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OPTIMIZATION OF CONDITIONS FOR THE ACTIVATION OF XANTHINE OXIDASE AND THE FORMATION OF NITRIC OXIDE IN FRESH SHEEP MILK

Abstract. For the detection of sheep milk XO activity a various natural and artificial antioxidants were examined. Among the natural antioxidants L-cysteine was more effective in the stabilization of XO in heated milk. XO of sheep milk activated by heat treatment in the presence of cysteine and molybdenum became able to convert nitrate and nitrite to nitric oxide (NO). Therefore, L-cysteine was used for double purposes: as the protector of enzyme active center against the oxidation during heat treatment of milk and as a reagent for S-nitrosothiol formation. Hypoxanthine, a natural substrate of XO, was the effective electron donor for NaR and NiR activities. Heat treatment of the milk in the presence of exogenous lecithin increased the activity of NaR and NiR of XO and CysNO formation. Thus, during the heat treatment: a) excess of exogenous phospholipids disintegrate the structure of MFGM and b) enzyme molecules denatured partially and their active center became available for exogenous cysteine, molybdenum, hypoxanthine and nitrate or nitrite.

Key words: milk, xanthine oxidase, NO, NO_3, NO_2, antioxidants, molybdenum, tungsten, phospholipids.

Introduction. At present, the pollution of environment with nitrates is a big problem in the world. In the environment and in human and animal body a microorganisms may convert nitrates to nitrites.

It is well known that nitrates (NO_3-) and nitrites (NO_2-) cause various diseases, including cancer. It is well known that nitrites irreversible bind to haemoglobin forming methhaemoglobin which losses the ability to transport oxygen [1]. Deficiency of oxygen causes asphyxiation and it is particularly hazardous to health for babies. Furthermore, nitrites easily bind to primary amines, such as cadaverine, putrescine, spermidine and form potential carcinogens – nitrosamines [2, 3].

In 1980 one of the authors of this article for the first time observed that purified and homogeneous xanthine oxidase (XO) of cow's milk has the ability to reduce NO_3^- and NO_2^-[5]. Later, other groups of scientists have found that animal XO converts NO_3^- and NO_2^- to physiologically important gas – nitric oxide (NO) [6, 7, 8]. It is generally recognized that NO is one of the major biological messenger molecules, regulating blood pressure and blood flow, neurotransmission and brain function, immune system function, wound healing inhibition of platelet aggregation. NO is also involved in defense mechanisms against pathogens and some kinds of cancer cells [9]. In 1992 the nitric oxide was recognized as a molecule of year and in 1998 scientists studying its properties have been awarded the Nobel Prize [10].

Because of the ability to form nitric oxide the XO of milk harbors an antimicrobial activity. It is known that NO as the oxidant is a strong antibacterial agent. Antibacterial functions of XO are associated with peroxynitrite (ONOO-) which is the product of the reaction between NO and $\cdot O_2$ (superoxide anion). XO is also involved in protective an antiviral responses by catalyzing the conversion of retinaldehyde to retinoic acid. Retinoic acid derivatives can inhibit viral replication and, thus, preventing the spread of viral disease [11, 12].

Xanthine oxidoreductase or dehydrogenase/oxidase (XO; EC 1.1.3.22) – molybdenum and iron-containing flavoprotein. Each of the two subunits of XO contains one molybdenum center, two iron-sulfur center and one FAD. XO of the breast milk of women shows a molecular mass of 160 kDa subunit, XO of the cow's milk - 150 kDa. It is believed that the main biological function of XO is the catalyses the final step of purine oxidation in eucaryotes, it catalyzes the sequence of hydroxylations that convert hypo-xanthine to xanthine, then to uric acid [13]. However, the enzyme has broad substrate specificity and is capable of reducing oxygen to generate the reactive oxygen species (ROS), superoxide and hydrogen peroxide, as well as oxidative transformation of pteridines and some aliphatic and aromatic aldehydes. Xanthine oxidase (XO) is not strongly specific to the oxidation of hypoxanthine or xanthine, it may catalyze the oxidation of about thirty nitrogen containing heterocycles and aldehydes [13]. Therefore because of its multifunctional enzymatic reactions XO is considered as a potential enzyme detoxifying different xenobiotics [14]. It is known that numerous hetercyclic xenobiotics (including pesticides) are carcinogens. Thus, in the case of contamination of milk with harmful xenobiotics the active XO makes possible their biotransformation into harmless forms. XO reduces nitrite (and nitrate), yielding reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite [15].

