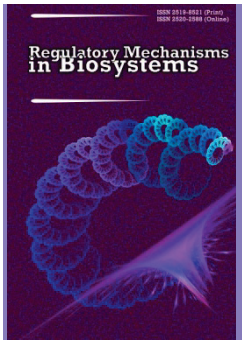

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2023, 14(1), 48–54
doi: 10.15421/022308

Dynamics of 17β-estradiol under influence of technological operations during production of dairy products

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Article info

Received 04.01.2023
Received in revised form 05.02.2023
Accepted 07.02.2023

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Kochetova, H. S., Kukhtyn, M. D., Salata, V. Z., Horiuk, Y. V., Kladnytska, L. V., Matviishyn, T. S. (2023). Dynamics of 17β-estradiol under influence of technological operations during production of dairy products. Regulatory Mechanisms in Biosystems, 14(1), 48–54. doi:10.15421/022308

Safety of milk can decline because of high concentrations of steroid hormones like 17β-estradiol, which is associated with the development of some oncological diseases and reproductive disorders. We studied the effects of thermal processing of raw milk and technologies of production of butter and yogurt on the concentration of 17β-estradiol. For this purpose, we determined the amount of 17β-estradiol in raw milk, after pasteurization under different regimes, boiling and during the production of butter and yogurt. Content of 17β-estradiol was determined using the method of immunoenzymatic analysis. We determined that low-temperature processing of milk at the temperature of 77.0 ± 1.0 °C for 1 min caused no changes in the structure of the estrogenic hormone 17β-estradiol, resulting in practically no changes in its amount in pasteurized milk. We determined that 17β-estradiol in milk is a temperature-stable hormone with no tendencies towards significant decrease when subject to high-temperature processing (85.0 ± 1.0 °C for 1 min) and during boiling, because the amount of the hormone decreased on average by 5%. Therefore, we may state that after pasteurization or sterilization, the concentration of 17β-estradiol in drinkable milk would not be significantly different from its initial amount in raw milk. We determined significant increase in 17β-estradiol in butter (3896.1 ± 67.5 pg/g), as compared with the concentration in raw milk (189.4 ± 12.5 pg/mL), and its insignificant content in buttermilk was insignificant (29.3 ± 1.8 pg/mL). The concentration of 17β-estradiol in milk decreased by 25% during 9-month storage at the temperature of –18 °C and by 20% at the temperature of –9 °C. This process can be applied to butter made from milk of cows at late stages of lactation, which contains high level of estrogen. We determined that the steroid hormone 17β-estradiol did not break down under the influence of dairy acid that accumulates as a result of lactic acid fermentation, both with the participation of mixed microflora of raw milk and pure lactic-acid bacteria of fermentation starter for yogurt. The prospects of the studies are the development of a safe maximum allowable level of 17β-estradiol in raw milk and methodological evaluation at a milk-processing factory.

Keywords: estrogen hormones; safety; raw milk; pasteurization; thermal processing.

Introduction

Ensuring food safety is a basic requirement for its production, storage, and trade (Kukhtyn et al., 2020). Taking into account the nutrition value, benefits and scales of consumption of milk and dairy products, the evaluation of quality and safety of this category of products is crucial (Yukalo et al., 2019a). Dairy products are considered a source of various environmental contaminants such as heavy metals (Kukhtyn et al., 2021), pesticides, antibiotics (Bosma et al., 2020), microorganisms (Kang et al., 2020), steroid hormones (Jiang et al. 2018; Snoj et al., 2017), etc. Among them, a certain content of esterogenic hormones in milk and dairy products is considered a physiologically natural process (Jouan et al., 2006; Pu et al., 2019; Wang et al., 2020), because they carry out a number of important functions in the body (synthesis of protein, transmission of signals between receptors, growth, regulation of reproduction) (Malekinejad et al., 2006; Hirpessa et al., 2020; Mo et al., 2021). Esterogenic hormones are synthesized by the glands of internal secretion and partially by the mammary gland (Janowski et al., 2002; Feng et al., 2016) and are removed with urine and milk (Hirpessa et al., 2020). The cow milk contains certain natural esterogenic hormones: 17β-estradiol, 17α-estradiol, estriol and estrone (Domenech et al., 2011; Varriale et al., 2015), of which 17β-estradiol is potentially the strongest (Snoj et al., 2017; Lyu et al., 2022). Furthermore, dairy products can include man-synthesized hormones if they are used as growth stimulators and to increase milk production (Capriotti et al., 2015; Snoj et al., 2017; Wang et al., 2020). Low concentrations of natural estrogens are found in milk of non-heifers, whereas hormone concentration increases during pregnancy, peaking in the third trimester (Malekinejad et al., 2006; Tong et al., 2016; Palacios et al., 2020). Since milk is obtained mostly (80%) from heifers (Qaid & Abdoun, 2022), high concentrations of esterogenic hormones, particularly 17β-estradiol, raise concerns among scientists because of possible negative impact on the health of consumers. Some researchers (DeMaleki et al., 2010; Ganmaa et al., 2012; Pettersson et al., 2012; Wang et al., 2015) assume possible (direct or indirect) correlation between consumption of milk and dairy products and reproductive disorders or incidence of oncological diseases. According to some studies (Maruyama et al., 2010),

