

## RESEARCHES REGARDING THE DECREASE OF THE OXIDATIVE LEVEL OF CAW MILK AFTER THE ACTION OF DIFFERENT ANTIOXIDANT AGENTS

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### Abstract

*In this paper we tested the capability of antioxidants agents (alone or in combination) to reduce oxidation (measured by the size of reports coenzimelor oxidoreductazelor anaerobic  $NAD^+ / NADH + H^+$  and aerobic  $FMN / FMNH + H^+$  milk cow).*

*Protecting dairy cows at risk of oxidative - after entering the action of oxidants agents - has been highlighted through the study of molecular absorption spectra with the maximum recorded absorbție for coenzimele oxidoreductazelor anaerobic and aerobic in this milk.*

*The study of redox processes of the cow's milk can lead to improved technology classical processing milk consumption, both by extending the duration of preservation of milk and food by eliminating the risk due to toxicity of hydrogen peroxide.*

**Key words:** oxidative level, cow milk, coenzymes of oxidoreductases, antioxidants

### INTRODUCTION

Cow's milk immediately after calving, colostrum, is much different from normal milk. With its more rich in proteins and salts, milk at the end of January - early February are different compositional versus normal milk from summer time due to fodder for animal feed and immobility of animal [6].

Milk is an easily perishable product. His conservation depend by the temperature of storage, transport (heat being a negative factor), depend by the diversity and number of microorganisms contained in particular, those contributing to the degradation of lactose, thereby contributing to increasing acidity by increasing the concentration of lactic acid [5].

In order to prolong of storage time and transportation may be used successfully redox processes in milk. The results of analysis can be registered on the block diagram of the technology of normalized milk, thus achieving a new technology, in economic performance, by eliminating the technological chain of the cooling tank from the collection points (or at the collection centers milk) but also in terms of food security.

### MATERIAL AND METHOD

There are many manufacturers that still use to extend the duration of conservation, hydrogen peroxide (2 - 4 cm<sup>3</sup> peroxide solution 3% per liter of milk), which influence redox processes that exist normally in balance in the milk.

This experience has targeted achieving the following objectives:

- Determining the best antioxidant able to protect milk against oxidative fermentation;

- introduce this antioxidant in to technology of the normalized milk.

- study the influence of hydrogen peroxide on the concentration of vitamins and oxidoreductase from milk, in order to determine the effect of extending the storage time of milk. Hydrogen peroxide has the effect of inhibition of pathogenic microorganisms for a period of up to 7 hours [4], but after this period must be eliminated, because of toxic effects that may occur. Lactic bacteria are not capable to develop the enzymes that can degrade hydrogen peroxide, for this reason a number of experimental variants test the ability to eliminate of hydrogen peroxide by using antioxidants.

Like as antioxidants that have been used is a number of vitamins (variants V1-V4 and V7) and selenium (V5).

For all variants used the same fresh cow milk (from 2 hours of milking), fat 3.5% (determined by the Gerber method), density (at 20°C) of 1.029 g/cm<sup>3</sup> (determined by thermo-densitometer), 18<sup>0</sup> Thörner acidity (determined by the titration method with solution of NaOH 0.1 N) with a coefficient of impurification 1 (value determined using filter of milk), looking, color, taste, smell, like as fresh milk without sensorial changes [1].

For dilution (1:40) and cleaning vats, preparation of water samples was used two-distilled water.

Samples were previously prepared at a Sigma centrifugal machine at 7800 rotations per minute during 5 minutes.

For analysis was used a spectrophotometer UV / VIS type UNICAM2 with band width of 2 nm. We scan the nearly UV range (190-400nm) and visible range (400-700 nm). At the value of 325 nm wavelength, the Deuterium lamp change automatically with a Wolfram lamp.

The used hydrogen peroxide concentration was 3%, ultra pure (with concentrations of Al, As, B, Ba, Co, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sn, Mn at 1 ppm maximally, the dry residue 10ppt max.

The used L (+)-ascorbic Acid was as aqueous solution 5%, with a density of 1.65 g/cm<sup>3</sup>, molecular weight 176.13 g / mol, without smell, white color.

The used E vitamin (DL - alpha-tocopherol acetate) and A vitamin (retinol) were as a local prepared solution. The used concentrations were 0.5 mg / 100 mL milk for A vitamin and 30 mg / 100 mL milk for E vitamin.

The E Vitamin was used for to protect the fatty acids, polyunsaturated, to protect the carotenoid and A vitamin from milk and thiolic groups of enzymes and for synergism of function for ubiquinone (Q10 coenzyme) [10].

The Q10 Coenzyme (soluble in fat compounds of each cell metabolic pathways, essential for energy production, powerful antioxidant) has been introduced in a concentration of 15 mg/100 mL milk [8].