In cow's milk XO localized in the inner membrane layer of the fat globules (XO content is about 8% of total proteins of cove milk fat globules) [16]. However, XO of milk fat globule membranes (MFGM) contains tenth times less molybdenum or does not contain it which results in a very low enzyme activity [17-19]. Study of the cofactor composition of XO isolated from cow's and women's milk showed that more than 85-90 % of enzyme molecules don't contain molybdenum in its active center and are inactive molecules [18]. The occurrence of xanthine oxidase in animal milk was a matter of slight controversy for a long time, because in the milk xanthine oxidation never been observed, although the levels of XO in the milk are relatively high. Presumably, this is due to the fact that before inclusion of Mo atoms in the active center of the enzyme, Mo-free molecules of XO already is associated with inner membrane of milk fat globules. As a result, of XO molecules become molecules without molybdenum. It is proposed that XO is involved in secretion of milk fat globules in a process dependent on the enzyme protein rather than on its enzymic activity [15].

Thus, in the presence of molybdenum atom in the active centre XO is active but in the absence of the metal it becomes inactive. Like other molybdoenzymes, in the active center XO contains so called molybdocofactor which has a pterin nature (therefore, it is called also molybdopterin) [20]. Molybdenum atom is bound to the pterin via its vicinal thiol groups (figure 1). This pterin is synthesized *in vivo* independently on the presence of molybdenum, i.e. in the absence of molybdenum cellular XO contains a normal amount of the cofactor. The cofactor is buried deeply within the interior of enzyme molecule and a tunnel-like structure makes it accessible to the appropriate substrates [21].

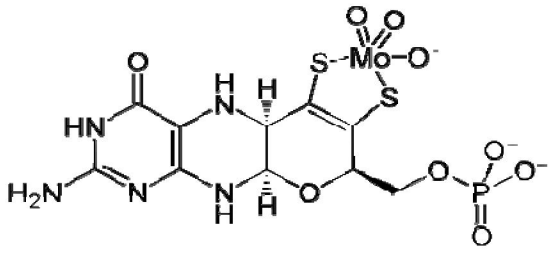

Figure 1 – Structure of molybdocofactor and its bond with molybdenum atom via vicinal SH-groups in the active center of XO [20]

Materials and Methods. *Preparation of the milk to the detection of the various enzymatic activities.* Before treatment in the milk of domestic animals added 10 μM ethylenediaminetetraacetic acid (EDTA) to bind heavy metals. For boiling fresh milk is poured into the narrow conical tubes in a volume of 2 ml. Then, for further determine of the enzymatic activity the tubes are placed in water bath with 35 °C temperature and kept for 10 min, then using the special reagents different activities of XO are determined.

Method for determination of the intrinsic activity of xanthine oxidase (XO). To determine the intrinsic activity of XO 200 μl aliquot of milk was mixed with 700 μl 0.1 M sodium phosphate buffer containing

10 μM EDTA, 5 μl phenylmethylsulfonylfluor (for inhibition of protease activity). To this mixture was added 100 μM 10 mM hypoxanthine. The mixture was incubated at 30°C for 10 min under aerobic conditions. The proteins in the mixture is precipitated with trichloroacetic acid. After centrifugation, the amount of uric acid (under the influence of XO converted hypoxanthine to uric acid) in the supernatant was determined by measuring the absorbance of the reaction mixture in a spectrophotometer at 295 nm [22]. The amount of uric acid also is determined by its chemical oxidation with potassium permanganate (K_2MnO_4) or hydrogen peroxide (H_2O_2), thereby forming allantoin [23]. Allantoin in biological liquids is easy to very accurately determined by a known method [24].