men who had been consuming at least 600 mL of cow milk per m^2 of body surface had higher content of steroid hormones (estradiol, estrone and progesterone) in the urine and blood serum. Those men had decreased testosterone and hypophyseal gonadotropins in blood serum approximately one hour after intake of milk. Also, we should mention (Pettersson et al., 2012; Wang et al., 2015) the negative effect of high concentrations of esterogenic hormones in dairy products on the development of the reproductive and nervous systems of pre-puberty children.

At the same time, other researches (Dong et al., 2011; Furnari et al., 2012; Grgurevic et al., 2016) are not that certain about the negative effects estrogenic hormones in dairy products have on consumers. In particular, (Davoodi et al., 2013) reports decrease in incidence of cancer of the rectum, ovaries, mammary gland and bladder among milk consumers. The studies (Radko & Posyniak, 2021) on rats fed with commercial milk and artificial estrogen-free milk (the control group) revealed no changes in blood and the reproductive system. Almost similar results were obtained by (Furnari et al., 2012), reporting that increases in the concentrations of 17β-estradiol and estrone, decrease in testosterone in the blood serum and uterotrophic changes in the womb occurred only after intake of milk with the estrogen concentration being 1,000 times higher than the average values for milk. Therefore, there is no unanimous opinion on effects the milk estrogens have on human health. Also, maximal thresholds of 17β-estradiol concentration in milk and dairy products have not been determined, though for such products as meat, it is regulated by the European Union Reference Laboratory (EURL) – CRL Guidance Paper (7 December 2007) – CRLs' View on State of the Art Analytical Methods for National Residue Control Plans. At the same time, it is indicated in the Codex Alimentarius Commission. As with the maximum residue limits (MRLs) and the Risk Management Recommendations (RMRs) for residues of veterinary drugs in foods, the CAC/MRL 2-2015 lists indicate that the amount of 17β-estradiol that is ingested with food must not exceed 50 ng/kg per day.

The data on presence of 17β-estradiol in milk also vary because its content depends on many factors, in particular phisiological condition of the animals (stage of lactation, estrous cycle, fat content in milk) (Antignac et al., 2003; Kukhtyn et al., 2022; Salata & Kochetova, 2022). It is reported (Domenech et al., 2011) that the milk sold in Iran contains 17β-estradiol ranging in the broad range of 77 to 930 pg/mL, with the average concentration of 330 pg/mL. Another study (Malekinejad et al., 2006) found that the concentration of 17β-estradiol in raw milk from cows during the first trimester of pregnancy equaled on average 10 pg/mL, and in milk from cows in the third trimester, the amount of the hormone increased to 60 pg/mL. In the samples of pasteurized and sterilized non-fat milk, the average content of 17β-estradiol accounted for 10 pg/mL (Qaid & Abdoun, 2022). According to Janowski et al. (2002), Malekinejad et al. (2006), the concentration of 17β-estradiol in milk was 15–50 pg/mL. Because the steroid hormone 17β-estradiol is a lipophile, its concentration in all high-fat dairy products was several times greater than in defatted products (Wang et al., 2015). The researchers (Palacios et al., 2020; Kilic-Akyilmaz et al., 2022) who studied the effects of pasteurization on the content of 17β-estradiol in milk reported insignificant changes in this hormone in pasteurized milk. At the same time, the scientific literature lacks data on the influence of milk processing technology on the concentration of esterogenic hormones in products.

Therefore, taking into account that the main source of animal-derived estrogens (60–70%) in human diet is milk and dairy products (Domenech et al., 2011; Qaid & Abdoun, 2022), studies of how the technologies of milk processing affect the concentration of 17β-estradiol in the final products would suggest technological operations that could decrease its content.

The objective of our study was determining the quantitative changes in 17β-estradiol during thermal processing of raw milk and technological process of production of milk and yogurt.

