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Article *in* European Food Research and Technology · May 2009

DOI: 10.1007/s00217-009-1039-7

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Eur Food Res Technol (2009) 229:175–178
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SHORT COMMUNICATION

First tests of standardized biocrystallization on milk and milk products

Johannes Kahl · Nicolaas Busscher · Paul Doesburg · Gaby Mergardt · Machteld Huber · Angelika Ploeger

Received: 9 April 2008 / Accepted: 16 February 2009 / Published online: 8 March 2009

Abstract The biocrystallization method has been standardized for plant products. The standardized biocrystallization method is tested on milk and butter samples from controlled feeding trials. When computerized image analysis is applied, samples from different origins (feeding regimes) can be distinguished significantly.

Keywords Food quality · Biocrystallization · Milk · Butter

Introduction

Crystallization patterns emerge when an aqueous dihydrate copper chloride ($CuCl_2$) solution with the sample (e g., food products) is crystallized on a glass plate [1–4]. The emerging patterns are characteristic of the sample material [5, 6]. A texture analysis software program was developed for the evaluation of the patterns [7, 8], thus allowing the characterization and standardization of the method, as documented for plant products by Kahl [9] and Busscher et al. [10]. Because the method has also been successfully applied to milk and milk products [11, 12], the present study is intended to show that standardized biocrystallization can also be applied to dairy products. The samples are derived from herds of cows with different feeding regimes and from standardized processing steps.

J. Kahl (✉) · N. Busscher · G. Mergardt · A. Ploeger
Department of Organic Food Quality and Food Culture,
University of Kassel, Nordbahnhofstr. 1a,
37213 Witzenhausen, Germany
e-mail: kahl@uni-kassel.de

P. Doesburg · M. Huber
Department of Healthcare and Nutrition,
Louis Bolk Instituut, Driebergen, The Netherlands

Materials and methods

Pretest

For the pretests, according to the characterization of the biocrystallization method, milk and butter samples were purchased from the local market.

Homogenization

Milk samples for testing the influence of homogenization on the patterns were collected directly after the milking of the cows. The samples were cooled and transported to the Netherlands Institute of Dairy Science and divided into subsamples, undergoing homogenization at 200 bar (preheating to 45 °C). After the treatment, subsamples were coded and transported to the laboratory for crystallization.

Feeding regime

Milk and butter samples (three repetitions) for the tests were from two herds of cows with different feeding regimes and were derived from Agroscope Liebefeld-Posieux Research Station ALP (Switzerland) in spring and fall, 2006 [13]. The samples were coded. Milk and butter bulk samples were obtained from Holstein cows ($n = 10$) fed with pasture and sunflower seeds during 2 weeks. Another group of cows ($n = 10$) were fed a conventional diet, composed of pasture and corn silage. The raw milk was collected separately from the two groups. The milk samples were subjected to analysis fresh, stabilized with Bronopol

and frozen. The butter samples were produced at the pilot plant of ALP in May and September 2006.

Shelf life

In fall 2006, the butter samples were stored at 4–6 °C and at −18 °C (as reference) and crystallized in weeks 1, 2, 4, 6 and 8.

Sample preparation of milk

After the sample tubes (200 mL) are moved gently in a circular, horizontal manner, 50 mL of the milk sample is transferred to a 100 mL Erlenmeyer flask. The flask is left to stand for 30 min in a water bath (the frozen sample 90 min). After reaching 20 °C, 2 mL was mixed with 43 mL milli-Q water (Millipore) and 15 mL 10% $CuCl_2$ solution and moved at 100 rpm for 30 min (Heidolph Unimax 2010). For each plate, 6 mL of this solution is used.

Sample preparation of butter

Because no biocrystallization procedure for butter samples existed, a method had to be developed. 100 g of a butter sample is transferred into a 250 mL Erlenmeyer flask and 100 mL milli-Q water (Millipore) is added at 50 °C. The extraction takes place in a water bath at 50 °C on a Heidolph shaker (Unimax 2010, 175 rpm). The butter samples are extracted at different temperatures (water bath).

The mixture is transferred into a separation funnel for separating the two phases. The watery phase is placed in a laboratory centrifuge (Universal 32R, supplier: Hettich/D) and run at 4.000 rpm for 10 min. After centrifugation, 10 mL of the extract is added to 57.75 mL milli-Q water (Millipore) and 11.25 mL of a 10% $CuCl_2$ solution. The solution is moved at 100 rpm for 30 min (Heidolph UniMax 2010). For each plate, 6 mL of this solution is used (mixing ratio sample/$CuCl_2$). To test the influence of different mixing ratios on the patterns, the butter samples are crystallized with 16 different mixing ratios.

Crystallization and texture analysis

For every sample run, a wheat standard is applied [9] and two crystallization chambers with 43 places are used in parallel. Construction and function of the crystallization chambers used here are documented in Kahl et al. [14], Kahl [9], and Busscher et al. [10]. Experiments are performed at a medium evaporation time of 12–15 h, at 26 °C and with 53% humidity in the outside chamber, and at 30 °C with 53% humidity over the plates. The result is a plate repetition of 3–6 plates per sample preparation, resulting in 6–12 plates per sample in total. For the evaluation of the trials, all plates per sample are grouped together. For the evaluation of the patterns, a texture analysis is used [7]. The calculated variable diagonal moment is applied by plotting it relative to circular regions of interest (ROIs), that is, circular areas comprising 20–100% around the center of the patterns. This plotting is also done for the results of the statistical evaluation (F- and P-values). Diagonal moment basically measures the differences in correlation of high and low gray levels [15].

