for 4 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 42 °C for 30 s, and elongation at 72 °C for 30 s, and a final extension at 72 °C for 4 min.

All amplicons were analysed in a 1.0 or 1.5% 1 × TBE agarose gel electrophoresis containing 10 mg/ml ethidium bromide, visualized under UV illumination and photographed.

2.5. Statistical analysis

The results were verified for normal distribution and homogeneity of variance. The significance of differences between means was analysed by Duncan's test. The interactions between factors were determined by ANOVA for the completely randomized design. The results were processed in Statistica 10.0 PL software (Statsoft 2011, Krakow, Poland) at $P < 0.05$ for $n = 3$ (physicochemical and biochemical parameters) and at $P < 0.05$ for $n = 3$ (microbiological analysis in duplicate). All data were presented as means ± standard error of mean. Each repetition of the experience has been manufactured the same day from the same milk.

3. Results

3.1. Chemical composition of cheese-like products and Gouda cheeses

The average water content of Gouda cheeses was determined at 42.00%, and it did not differ significantly ($P > 0.05$) from that of Gouda-type cheese-like products (44.0%). The protein content of ripened cheeses and Gouda-type cheese-like products was similar ($P > 0.05$) at 24%. The analysed product groups differed significantly ($P < 0.05$) in their average fat content which reached 27% in ripened Gouda cheeses and 28% in Gouda-type cheese-like products (Table. A.1). The average content of sodium chloride was determined at