Materials and methods

We analyzed the samples of raw milk with different concentrations of 17β-estradiol after thermal processing and the processing into dairy products, in particular, 5 samples of milk after pasteurization at various temperatures, 10 samples during the production of milk, 4 during storage of butter at different temperatures; 5 during the production of yogurt, 5 during souring of milk. For the production of yogurt, we used fermentation starter composed of cultures of lactic-acid bacteria *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

At the first stage of the study, we analyzed the effects of various pasteurization regimes on the dynamics of 17β-estradiol in pasteurized milk. At the second stage, we monitored changes in the concentration of 17β-estradiol in relation to the technologies of production of high-fat dairy products – butter with various contents of fat. We studied the influence of various regimes of refrigerator storage of butter on 17β-estradiol in the conditions of the standard regimes of butter storage according to DSTU 4399:2005. Butter. Specifications. National Standard of Ukraine, specifically at the temperature of 0 °C for 3 months, minus 9 °C for 9 months, and at minus 18 °C for 9–12 months. At the third stage, we examined the influence of lactic-acid fermentation with participation of fermentation-starter microorganisms on the content of 17β-estradiol in fermented milk products. Raw milk samples were collected in sterile laboratory flasks during the transportation to a milk-processing factory from different dairy farms. The samples were delivered to the laboratory for examination in a refrigerator bag.

The amount of 17β-estradiol in the samples was determined using an immune-enzymatic analysis with a Ridascreen®17β-őstradiol (R-Biopharm, Germany) test system, according to the manufacturer's recommendations. Prior to the studies, the milk samples were heated in a thermostat up to the temperature of 20–25 °C and homogenized using an IKA (T 18 Basic) homogenizer with nozzles (S 18 N-10 G) in order to obtain uniformity. To chart a calibration scale, we used a solution of 17β-estradiol in the concentrations of 0, 50, 200, 800, 3,200 and 12,800 pg/mL. Into a micro-titer plate, sensitized by antibodies to 17β-estradiol, we introduced 20 μL of the solutions and the examined samples and also 50 μL of diluted preparation of antibodies and conjugate of 17β-estradiol into each well. We incubated the plate for 2 h at the temperature of 20–25 °C. Then, using a device (Biorad PW 40), we rinsed the plate wells with distilled water. Into each well, we added 50 μL of the solution of substrate and chromogen and incubated again for 30 min at 20–25 °C. After the incubation, we added 100 μL of stop reagent into each well. The optical density was measured using a Sunrise immune-enzymatic reader (Austria) at the wavelength of 450 nm. The computer analysis of the measurements was carried out in Rida®Soft.

The data were statistically analyzed using the ANOVA disperse analysis. The data are presented as $x \pm SD$ (mean ± standard deviation). The significance of the obtained data was evaluated by the F-criterion with the significance levels of $P < 0.05$ (taking into account the Bonferroni's correction).

Results

The low-temperature (smooth) regime of thermal processing of raw milk is most often used for pasteurization of raw milk, i.e. at the temperature of 77.0 ± 1 °C for 1 min. At the same time, we used the samples of raw milk with various initial contents of 17β-estradiol, from cows at different lactation stages. In the samples of the first group, 17β-estradiol accounted for 17.3 ± 1.5 pg/mL; it was 57.6 ± 3.9 pg/mL from the second and 409.5 ± 34.1 pg/mL from the third.

After low-temperature pasteurization regime (Fig. 1), we observed no significant changes in 17β-estradiol in pasteurized milk, compared with raw milk. Particularly, when the hormone in raw milk was in the lowest concentration of 17.3 ± 1.5 pg/mL, its content in pasteurized milk decreased to 16.0 ± 1.2 pg/mL, i.e. only by 1.3 pg/mL ($P > 0.05$). In the second variant of the experiment, having the initial hormone content of 57.6 ± 3.9 pg/mL, its decrease in pasteurized milk was also insignificant, accounting only for 2.8 pg/mL ($P > 0.05$). In the third variant of the experiment, where the initial 17β-estradiol in raw milk was the highest, its amount decreased by 17.8 pg/mL ($P > 0.05$) after pasteurization, equaling 391.7 ± 31.4 pg/mL. Therefore, the results of the experiment suggest that low-temperature processing of milk at 77.0 ± 1.0 °C for 1 min caused no changes in the structure of estrogenic hormone 17β-estradiol, having almost zero effect on its amount in pasteurized milk.

