Technology of butter fortified with phytosterols

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Abstract. The aim of the resent study was to determine the appropriate moment to enrich the butter with phytosterols. The butter was enriched in phytosterols at three different stages of the technological process – before cream pasteurisation (batch B), before cream ripening (batch C) and before working the butter into a continuous fat phase (batch D). A control sample with no added phytosterols was prepared (batch A). The titratable acidity of cream before and after biological ripening and of the resulting butter was determined. No significant differences between samples were found (p>0.05). The fat, moisture and phytosterol content of butters, produced from different batches, were evaluated. Any significant differences between samples were found (p>0.05). The number of lactic acid microorganisms in the final product was similar in all analysed samples (p>0.05). The butters enriched with phytosterols were characterized by high sensory scores comparable with those of the control sample (p>0.05). This study suggested that the addition of phytosterols in butter could be performed at different stages of the technological process with no reflect on the phytosterol content in the final product. From a safety point of view, it is appropriate to add the phytosterols before pasteurisation process. The obtained butter is considered as functional product with potential health effects.

Key words: butter; phytosterol esters; enriched dairy products; functional dairy products; spectrophotometric determination.

1. Introduction

The biggest group of functional foods on the European Union market is for the dairy sector [1]. Milk and dairy products are excellent sources of different nutritional compounds such as high valuable protein, calcium, phosphorus, vitamin A etc. Milk fat, as a typical animal lipid, contains a lot of saturated fatty acids and cholesterol which are associated with some negative health effects for the human being [2]. Butter, as largely consumed dairy product, is abundant in milk fat, but is rich in cholesterol. It is reasonable to enhance its lipid profile in order to give him a value-added healthy profile.

Plant sterols (phytosterols) represent a healthy variant of animal sterols [3]. The European Food Safety Authority recognizes the incorporation of stanols/sterols as an ingredient in foods as safe [4]. Phytosterols (plant sterols and stanols) possess low density lipoprotein-cholesterol (LDL-C) and total cholesterol lowering effects when consumed by fermented milk-enriched products [5]. Some authors summarized the role of phytosterols-enriched dairy products in the control of cardiovascular diseases [6]. Oxidation and serum lipids can be regulated by dairy products supplemented with dietary phytosterols [7, 8]. Some anticancer properties can be awarded to phytosterols [9].
It is reasonable to supplement dairy products with phytosterols in the range from 0.2 to 2% [10]. According to the EU stipulates phytosterol-enriched dairy products must be easily divided in portions of maximum 3 g (in case of one portion) and maximum 1 g (in case of three portions) of added phytosterols per day [11, 12]. The possibility to add free and encapsulated phytosterols for the formulation of functional foods was revised by [13]. A research team fortified milk with phytosterols in the form of emulsion [14]. Some authors added encapsulated betasitosterol using nanostructured lipid carriers in the purpose to be added into butter [15]. Other researchers focused attention to the oxidation of phytosterol esters in culinary processing (pan-fried at 160–200°C for 5–10 min) but addition of antioxidants could be done in order to prevent phytosterol oxidation [16, 17]. This fact led to recommend strongly the use of dairy products as vehicle for phytosterols fortification as compared to other possibilities which involve high temperature processing (>100°C) [18]. It is justified to obtain cholesterol-lowering dairy products by the addition of phytosterols esters in high-fat products because they dissolve poorly in water based products [2].

Although these data, there are few researches on the possibility to fortify the butter with phytosterols. In the current study, we hypothesize that the fortification of butter with phytosterols will improve its nutritional value and healthy properties.

Therefore, the aim of the present study was to determine the appropriate moment to fortify the butter with phytosterols in order to obtain minimum losses of phytosterols in the final product.

2. Materials and methods

2.1. Raw materials
Raw cream, obtained from raw cow milk characterized by sensory, physicochemical and microbiological parameters, according to the requirements of European Union (EU) Regulation № 853/2004, was used.

A starter culture for butter production, containing the specific strains (Lactococcus lactis ssp. cremoris, Lactococcus lactis ssp. lactis, Streptococcus thermophilus), was kindly provided by Lactina Ltd., Bulgaria.

Phytosterol preparation (soybean HSF Phytosterol Ester™, extracted from non-GMO soy oil) containing β-sitosterol (≥40%), stigmasterol (15-30%), campesterol (15-30%) and brassicasterol (<10%), was kindly provided by Xian Healthful Biotechnology Co., Ltd.

