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RESEARCHES REGARDING THE ANTIOXIDANT EFFECTS FOR THE MILK XHANTINE OXIDASE CONCENTRATION

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Abstract

The cow milk can be protected against the oxidation and lactic fermentation started through different methods: thermal methods (boil or cooling), physical methods (the pressure variation, the physically state change), chemical methods (treatment with antioxidants, treatments with preserve material), and different methods for preserve. Follow the thermal treatments, more proteins, aminoacids, vitamins are destroit. For this reason the technological must search more and competitive recipes-that keep the nutritional potential. The best variants for these cases were always the natural treatments with antioxidants. In this work we present the changes of redox equilibrium from cow milk in the case of added some used antioxidants. We use the absorption spectra for registered the changes for Xhantine oxidase level of cow milk.
***Keywords**: cow milk, antioxidants, Xhantine oxidoreductase*

Introduction

Xhantine oxidase from milk reactions with acceptors and donors of electrons. The enzyme contains the 2 active centers, to each having a FAD remainder, of four iron ions and ion of Molibden. (Enroth, 2000).

Through they specificity, the enzyme guide the oxidation for xhantine and hipoxhantine to uric acid, reducing the oxygen from air to the peroxide through transport of electrons in 2 steps (figure 1 and 2).

Experimental

Where through exist more milk producers what still else use - for the extension of the duration of preservation - peroxide of hydrogen (2 - 4 cm^3 the peroxide 3% to 1 milk liter), and this peroxide influence the process redox that are in a equilibrium in milk it was mounted a series

of experiences for to establish the way in which the added peroxide and antioxidants affect the xhantine oxidoreductase from milk.

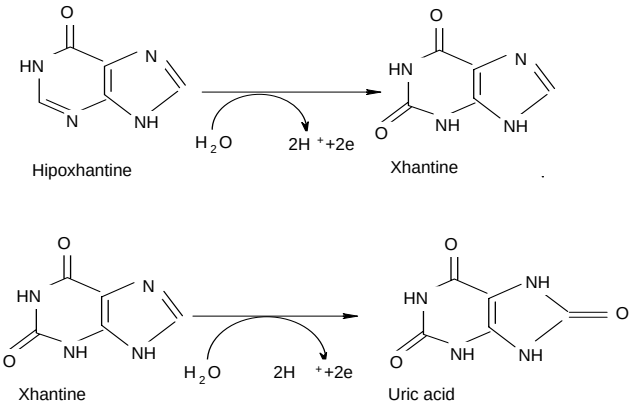

Fig 1.The oxidation of xhantine to uric acid (Belitz, 1999)

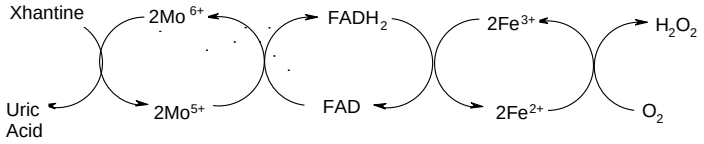

Fig. 2. The electronic transfer system (Belitz, 1999)

The experimental variants are:

- RV-cow milk 3.5% fat, unadditivated (Reference Variant);
- V1-pasteurisated cow milk 3.5% fat;
- V2- cow milk 3.5% fat with A vitamin;
- V3 - cow milk 3.5% fat with C vitamin;
- V4 - cow milk 3.5% fat with E vitamin;
- V5 - cow milk 3.5% fat with Q10 coenzyme;
- V6 - cow milk 3.5% fat with Selenium;
- V7 - cow milk 3.5% fat with ascorbic acid 5%;
- V8 - cow milk 3.5% fat with peroxide of hydrogen

For all the variants we use same fresh milk of cow (to 2 hours from milking), with 3.5 % fat (measure through GERBER method), density (to 20^{o}C) of 1.029 g cm^{3} (determined with specific densimetry), acidity of 18^{o} Thörner (measure through titration method with solution of NaOH 0.1n, with a coefficient of valuable impurity 1and with appearance, colour, taste, specific smell, lacteal fresh, without sensorial changes (Berglund, 1996; Ciobanu, 2002; Preda, 1998).

For dilution (1 : 40) and the purification vats, preparations used proofs the bidistiled water. The samples were centrifuge preliminarily to a centrifugal machine Sygma type, to 7800 rot/min, for 5 minutes.

For the analyses we used a UV/VIS spectrophotometer UNICAM2, with the length band of 1 mm. We scanned the area of visible (400 nm), to the value of 325 nm automatically changed the lamp of Deuterium with a lamp with Wolfram.

We used peroxide of hydrogen 3 Ultrapure (with concentration of Al, As, B, Ba, Co, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sn of max. 1 ppm and dry residue of max 10ppt.).