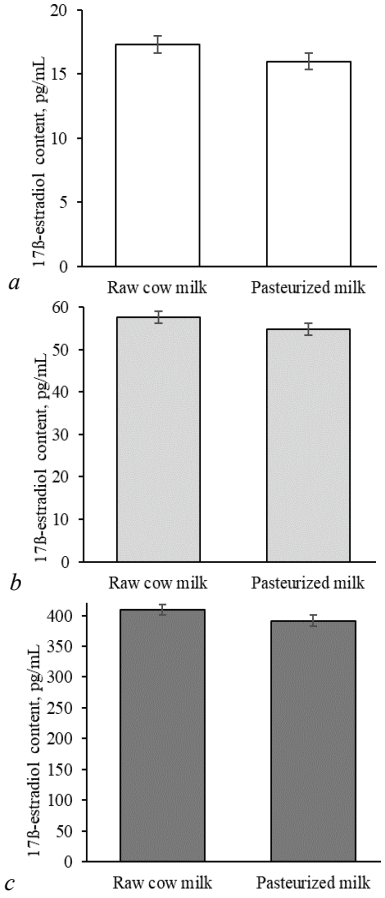

Fig. 1. Influence of milk pasteurization at the temperature of 77.0 ± 1 °C for 1 min on the content of 17β-estradiol: initial content of 17β-estradiol *a* – 17.3 pg/mL, *b* – 57.6 pg/mL, *c* – 409.5 pg/mL; x ± SD, n = 5; contents of 17β-estradiol had no statistical differences in the samples according to ANOVA (P > 0.05)

For lower-grade raw milk – according to the amount of mesophilic aerobic and facultative anaerobic microorganisms – the factory uses "severer", higher, temperatures of thermal processing so as to most efficiently kill vegetative forms of microorganisms. Therefore, we studied how 17β-estradiol in drinking milk is affected by 1 min pasteurization at the temperature of 85.0 ± 1.0 °C (Fig. 2).

We determined (Fig. 2) that 1 min pasteurization at 85.0 ± 1.0 °C had no significant effect on the dynamics of 17β-estradiol decrease in milk. In particular, the results were almost identical to milk that had been subjected to 77.0 ± 1.0 °C thermal processing. Therefore, in the raw milk with the lowest concentration of 17β-estradiol, it decreased only by 2.1 pg/mL (P < 0.05) after the pasteurization, accounting for 15.2 ± 1.2 pg/mL on average. In the second variant of the experiment with the initial 17β-estradiol content of 57.6 ± 3.9 pg/mL, the amount of the hormone in pasteurized milk equaled 53.1 ± 3.0 pg/mL, i.e. 4.5 pg/mL (P < 0.05) lower on average than in raw milk. In the third variant, with the highest initial amount of the estrogenic hormone, 17β-esradiol concentration after pasteurization was 390.4 pg/mL on average, which was 19.1 pg/mL (P < 0.05) lower than its content prior to pasteurization.

Therefore, the results indicate that 17β-estradiol in milk is stable to the temperature and has no tendency towards decrease when subject to high-temperature processing. In general, the study suggests that even high-temperature heating regimes do not decrease the hormone content in pasteurized milk by more than 5% of its initial concentration.

Other than pasteurization, raw milk can sterilized at the temperature of 100 °C and more in order to completely remove all forms of microorganisms. Sterilized milk has a number of advantages over pasteurized regarding its possible long shelf life (half a year and more). Therefore, we studied the effect of 5 min boiling of raw milk on 17β-estradiol (Fig. 3).

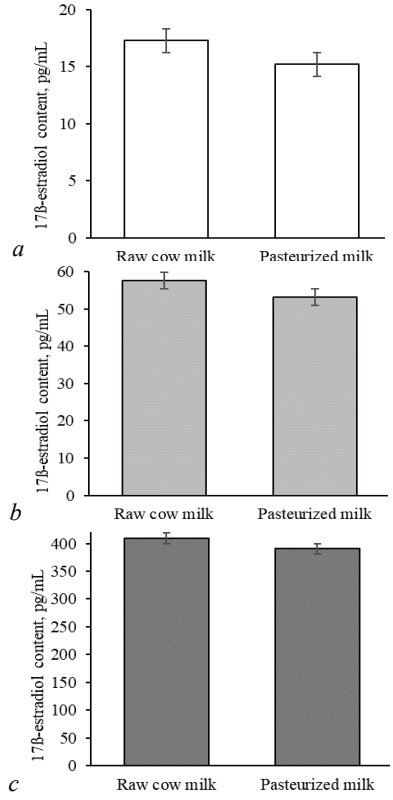

Fig. 2. Influence of milk pasteurization at the temperature of 85.0 ± 1 °C for 1 min on 17β-estradiol: initial content of 17β-estradiol *a* – 17.3 pg/mL, *b* – 57.6 pg/mL, *c* – 409.5 pg/mL; x ± SD, n = 5; no statistical differences were found according to ANOVA (P > 0.05)