Method for the determination of nitrate- and nitrite-reducing activity of XO. Nitrate-reducing activity of XO determined by the disappearance of the added nitrate (NO_3^-) or by the appearance of nitrite (NO_2^-) in the reaction medium [5]. Nitrite-reducing activity of XO is determined by the disappearance of the nitrite to the reaction medium or by the appearance of nitric oxide (NO) [25].

Results and Discussion. *Proposed mechanisms of milk XO activation.* As mentioned above, milk XO in exists in molybdenum-free form and it is localized in the inner layer of MFGM. Therefore, the activation of milk XO requires the incorporation of exogenous molybdenum into its active center [4]. It is generally known that XO belongs to heat-stable enzymes – it remain active at 75-80°C temperature in several minutes [5]. However, at this temperature, the enzyme molecules undergo partial reversible denaturation. Therefore, one of possible ways for the availability of XO molecules for exogenous molybdenum is the disintegration of milk fat globule membranes and partial denaturation of enzyme molecules. Thus, during the heat treatment: a) excess of exogenous phospholipids disintegrate the structure of MFGM and b) enzyme molecules denatured partially and their active center became available for exogenous cysteine, molybdenum, hypoxanthine and nitrate or nitrite. Cysteine not only protects SH-groups of Mo-co against oxidation but also promotes the binding of molybdenum to the cofactor.

Effects of different antioxidants in the stabilizing of heat-denatured milk XO. Our preliminary experiments showed that the optimal heating temperature and its duration for detection of all activities in fresh milk XO were 80°C and 7 min (data not shown). Besides, the presence of 0.1 mM concentrations of cysteine and exogenous molybdenum was also necessary for the detection. Thus, to detect the activity of MFGM-bound enzyme is really required heat treatment and the addition of cysteine as an antioxidant and molybdenum to fresh sheep milk. In the next experiments we examined various natural and artificial antioxidants for the detection of milk XO activity. An natural antioxidants – cysteine, glutathione, lipoic acid and ascorbic acid, and artificial antioxidants – dithiotreitol, unithiol and mercaptoethanol (2,3-dimercapto-1-propanesulfonic acid) were used in different concentrations (table 1).

Results present in the table 1 show that among the natural antioxidants L-cysteine was more effective in the stabilization of XO in heat treated milk. In the same concentrations the effectiveness of cysteine containing antioxidant – glutathione was considerably lower. Apparently, this is due to the fact that tripeptide molecule of glutathione contains only one cysteine residue. Although both the oxidized (disulfide) and reduced (dihydro) forms of lipoic acid (dithiol) show antioxidant properties [26], its stabilizing effect for milk XO was lower than glutathione. Among the artificial antioxidants monothiol mercaptoethanol was more effective in the stabilization of heated milk XO. Although dithiotreitol and unithiol contain two sulfhydrile groups they don't stabilize heat treated milk XO. Recently we found that dithiotreitol in the presence of sulfanilamide forms stable blue complex with molybdate ion (on the basis of this finding we developed new fast method for molybdenum determination in the biological materials). Therefore, dithiotreitol associated with molybdenum does not show a stabilizing or antioxidant effect. The absence of such a stabilizing effect of unitiol remains unclear.

Effect of different electron donors on associated activities of fresh sheep milk. In the experiment for comparison various electron donors were used for own XO, NaR and NiR activities of fresh milk enzyme. Fresh sheep milk heated at 80°C in 7 min in the presence of 10 μM EDTA, 0.1 mM cysteine, 0.1 mM Na_2MO_4 (or Na_2WO_4). After cooling to the milk aliquots (200 μl) were added different electron donors separately (their concentrations are shown in the Table) and incubated foe 15 min at 35°C. The final volume of reaction mixture was brought to 0.5 ml with 0.1 M Na-phosphate buffer (pH 6.0). After incubation the volume of the mixture was adjusted to 3 ml. The mixture was precipitated with acetic acid at pH 3.5 and centrifuged at 15000g in 15 min. In the supernatant the associated activities of milk XO were determined. In fresh untreated sheep milk none of associated activities was detected.