All used solvents were analytical grade.

2.2. Butter preparation
The butter was prepared according to a classic technology for sour cream butter preparation. The cream fat content was standardized with skim milk to 30 ± 0.5% fat content. Different batches were examined – control sample with no added phytosterols (batch A), 0.26% phytosterols added before cream pasteurization (batch B), 0.26% phytosterols added before cream ripening (batch C) and 0.26% phytosterols added before working the butter into a continuous fat phase (batch D). The samples were heated to 90±5°C and held at this temperature for 30 s. After cooling at 25±5°C, the samples were inoculated with direct vat set (DVS) butter starter culture in the amount of 1 g/l. The samples were stirred and incubated at 25±5°C for 12 h (biological maturation). The fermentation process was performed. After souring of the cream, the samples were cooled down to 6±2°C and stored at this temperature or 12 h for temperature treatment (physical maturation). The cream was churned after temperature treatment and after souring in a butter churn for batch production. The buttermilk was separated and the butter grains were washed two times with water with a temperature 6±2°C. Finally, the butter grains were pressed and mashed in order to remove the rest enclosed water between them by the working process. The ready butter samples were packed in sterile plastic packaging and stored at 4±2°C for further analysis.
2.3. Chemical analysis

2.3.1. Chemical analysis of cream and butter
Titratable acidity (TA) of cream was measured according to Bulgarian National Standard (BNS) 1111-80 and expressed in Thörner degree, °T. Titratable acidity (TA) of butter was measured according to BNS 1111-80 and expressed in Ketstorfer degree, °K. Cream and butter fat contents were evaluated according to BNS ISO 19660:2019 and ISO 8851-3:2004, respectively. Butter moisture was determined in compliance with BNS EN ISO 3727-1:2002.

2.3.2. Phytosterol determination of cream and butter
Phytosterol content was determined by a spectrophotometric method according to a previously established method [19]:

1) Preparation of Liebermann-Burchard reagent (LB): an Erlenmeyer flask was put in a crystallizer filled with glass water, and then 10 ml of acetic anhydride were transferred in the Erlenmeyer flask and after 30 min, 1 ml of sulfuric acid was added with attention to the acetic anhydride. 2) Standard calibration curve: 25.8 mg of the 97% concentrated phytosterol sample was weighed with a precision balance. The weighed mass was placed in a 50 ml volumetric flask and made up to the mark with chloroform. A standard range of values was produced. The tubes of the range were assayed with a spectrophotometer at a wavelength of 625 nm. 3) Phytosterol extraction and determination: an extraction of phytosterols from cream and butter was carried out with 6.25 g of sample and 30 ml of chloroform placed together for 30 min. The extract was cooled to room temperature (25°C) and filtered through cotton wool. The residue (cotton and plant tissue) was re-extracted twice using 30 ml of chloroform for 15 min. The filtered fractions were collected in the extract solution and dried under reduced pressure at 40°C. The residue was resuspended in 20 ml of chloroform, and the volume is brought to 50 ml with the same solvent (SS). A small amount of SS was taken and transferred to a 10 ml graduated flask to which 2 ml of LB reagent was added, and the volume was adjusted to the mark with chloroform. The phytosterols were assayed with a spectrophotometer at 625 nm after 5 min. The blank sample was a pure chloroform sample.

2.4. Microbiological analysis
Total Lactococcus count - sample preparation was performed in accordance with ISO 6887-5:2020. Suitable dilutions were subsequently inoculated on selective culture medium M17, according to ISO 17792:2006.

2.5. Sensory analysis
Sensory analysis test was performed according to the requirements of the Bulgarian National standard BNS 15612:83 which includes the evaluation criteria appearance and cut surface, consistency at 10-12°C, colour, flavour and aroma. Each characteristic was evaluated by a 10-points scale. The butter samples were evaluated on the 1st day of storage by 15 experienced panelists.

2.6. Statistical analysis
Statistical analysis was performed using the program Microsoft Excel 2010 (ANOVA). Multiple comparisons were made by LSD method. The results were presented as mean value ± SD (n=4, two batches with two repetitions).