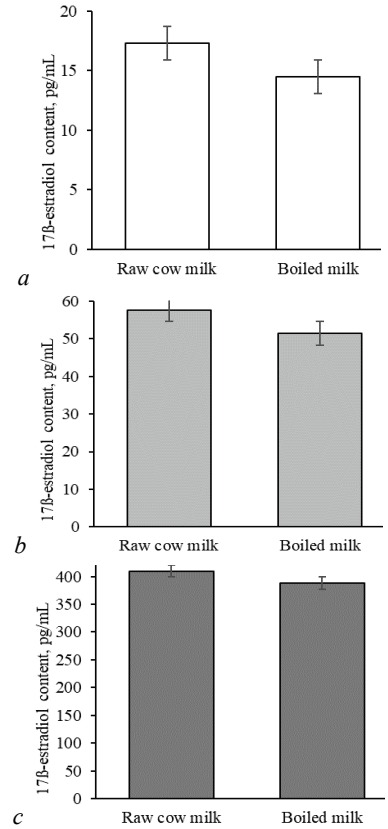

Fig. 3. Influence of 5 min boiling of raw milk on 17β-estradiol: initial content of17β-estradiol *a* – 17.3 pg/mL, *b* – 57.6 pg/mL, *c* – 409.5 pg/mL; x ± SD, n = 5; no statistical differences were found according to ANOVA (P > 0.05)

The data in Figure 3 shows that boiling of raw milk for 5 min significantly did not affect the concentration of 17β-estradiol. The general tendency was the same as during the pasteurization at low and high temperatures. However, after boiling, the amount of hormone in the first variant of the experiment decreased by 2.8 pg/mL on average ($P < 0.05$), compared with 2.1 pg/mL after pasteurization at the temperature of 85.0 ± 1 °C.

In the second variant of the experiment with 57.6 ± 3.9 pg/mL initial amount of 17β-estradiol, the content of the hormone decreased in boiled milk by 6.2 pg/mL on average ($P < 0.05$), against 4.5 pg/mL in milk pasteurized at 85.0 ± 1.0 °C. At the same time, in the third variant of the experiment, with the highest content of esterogenic hormone in raw milk, the decrease in boiled milk accounted for 21.3 pg/mL on average ($P < 0.05$). However, we observed no difference between 17β-estradiol in boiled and pasteurized milk at the highest temperature.

Therefore, the study suggests that despite the fact that boiling – as a thermal process – causes more intensive changes in the physical-chemical composition of milk, we saw no significant ruination of esterogenic hormone 17β-estradiol.

We examined butter made in the conditions of two production technologies: extra with 82.0% fraction of milk fat and village oil with 72.5% fat content (Fig. 4). We determined that after using the technologies of separation of milk and cream, the concentration of 17β-estradiol in cream was on average 7-fold higher ($P < 0.05$) than in milk. After churning cream, in ready goods with massive fat contents of 72.5% and 82.0%, the amounts of 17β-estradiol equaled 3361.0 ± 54.3 and 3896.1 ± 67.5 pg/mL, respectively. That is, the content of 17β-estradiol in butter increased 2.6 and 2.9-fold ($P < 0.01$), compared with its concentration in cream, depending on fat content in it.

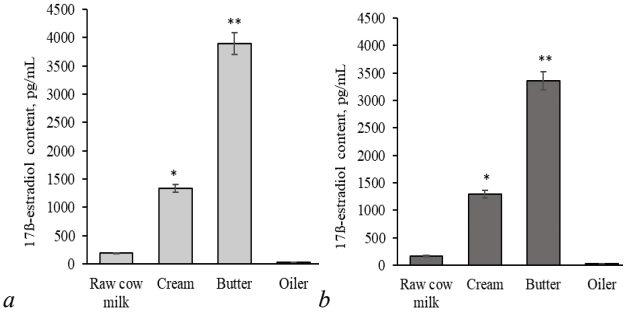

Fig. 4. The dynamics of change in 17β-estradiol in the conditions of production of butter: *a* – butter with 82.0% fat content, *b* – butter with 72.5% fat content; x ± SD, n = 10; * – $P < 0.01$ compared with the concentration in cream, ** – $P < 0.001$ compared with the concentration in milk

At the same time, the samples of buttermilk were observed to contain less 17β-estradiol than the butter samples and even raw milk. In particular, the hormone concentration in buttermilk from two types of butter was within 27.6–29.3 pg/mL, indicating poor solubility of 17β-estradiol in aqueous solutions and its hydrophoby.

Thus, to generalize the results of this experiment, we can note that in the conditions of butter production using the method of churning cream, the main fraction of 17β-estradiol was concentrated in butter, while buttermilk obtained on average 0.8% of the hormone of its overall amount in butter.

We observed a tendency towards decrease in the content of 17β-estradiol in butter after maintenance at 0 °C for 3 months, minus 9 °C for 9 months, and minus 18 °C for 9–12 months (Fig. 5), but the most intensive decrease was produced by the lowest-temperature regime (–18 °C). In particular, after 3-month maintenance at 0 °C, as allowed according to the standard, the content of 17β-estradiol decreased only by 3.6%. Over twice as long period (6 months), decrease in the hormone was insignificant, up to 5%.