The used ascorbic L (+) acid has a density of 1.65 g/ cm^{3}, molecular weight 176.13, deprived the smell, of white colour, soluble in the water were used in the recipe of the solution 5%.

The E vitamins (D, L the alpha-tocopherol acetate) and A vitamin (retinol) used were in the liquid state (as the before preparated solution of the alpha acetate). The used levels were of 300,000 units for the case of A vitamin and 30 mg for 100 ml milk in the case of the E vitamin.

The E vitamin had been used for the polyunfatted acid protection, for protect the vitamin and carotenoids from milk and the thiolic group of any enzymes and for heighten the ubiquinone function (of the Q_{10}coenzyme) (Florea, 2001; Leonte, 1998).

The Q_{10} coenzyme, fat-soluble compound of each main cell from the metabolic paths of produce the energy, hard antioxidant, entered in a concentrate of 15 mg for 100 ml milk.

The selenium had been used in milk for him antioxidant activity, for increase of the organisms reluctance for milk consumer to action of free radicals (Hunt, 1993); it was used in a dose of 50 μg for 100 ml milk. They took all the measures for the stultification of the variations of temperature, the limitation to maximum the influence of interferential substances, the of an assurance frames of high

repeatability of the results, the assurance of optimum conditions for the limitation average errors of analysis.

To analysis and the interpretation of results we use MS OFFICE 2000: MS WORD2000 and MS EXCEL2000

Results and Discussions

The Xhantine oxidoreductase (XOR) with the both forms, XO - Xhantine oxygenase and XDH - Xhantine dehydrogenase, is very important parameter for establish the final electrochemical potential for the milk. Reported by the final electrochemical potential the technologists can establish the direction for processing for milk. The obtained results are presented in figure 3.

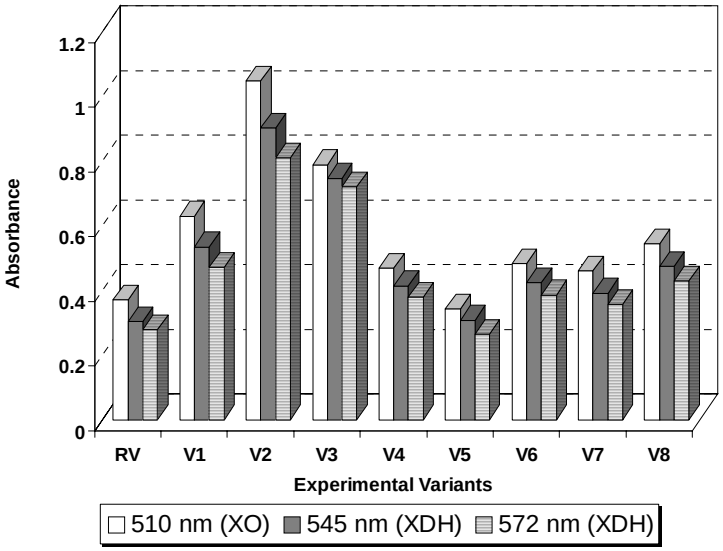

Fig. 3. Correlation between Absorbency with wavelength for Xhantine Oxidoreductase for each experimental variant

After obtained results, the hydrogen peroxide form V8 partial obstructs the enzyme (Xhantine oxidoreductase); for this reason, the system from figure 2 brings a very little amount of uric acid. The pasteurisation task give the obstruct for the enzymatic system and values for V1 were more big.

The C and A (retinol acetate) vitamins have a synergetic effects for active centre from Xhantine oxidoreductase; she protect the milk environment against oxidise and the saprophyte flora of micro-organisms can produced more enzyme. For this reason, the XO and XDH concentration for V2 and V3 are bigger.

The ascorbic acid 5% (the best scavenger for the traces of hydrogen peroxide from milk) (Savescu, 2005) and the treatment with tocopherols were proved same effects, in to V4 and V7 she act for eliminate the hydrogen peroxide from environment, and the electronic transfer system can be faster. Q_{10}-coenzyme can maintain the equilibrium for the XO and XDH amounts; V5 is closely for RV. The Selenium not proves a great difference for the XO and XDH amounts.

Conclusions

The Xhantine oxidoreductase (XOR) with the both forms (XO-Xhantine oxygenase and XDH-Xhantine dehydrogenase) is very important parameter for establish the final electrochemical potential for the milk. Reported by the final electrochemical potential the technologists can establish the direction for processing for milk. For the best control on the redox system of milk, it is very important the antioxidants choose. After this study, the variant with Q_{10}-coenzyme (V5) was closely for RV, the redox equilibrium for the XOR was unchanged. For increase the XOR activity, V2 and V3 was better.

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