Statistical evaluation

The statistical evaluation is carried out by means of a "linear-mixed effects" model Programme R [8].

Results

When the described procedures are applied, milk and butter samples can be crystallized at all different mixing ratios.

Homogenization

The homogenization of the milk samples affects the biocrystallization pattern when compared to the pattern from raw milk samples (Fig. 1). Visually, the crystallization structure loses complexity when the milk sample undergoes homogenization.

This is comparable with the findings from Merten and Lagoni [11] and Merten et al. [12]. With the texture analysis, patterns from the different treatments can be differentiated significantly ($P < 0.01$, ROI < 80%).

Feeding regime, milk

Milk patterns from the different feeding regimes (spring 2006) show significant differences ($P < 0.05$, ROI > 40%), independent of the stabilization. Freezing the milk samples influences the patterns that can be evaluated by texture analysis as statistically significant ($P < 0.05$) of the spring 2006 sample. The effect depends on the ROI. The effect is still measurable, but not significant in the samples from fall 2006. Because the milk obtained from cows fed on a conventional diet, consisting of pasture and corn silage, is influenced more strongly by the freezing process than the milk from cows fed with pasture and sunflower seeds, the difference between both milk samples from the different feeding regimes increases when the samples are frozen (Fig. 2). When the two samples were sent in the fall, the texture analysis variable shows no significant difference between the feeding regimes except when the samples are stabilized with *Bronopol*. Here, the difference is significant ($P < 0.05$, ROI > 70%).

Fig. 1 Crystal patterns from raw milk sample (*left*) and homogenized milk sample (*right*)

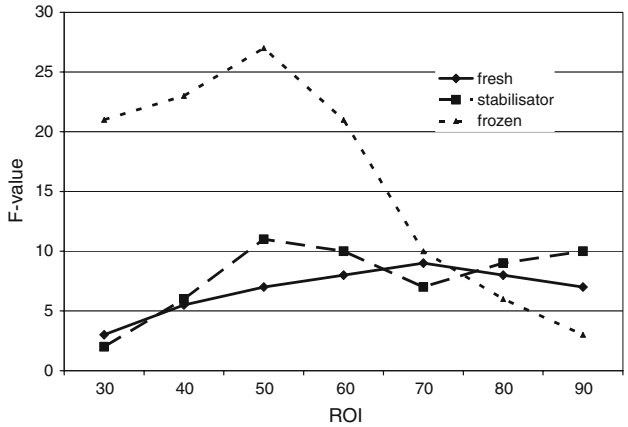

Fig. 2 *F*-values (lme ANOVA) and ROIs for the difference between milk samples of different feeding regimes from spring 2006 according to different sample treatments during transport

Feeding regime, butter

The butter patterns were influenced by different temperatures during extraction. The texture analysis variable increases with higher temperatures. The increase depends on ROI and on the feeding regime. Furthermore, the butter patterns are influenced by different amounts of sample per plate. This effect was described by Gallinet and Gauthier-Manuel [3]. The texture analysis variable increases with higher amounts of the sample. The increase does not depend on ROI. The differences between the butter samples of the two different feeding regimes are significant ($P < 0.05$, ROI 60%). The influence of the mixing ratio on the patterns is stronger for the butter sample produced from the milk of cows fed with a conventional diet, composed of pasture and corn silage, than for the butter sample produced from the milk of cows fed with pasture and sunflower seeds.

When the two different butter samples are crystallized, the difference between the two feeding regimes is significant ($P < 0.001$, $30\% < \text{ROI} < 70\%$). When the samples of the different feeding regimes were sent in fall 2006, the difference was still significant ($P < 0.05$, $30\% < \text{ROI} < 70\%$).

Shelf life

When the two butter samples from the different feeding regimes are stored for 8 weeks at 4–6 °C, a significant change in the patterns of both samples can be observed. The storage influences the patterns from the samples of milk of cows fed with a conventional diet more than those from other samples.

After 8 weeks of storage, the patterns from butter, derived from cows fed with conventional diet, stored at 4–6 °C show a significant difference from those stored as reference material at −18 °C ($P < 0.001$, $30\% < \text{ROI} < 70\%$). The difference between the treatments (feedings regimes) can still be detected as significant ($P < 0.05$), independent of ROI.

The results submitted here show that the standardized biocrystallization method can be applied to dairy products. Subsequent studies will have to test how far the method is able to differentiate samples of unknown origin according to treatment (feeding, processing). This will have to be done on problem complexes larger than those studied for this research. The potential of the method for a classification of dairy products has to be tested using larger training data sets from samples of different farming systems, comprising several farms or dairies, respectively.

Acknowledgments The authors would like to thank Brita Rehberger, Agroscope Liebefeld-Posieux Research Station ALP (Switzerland), Fr. A. de Vries and Dr. L. P .L. van de Vijver (The Netherlands) for providing the samples. J. Kahl, N. Busscher, and G. Mergardt gratefully acknowledge the funding from the European

Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project QUALITYLOWINPUT-FOOD, FP6-FOOD-CT-2003-506358. The views expressed in this publication are the sole responsibility of the author(s) and do not necessarily reflect the views of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use that might be made of the information contained herein.

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