Decrease in the regime temperature of butter storage to –9 °C had a more intensive effect on decrease in 17β-estradiol, compared with the temperature of 0 °C. Therefore, after three months of the product storage, the amount of the hormone decreased on average by 12.2%, which was almost 3.4 times ($P < 0.05$) more intensive dynamics than at the temperature of 0 °C. The continued storage of butter for three more months (6 months from the start) also caused a decrease in 17β-estradiol, on average by 1.5 times ($P < 0.05$) to 18.0%. At the same time, the concentration of the hormone in butter in that period was 3.8 times ($P < 0.01$) lower compared with maintenance at 0 °C. However, further storage up to 9 months had no significant effect on 17β-estradiol in butter, because its content decreased by 20.5% of the initial amount in fresh product.

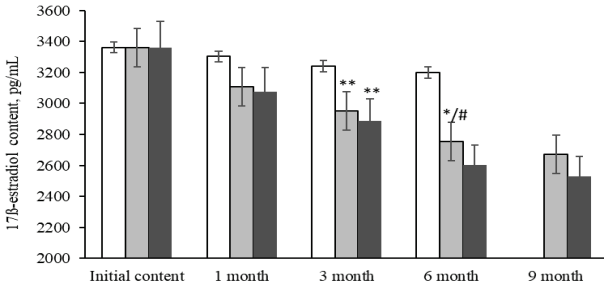

Fig. 5. Changes in 17β-estradiol in butter during storage in refrigerator: maintenance at the temperature of 0 °C (solid line), maintenance at the temperature of –9 °C (dash line) and maintenance at the temperature of –18 °C (dotted line); x ± SD, n = 4; * – $P < 0.05$ compared with the changes over 3 months of storage, ** – $P < 0.01$ compared with maintenance at 0 °C, # – $P < 0.01$, compared with maintenance at 0 °C

In the conditions of storage at –18 °C, decrease in esterogenic hormone was the most intensive. Therefore, after three months of storage, the concentration of 17β-estradiol decreased on average by 14.1%, which was 3.9 times ($P < 0.01$) more intensive than after maintenance at 0 °C and only 1.2 times lower compared with –9 °C maintenance. In the ninth month of storage, the content of 17β-estradiol was lower on average by 24.7%, against 20.5% at –9 °C storage.

Thus, this experiment suggests that by storing butter at –18 °C for 9 months, there could be achieved 25% decrease in 17β-estradiol, and 20% decrease at –9 °C. Therefore, butter with significant content of 17β-estradiol will undergo a significant decrease in estrogenic hormone during frozen storage regime. This phenomenon can be used for butter made of milk from cows at the late stages of lactation, when it contains high level of estrogen.

The next stage of the study was determining how 17β-estradiol is affected by lactic-acid fermentation. The study was carried out in two variants: in the first, we determined quantitative changes in the hormone in milk during self-souring. In the second variant, we analyzed changes in 17β-estradiol during the technological process of yogurt production, i.e. in the conditions of pure cultures of lactic-acid microorganisms. In the experiment, we used raw milk with different initial contents of 17β-estradiol. The results of the study of the first variant of the experiment are presented in Figure 6. During fermentation, in which raw milk microflora is involved, 17β-estradiol underwent no significant changes in all the variants of the experiment. The amount of esterogenic hormone in sour milk after 48 h fermentation decreased on average by 2–7 pg/mL, though the data are not significant compared with the initial 17β-estradiol. At the same time, the titrated acidity of milk was 48 ± 4 °T 12 h after souring, 78 ± 5 °T after 24 h, and 124 ± 7 °T after 48 h. That is, the milk acidity increased, while no change in the content of hormone was observed.

The results of the study of changes in 17β-estradiol during the yogurt production technology are presented in Figure 7. The fermentation lasted for 6 h until the titrated acidity was within 80–85 °T. After pasteurization and homogenization of milk (Fig. 7), the concentration of 17β-estradiol decreased on average by 2.7 pg/mL ($P < 0.05$) in the samples with its insignificant content and by 7.7 pg/mL ($P < 0.05$) in the samples where its content was high. During fermentation of milk, the amount of the hormone in the both variants decreased insignificantly, by 1.7 and 8.2 pg/mL respectively. In general, during yogurt production, the concentration of the hormone decreased by 4.4 pg/mL in the variant with its insignificant initial amount and by 15.9 pg/mL ($P < 0.05$) in the variant with significant amount of 17β-estradiol. This process was mostly related to homogenization. Therefore, during the lactic-acid fermentation, both with participation of raw milk microflora and using pure cultures of lactic-acid bacteria, we saw no significant changes in 17β-estradiol in finished products.