Table 1 – Effects of different antioxidants on the stability of XO during the heat treatment of fresh sheep milk in the presence of Na_2MoO_4

Antioxidant	Concentrations, mM	Associated activities		
		XO	NaR	NiR
Cysteine	0.05	9.5 ± 1.4	75.2 ± 8.4	42.1 ± 6.3
	0.1	13.5 ± 1.7	93.5 ± 10.2	53.2 ± 6.8
	0.5	11.5 ± 2.4	78.7 ± 8.5	39.7 ± 7.1
Glutathione	0.05	4.8 ± 0.9	66.3 ± 1.7	35.2 ± 4.2
	0.1	10.3 ± 1.6	78.6 ± 3.9	48.6 ± 8.7
	0.5	12.6 ± 2.1	94.3 ± 9.7	54.3 ± 8.2
Ascorbic acid	0.05	7.6 ± 1.0	57.7 ± 9.7	42.7 ± 7.2
	0.1	9.5 ± 0.9	78.4 ± 4.9	48.6 ± 5.2
	0.5	10.3 ± 1.6	84.2 ± 9.3	50.2 ± 7.2
Lipoic acid	0.05	7.3 ± 1.6	69.7 ± 9.2	40.4 ± 6.2
	0.1	9.5 ± 1.3	77.5 ± 15.0	51.2 ± 6.4
	0.5	8.9 ± 1.2	74.3 ± 9.9	48.2 ± 8.7
Dithiotreitol	0.05	0	0	0
	0.1	0	0	0
	0.5	0	0	0
Unithiol	0.05	0	0	0
	0.1	0	0	0
	0.5	0	0	0
Mercaptoethanol	0.05	9.2 ± 1.6	73.8 ± 9.2	41.3 ± 5.9
	0.1	10.7 ± 1.7	89.7 ± 11.3	53.0 ± 6.2
	0.5	9.2 ± 1.3	71.3 ± 9.7	40.4 ± 8.3

XO: nmoles of uric acid/0.1 ml/min; NaR: nmoles of NO_2- formed/0.1 ml/min; NiR: nmoles of NO_2- reduced/0.1 ml/min.

Table 2 – Effects of different electron donors on nitrate- and nitrite-reducing activity and own activities of sheep milk XO in the presence of cysteine

Electron donors	Associated activities		
	XO	NaR	NiR
10 µ NADPH	0.0	0.0	0.0
10 µ NADH	18.3 ± 2.7	114.7 ± 18.7	68 ± 9.4
10 µ $FADH_2$	2.4 ± 0.6	21.3 ± 1.6	27.2 ± 4.2
0.1 mM hypoxanthine	–	92.8 ± 8.5	57.6 ± 7.6
*Reduced methylviologen (MVH)	–	128.6 ±21.3	98.6 ±14.3

XO: nmoles of uric acid/0.1 ml/min; NaR: nmoles of NO_2- formed/0.1 ml/min; NiR: nmoles of NO_2- reduced/0.1 ml/min; MVH: Reference 4.

Results in the table 2 show that the highest levels of the associated activities of milk XO observed with artificial electron donor – methylviologen reduced by dithionite ($Na_2S_2O_4$). Among the physiological electron donors the highest associated activities were obtained using reduced NADH. Natural substrate of XO – hypoxanthine was also effective in the donating of electrons for NaR and NiR activities of the enzyme in fresh sheep milk. Thus, the electron transfer may also occur from the hypoxanthine (the reducing substrate) to nitrite and nitrate (the oxidizing substrates). Because in the presence of tungstate in the reaction mixture the associated activities were not detected, during nitrate reduction by hypoxanthine, the

XO molybdenum centre may participate directly in both the oxidative (hypoxanthine oxidation) and the reductive (nitrate and nitrite reduction) half-reactions.