3. Results and discussion

3.1. Chemical analysis
The changes in the amount of lactose and its biochemical transformation to lactic acid during the fermentation process in the cream were monitored by titratable acidity, expressed in °T (Table 1).
Table 1. Chemical analysis of cream and butter of different batches.

| Batch | TA of raw cream, °T | TA of cream before temperature treatment, °T | TA of cream before churning, °T | TA butter, °K | Butter fat, % | Butter moisture, % | Phytosterols, % |
|-------|---------------------|---------------------------------------------|--------------------------------|--------------|--------------|------------------|-----------------|
| A     | 69 ± 1a             | 74 ± 1a                                     | 1.06 ± 0.10                  | 1.5a         | 1.5a         | 21.5 ± 1.0a      | 0               |
| B     | 68 ± 1a             | 76 ± 2a                                     | 1.18 ± 0.13                  | 0.13a        | 1.0a         | 21.5 ± 1.0a      | 0.23±0.02a      |
| C     | 16 ± 1              | 68 ± 1a                                     | 1.10 ± 0.11                  | 0.11a        | 1.0a         | 19.5 ± 1.0a      | 0.22±0.02a      |
| D     | 67 ± 1a             | 74 ± 1a                                     | 1.04 ± 0.12                  | 1.5a         | 19.0 ± 1.5a  |                  | 0.26±0.01a      |

*Means with different letters within a column are significantly different (p<0.05)

A - control sample with no added phytosterols; B - 0.26% phytosterols added before cream pasteurization; C – 0.26% phytosterols added before cream ripening; D - 0.26% phytosterols added before working the butter into a continuous fat phase; TA – titratable acidity.

The titratable acidity of cream before and after biological ripening as well as the titratable acidity of the resulting butter was determined. It was found that the phytosterol addition in the analyzed variants - before cream pasteurization, before cream ripening and before working, did not influence butter-enriched titratable acidity values (p>0.05).

The fat, moisture and phytosterol content of butters produced from different batches were evaluated. Any significant differences between control and enriched samples were found (p>0.05) (Table 1). The obtained data demonstrated the possibility to add phytosterols to butter at different stages of the technological process without losing the valuable functional ingredients.

3.2. Microbiological analysis

The total number of viable lactic acid microorganisms is presented on Figure 1.

![Figure 1. Microbiological analysis of butters.](image)

*Means with different letters within a column are significantly different (p<0.05)

A - control sample with no added phytosterols; B - 0.26% phytosterols added before cream pasteurization; C – 0.26% phytosterols added before cream ripening; D - 0.26% phytosterols added before working the butter into a continuous fat phase; TA – titratable acidity.
The number of lactic acid microorganisms in the final product was similar in all analysed samples \((p>0.05)\). Similar results were obtained by other scientific teams [20]. These values demonstrated that the microorganisms from the starter culture were not negatively influenced by the addition of phytosterols to butter. These data correlated with the previously mentioned results about butter titratable acidity.

3.3. Sensory analysis

Foods sensory profile is very important for the consumer acceptability. Phytosterols-enriched butter were analysed and their sensory profiles were established (Figure 2).

![Figure 2. Sensory analysis of butters.](image)

Mean with different letters within a column are significantly different \((p<0.05)\)
A - control sample with no added phytosterols; B - 0.26% phytosterols added before cream pasteurization; C – 0.26% phytosterols added before cream ripening; D - 0.26% phytosterols added before working the butter into a continuous fat phase; TA – titratable acidity.

The butters fortified with phytosterols were characterized by high sensory scores comparable with those of the control sample. These results contradict the data obtained by authors who found a lower overall acceptability of butter enriched with phytosterols [21]. This can be explained by cholesterol reduction and evening primrose oil addition in their study which reflected on the overall sensory profile.

A research team stated a softer consistency in the phytosterol-enriched butter which was due to an emulsion softer than the original butter. In our case, the consistency of butter enriched in phytosterols, showed no significant difference in comparison to the control sample \((p>0.05)\) [22]. This can be explained with the fact that in the present study a minimum recommended concentration of fortification was applied.

4. Conclusions

This study suggested that the addition of phytosterols in butter could be performed at different stages of the technological process with no reflect on the phytosterol content in the final product \((p>0.05)\). No significant differences between fat and moisture content of butters produced from different batches were found \((p>0.05)\). No changes of the microbiological profile were established \((p>0.05)\). The obtained butters were characterized by similar sensory profiles \((p>0.05)\) in the range of used phytosterols quantities. From a safety point of view, it is appropriate to add the phytosterols before pasteurization process. The obtained butter is considered as functional product with potential health effects.
The effects of phytosterol fortification on the rheological properties of butter fortified with phytosterols and the oxidative stability of the functional ingredient during storage will be evaluated.

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