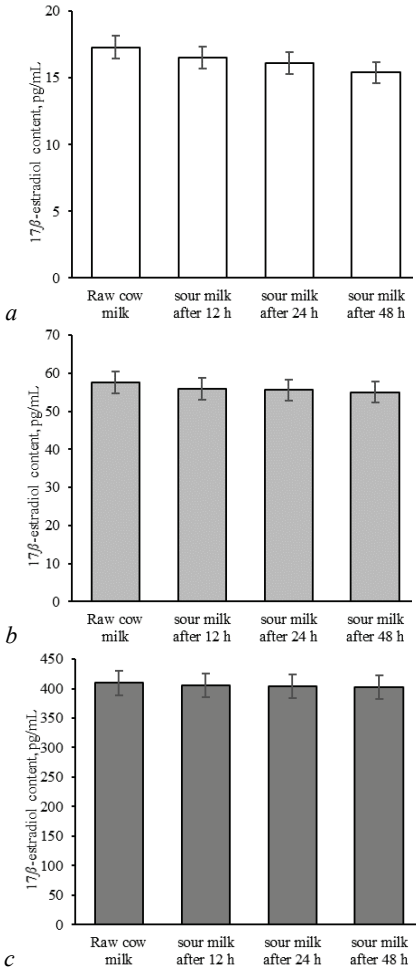

Fig. 6. The influence of lactic-acid process on changes in 17β-estradiol during self-souring of milk: initial content of 17β-estradiol *a* – 17.3 pg/mL, *b* – 57.6 pg/mL, *c* – 409.5 pg/mL; x ± SD, n = 5; contents of 17β-estradiol in the samples had no statistical differences according to ANOVA (P > 0.05)

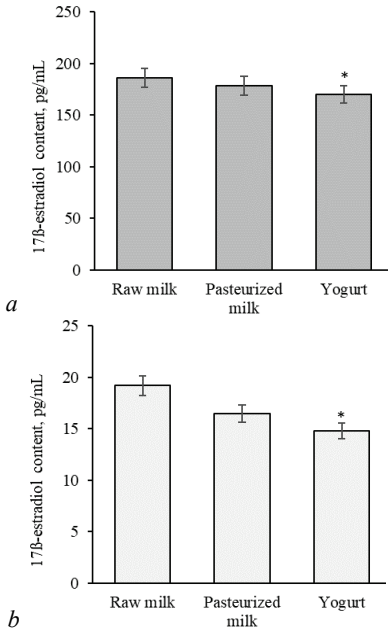

Fig. 7. Changes in the content of 17β-estradiol in the conditions of yogurt production technology: initial content of 17β-estradiol *a* – 186.1 pg/mL, *b* – 19.2 pg/mL; x ± SD, n = 5; * – P < 0.05 compared with the content in raw milk

Discussion

Milk and dairy products play an important role in healthy diet throughout life (Yukalo et al., 2019b). However, the recent research has cast doubt on the safety of milk because of high concentrations of steroid hormones such as 17β-estradiol, which is attributed to the development of some oncological diseases (DeMaleki et al., 2010; Ganmaa et al., 2012; Pettersson et al., 2012; Wang et al., 2015). Taking this fact into account, the search for technological means of most effectively reducing 17β-estradiol concentration during milk processing is considered to be especially relevant. In our study, we saw that the thermal low-temperature pasteurization (77.0 ± 1.0 °C for 1 min) did not significantly change the hormone concentration in processed milk. Subject to high-temperature regime of milk pasteurization (85.0 ± 1.0 °C for 1 min) and boiling for 5 min, 17β-estradiol in processed milk decreased at most by 5%. This is related to the fact that 17β-estradiol is a thermostable hormone (Domenech et al., 2011; Varriale et al., 2015; Pu et al., 2019). Therefore, it undergoes no significant destruction under high-temperature regimes that are used in the dairy industry for pasteurization and sterilization of raw milk. Therefore, we may state that after pasteurization or sterilization, the concentration of estradiol in dairy products would not be significantly different from its initial amount in raw milk. Presence of high and low concentrations of estrogens, including 17β-estradiol, in pasteurized drinking milk was described by other researchers (Malekinejad et al., 2006; Domenech et al., 2011; Qaid & Abdoun, 2022), though they did not indicate the concentration of hormones in raw unprocessed milk. According to the researchers (Malekinejad et al., 2006; Snoj et al., 2017; Kukhtyn et al., 2022), concentrations of 17β-estradiol and other estrogene hormones in drinking milk and dairy products mainly depend on mass fraction of fat and physiological condition of animals (Tong et al., 2016; Palacios et al., 2020; Salata & Kochetova, 2022) rather than on influence of thermal processing. In the studies (Kukhtyn et al., 2020) that analyzed how e boiling of beef meat (1 h) affects the concentration of synthetic hormone zeranol, there was determined no significant decrease of it, because its concentration decreased in processed milk only by 6.1 ± 0.2%. Similar results were obtained by (Mugo & Lu, 2022), who determined the dynamics of changes in steroid estrogens during thermal processing of meat.