Detection of the products of nitrate- and nitrite reduction by XO in heat treated sheep fresh milk. As mentioned above, it is found that animal XO converts NO_3^- and NO_2^- to physiologically important gas – nitric oxide (NO) [6-8]. NO effectively reacts with L-cysteine or reduced glutathione (GSH) at pH 7.0 and 7.4, to form orange-pink products of S-nitrosocysteine (CySNO) or S-nitrosoglutathione (GSNO). These products exhibited a peak absorbances at around 340 and 540 nm [25]. It is known that NADH is one of potential physiological electron donors for XO and it also has absorbance at 340 nm (reduced NADH exhibits strong UV absorption at 340 nm whilst the oxidized form has virtually no absorption at this wavelength). Therefore, to avoid mutual interference between the optical density of NADH and CysNO at 340 nm, instead of NADH we used hypoxanthine as an electron donor for NaR and NiR activities of milk XO (see above).

It was demonstrated that heat treatment (80°C, 10 min) of homogenic XO resulted in the release of molybdenum cofactor (Mo-co) from the active center of denatured enzyme molecule. During the heat treatment of XO ascorbic acid was the potential protector against the oxidation of released Mo-co. However, in the absence of ascorbic acid it quickly inactivated by oxygen (even in anerobic condions) [4]. Later we showed that glutathione and cysteine were the more powerful protectors for isolated Mo-co [27].

However ascorbic acid decomposes S-nitrosocysteine [28] and, therefore, we used L-cysteine as the protector against the oxidation of the cofactor in the active center of XO localized in MFGM. Thus, in our experiments L-cysteine was used for double purposes: as the protector of enzyme Mo-co against the oxidation during heat treatment of milk and as a reagent for S-nitrosothiol formation. For construction of calibration curve we used nitroprusside as a donor of NO. Increasing concentrations of nitroprusside from 10 nM to 1.0 μM mixed with constant 0.1 mM concentration of cysteine in milk serum. Micromolar concentrations of nitroprusside release nanomolar concentrations of NO [20]. The serum was obtained by heating fresh milk, precipitating its proteins with acetic acid and centrifuging. In order to obtain a transparent milk serum, its filtration was carried out through an "Merk Millipore" membrane (33 mm Millex Filter Units).

For NO determination sheep milk mixed in the ratio of 1:1 with 0.2 M chlorinated phosphate buffer (PBS), pH 6.5, containing 10mM NEM, 2.5 mM EDTA [28], 0.2 mM Na_2MoO_4 or Na_2WO_4 and 0.1 mM cysteine. After incubation in 15 minutes at a temperature of 36°C the milk proteins were precipitated by diluted acetic acid added to the reaction mixture until pH 4.0. After centrifugation in the supernatant absorbance at 340 nm and 540 nm were measured.

For many years tungsten was considered to be a biological antagonist of molybdenum and was used for study of the properties and functions of molybdenum in Mo-enzymes. This was due to the fact that tungsten is able to replace molybdenum in Mo-enzymes, forming catalytically inactive analogs [30]. Therefore, to make sure that it is the molybdenum enzyme that catalyzes the formation of NO, instead of molybdenum we incubated the milk in the presence of tungsten (table 3).

Thus, the results obtained (table 3) convincingly show that the heat treatment of fresh sheep milk in the presence of exogenous molybdenum actually activates XO and the enzyme becomes capable of converting nitrate and nitrite to nitric oxide. However, when nitrates were used as a substrate NO formation was very low. At the same time, using nitrites as substrate resulted in 10 times higher amount of formed NO (i.e. CySNO) in comparison with nitrate substrates. The levels of CysNO determined by absorbances at 340 and 540 nm were completely different. It is likely that this was due to the difference in the sensitivity of the absorption at the ultraviolet and visible wavelengths of the spectrophotometer.

Effect of exogenous phospholipid on the associated activities of sheep milk XO. Phospholipids (PLs) are formed by glycerol, phosphoric acid, fatty acids and a hydroxy compound (e.g., choline, ethanolamine, serine, inositol). It is well known that milk fat globule membranes (MFGM) are composed of four main species of PLs: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS). The most abundant PLs in milk fat, expressed as percentage of total, are PE (26.4 % – 72.3 % of total PLs), PC (8.0 % – 45.5 %), PI (1.4 % – 14.1 %) and PS (2.0 % – 16.1 %). Of the lipids, phosphatidylcholine is largely located on the outside while the neutral lipids, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol are concentrated on the inner surface [31]. Amphiphilic properties of PLs derive from the presence of both a hydrophobic tail and a hydrophilic head

Table 3 – Formation of CySNO in fresh sheep milk after heat treatment in the presence of cysteine and molybdenum or tungsten

Treatments of the milk	Substrate in reaction mixture	Absorbance at:	Amount of CySNO, *nanomoles
Control – PBS only without milk	NO_3-	340 nм	0.0
		540 nм	0.0
	NO_2-	340 nм	0.0
		540 nм	0.0
Control – milk without heat treatment	NO_3-	340 nм	2.7 ± 0.3
		540 nм	0.0
	NO_2-	340 nм	5.8 ± 0.2
		540 nм	0.0
Heating + Na_2MoO_4 at 80°C, 7 minutes	NO_3-	340 nм	12.6 ±0.8
		540 nм	3.2 ± 0.2
	NO_2-	340 nм	35.5 ± 3.5
		540 nм	7.3 ± 1.3
Heating + Na_2WO_4 at 80°C, 7 minutes	NO_3-	340 nм	0.0
		540 nм	0.0
	NO_2-	340 nм	0.0
		540 nм	0.0
Heating of milk without $MoO_4^=$ or $WO_4^=$	NO_3-	340 nм	0.0
		540 nм	0.0
	NO_2-	340 nм	0.0
		540 nм	0.0
*CysNO nanomoles/0.1 ml/min.			

make them natural detergents. Thus, under high temperature phospholipids may effectively disrupt hydrophobic bonds between fatty acids, e.g., the integrity of all membrane layers of MFGM. Therefore, the destruction of MFGM makes the enzyme molecules available for exogenous molybdenum and its substrates (hypoxanthine, nitrate and nitrite).

In the next experiments, we examined the effect of the lecithin on NaR and NiR activities of heat-treated fresh sheep milk. Egg yolk lecithin from AppliChem (Panreac, ITW Companies, Darmstadt, Germany) was used in the experiments. The main phospholipids of this lecithin are phosphotidylcholine and phosphatidylethanolamine, i.e. they are components of the outer and inner layers of the membrane. The lecithin was dissolved in isopropyl alcohol and then added to the milk before heat treatment. The other components required for the heat treatment of milk have already been written above. Hypoxanthine was used an electron donor for NaR and NiR activities.

Table 4 – Effect of increasing concentrations of the lecithin on nitrate- and nitrite-reducing activities and CysNO formation in sheep milk

Lecithin content	Associated activities		Amount of CySNO*	
	NaR	NiR	From NaR	From NiR
Control	92.8 ± 8.5	57.6 ± 7.6	13.2 ± 1.2	33.7 ± 5.2
0.01 mg/ml	91.2 ± 10.2	58.4 ± 6.8	14.3 ± 2.6	33.5 ± 7.3
0.05 mg/ml	92.7 ± 9.5	57.8 ± 9.3	14.4 ± 1.8	34.7 ± 7.1
0.1 mg/ml	95.8 ± 11.6	62.9 ± 9.7	18.4 ± 3.2	42.9 ± 8.2
0.5 mg/ml	122.5 ± 14.7	117.6 ± 12.8	33.6 ± 4.5	78.9± 12.2
1.0 mg/ml	128.6 ± 12.8	135.2 ± 18.6	36.7 ± 7.3	93.8 ± 11.70
*Results obtained from 340 nm absorbance.				