We also determined distribution of 17β-estradiol during production of butter, because earlier, estrogen concentration was determined (Kilic-Akyilmaz et al., 2022) to correlate with the content of dairy fat. The results revealed significant increase in 17β-estradiol in butter (3,896.1 ± 67.5 pg/g), compared with its content in raw milk (189.4 ± 12.5 pg/mL) and its amount in buttermilk was insignificant (29.3 ± 1.8 pg/mL). Therefore, we think that in order to decrease steroid estrogens in diet, consumption of dairy fat should be decreased. High-fat types of butter are a large source of daily ingress of 17β-estradiol into consumers' organisms. In our opinion, technology of milk production cannot be used so as to decrease estradiol in case of its high content in raw milk or cream. For this purpose, there should be a search for other possible processing technologies or storage regimes to reduce 17β-estradiol in raw milk, especially milk obtained from cows during third trimester of pregnancy.

Research (Kukhtyn et al., 2020) has shown that a cold regime of beef maintenance effected about 30% reduction of such estrogenic synthetic hormone as zeranol after 3 month storage. The indicated study also revealed that a rather effective and safe process that decreased 17β-estradiol by 25% is 9 month storage of butter at the temperature of –18 °C, as required by the standard (DSTU, 2006). This storage regime can be used for decreasing estrogenic hormones in butter made of milk from cows at late stages of lactation, with high concentration of 17β-estradiol (Tong et al., 2016; Palacios et al., 2020).

Also, we studied changes in estradiol content in raw milk during spontaneous souring and in the conditions of yogurt production technology, since the literature contains no sufficient data on how lactic-acid fermentation affects concentrations of estrogens. The reports (Snoj et al., 2017) indicate that amount of estrogen hormones in sour milk is no different from such in drinking milk. In our experiment, we took samples of milk with various amounts of the hormone, since its high concentrations should be seen to have appreciable changes if such occur. We determined that the amount of 17β-estradiol in sour milk after 48 h spontaneous fer-

mentation was not significantly different from its initial content (409.5 ± 13.7 pg/mL against 402.4 ± 10.9 pg/mL). During yogurt production with the participation of fermenting bacteria, the amount of the hormone after 6 h of fermentation accounted for 170.2 ± 7.3 pg/mL, compared with 178.4 ± 8.1 pg/mL in pasteurized milk. This indicates that steroid hormone 17β-estradiol did not breakdown during exposure to lactic acid that accumulates as a result of lactic-acid fermentation, involving mixed microflora of raw milk, as well as with participation of lactic-acid bacteria of yogurt fermentation starter.

Therefore, to sum up the research of whether various technological regimes can decrease 17β-estradiol in milk and dairy products, we may assume the following. Such technological operations of raw milk processing as low- and high-temperature pasteurization and sterilization have almost zero effect on 17β-estradiol in food products. Also, we determined that the highest amount of the estrogen hormone was in buttermilk with largest content of milk fat. At the same time, storage of butter in frozen state at the temperatures of –9 °C and –18 °C for 6–9 months led to approximately 20–25% decrease in 17β-estradiol. Therefore, we may recommend the producers to store butter in frozen state to reduce the hormone content, especially products made of milk from cows at the end of their lactation period.

Conclusions

The content of estrogen hormone 17β-estradiol undergoes almost no changes after low-temperature pasteurization (77.0 ± 1.0 °C) of milk for 1 min. High-temperature pasteurization, at 85.0 ± 1.0 °C, with maintenance for 1 min, and boiling caused an average 5% decrease in the hormone concentration.

Using cream churning technology of butter production, the main fraction of 17β-estradiol concentrated in butter, while buttermilk obtained on average 0.8% of its total amount in butter. Nine-month storage of butter at the temperature of –18 °C caused 25% decrease in 17β-estradiol, and 20% decrease occurred after storage at –9 °C. This process could be used for butter made of milk from cows at late lactation stages, which contains high estrogen level.

During lactic-acid fermentation with the participation of raw-milk microflora of, and also using pure cultures of lactic-acid bacteria, we observed no significant changes in the concentration of 17β-estradiol in food products.

Authors declare no conflict of interest.

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