The results presented in table 4 show that exogenous lecithin in relatively high concentrations (0.5–1.0 mg/ml) increases the activity of NaR, NiR and the formation of CysNO. Apparently, during heat treatment, exogenous lecithin does partially decompose the membrane of MFGM of milk and enzyme molecules become more accessible. Thus, the results obtained may be important in the cleaning of animal milk contaminated with nitrates or nitrites. Thus, our results suggest the possible use of XO activation by heat treatment to remove nitrates from milk. Usually fresh sheep's milk is consumed after heat treatment.

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ҚОЙДЫҢ САУМАЛ СҮТІНДЕГІ КСАНТИНОКСИДАЗАНЫ АКТИВТЕНДІРУ ЖӘНЕ АЗОТ ТОТЫҒЫНЫҢ ТҮЗІЛУ ЖАҒДАЙЛАРЫН ОҢТАЙЛАНДЫРУ

Аннотация. Қойдың сүтіндегі КО-ның активтігін анықтау үшін әртүрлі табиғи және жасанды антиоксиданттар тексерілді. Табиғи антиоксиданттардың арасында L-цистеин қыздырылған сүттегі КО-ны күштірек тұрақтандыратын болып шықты. Цистеин мен молибденнің қатысуымен қыздырылған кезде активтенген сүттің КО-сы нитрат пен нитритті азот тотығына (NO) айналдыратын қабілетке ие болды. Сондықтан, цистеин екі мақсатта: қыздыру кезінде ферменттің активті орталығын тотығудан қорғайтын протектор және S-нитрозотиолдардың түзілуіне қажет реагент ретінде пайдаланылды. КО-ның табиғи субстраты – гипоксантин НаР мен НиР-дің активтіктері үшін күшті электрондар доноры бола алатыны анықталды. Сүтті сырттан берілген лецитиннің қатысуымен қыздырғанда НаР мен НиР-дің активтігі және CysNO-ның түзілуі жоғарылады. Сонымен, қыздыру кезінде: а) сырттан берілген фосфолипидтер СМГМ-ның құрылысын бұзады және б) ферменттің молекулалары жартылай денатурацияланады және оның нәтижесінде олардың активтік орталықтарына сырттан берілген цистеин, молибден, гипоксантин, нитрат немес нитрит ене алады.

Түйін сөздер: сүт, ксантиноксидаза, NO, NO_{3-}, NO_2 , антиоксидантар,, молибден, вольфрам, фосфолипидтар.

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ОПТИМИЗАЦИЯ УСЛОВИЙ ДЛЯ АКТИВАЦИИ КСАНТИНОКСИДАЗЫ И ОБРАЗОВАНИЯ ОКСИДА АЗОТА В ОВЕЧЬЕМ МОЛОКЕ

Аннотация. Для обнаружения активности ксантиноксидазы (КО) в овечьем молоке были исследованы различные природные и искусственные антиоксиданты. Среди природных антиоксидантов L-цистеин был более эффективным в стабилизации КО прогретом молоке. КО молока, активированная прогреванием в присутствии цистеина и молибдена, обладала способностью превращать нитрат и нитрит в оксид азота (NO). Поэтому, цистеин был использован для двух целей: в качестве протектора активного центра фермента от окисления во время термообработки и в качестве реагента для образования S-нитрозотиолов. Природный субстрат КО – гипоксантин был эффективным донором электронов для НаР и НиР активностей. Прогревание молока в присутствии экзогенного лецитина повышало НаР и НиР активности фермента и образование CysNO. Таким образом, во время термообработки: а) экзогенные фосфолипиды разрушают структуру МЖГМ и б) молекулы фермента частично денатурируются и в результате их активные центры становятся доступными для экзогенных цистеина, молибдена, гипоксантина и нитрата или нитрита.

Ключевые слова: молоко, ксантиноксидаза, NO, NO_{3-}, NO_2, антиоксиданты,, молибден, вольфрам, фосфолипиды.

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