The Selenium (used in to milk for its antioxidant action, to increase of the milk consumer resistance to the free radicals) [2], was used in a dose of 50 µg/100 mL milk.

The redox agents from the experimental variants who had major differences of absorption compared with the simile of the witness caused imbalances in redox systems and it is not recommended like as protectors.

We took all the specific measures to minimize the variations of temperature (constant temperature by providing reagents, the environment, using spectrophotometer with thermostat). It is limited to a maximum influence for the interfering substances by removing lipids interference through defecation other organic compounds from the category of proteins, using the unique addition through verifying the molecular spectra in the literature cited in the new working conditions. Frameworks high repeatability of results was ensured by rigorously maintaining the same environment and working time and the same human operator. For two consecutive tests of absorption at same experimental variant on the same wavelength were admitted differences of up to ± 0003.

## RESULTS AND DISCUSSION

It was analyzed the variation of molecular absorption spectra of the maximum wave length in the near UV (200-400nm) and Visible (400-700nm).

Were traced graphics functions change for experimental variants, grouped by type of antioxidant used by the order of addition of peroxide and antioxidant.

From the study of molecular absorption spectra presented in graphs in Figures 1 and 2 results that the some experimental variants have major differences to reference witnesses (V1-V6, V9-V12, V16).

Analyzing on the experimental variants the report of the oxidized and reduced forms of coenzymes Flavine Mono Nucleotide (FMN-dependent specific for the aerobic oxidoreductases) have obtained appropriate values inserted in Table 1 and represented in Figure 1. The ratio of FMN/FMNH<sub>2</sub> forms was determined as the ratio of absorption of oxidized / reduced forms at A<sub>445</sub> / A<sub>570</sub>.

Table 1  
 The ratio of the concentrations of oxidized/reduced forms of FMN/FMNH<sub>2</sub> registered at the experimental variants that try the biggest differences from the witness variant

The experimental variant	The ratio of the oxidized/reduced forms of FMN/FMNH <sub>2</sub> at A <sub>445</sub> /A <sub>570</sub>	The differences (±) from the witness variant Mt
V8	1,555	-0,344
V13	1,833	-0,066
V14	1,712	-0,1869
V15	1,760	-0,1394
Mt	1,899	0

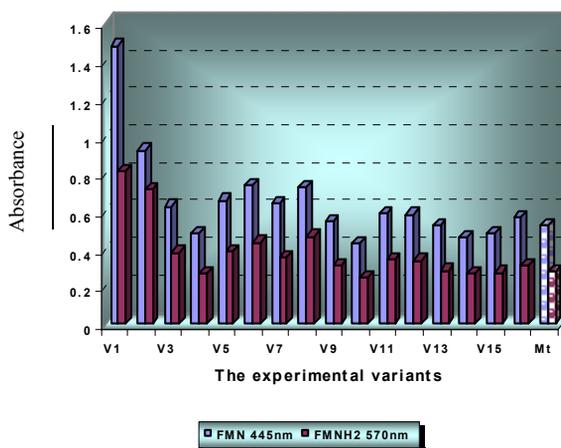


Figure 1 - Changes of absorbance for the oxidized and reduced forms of FMN to the experimental variants

Besides the fact that the FMN/FMNH<sub>2</sub> is best to V<sub>13</sub> (being the least difference to the value of a witness), have been almost identical values and concentrations of FMN (the molecular spectra of absorption at 445 nm) for V<sub>13</sub> and M<sub>t</sub> (figure 1). Thus, at 445nm were obtained 0.526 units of absorbance for the V<sub>13</sub> and 0,528 units of absorbance at M<sub>t</sub>, which means that the concentration values of oxidized forms FMN at V<sub>13</sub> and M<sub>t</sub> are almost identical, the V<sub>13</sub> has a surplus of slightly reduced form (at-570nm which have only the maximum reduced forms

FMNH<sub>2</sub>-V<sub>13</sub> presented a absorbed by 0.287 compared to 0.278 to witness).

Analyzing on the experimental variants the oxidized and reduced forms of coenzyme Nicotinamide Adenine Dinucleotide NAD-dependent (specific of anaerobic oxidoreductases) were obtained corresponding values, values inserted in Table 2 and Figure 2. The ratio of NAD/NADH + H<sup>+</sup> forms was determined as the ratio of oxidized/reduced forms at A<sub>270</sub>/A<sub>340</sub>.

Table 2  
 The ratio of the concentrations of oxidized/reduced forms of NAD/NADH+H<sup>+</sup> registered at the experimental variants that try the biggest differences from the witness variant

The experimental variant	The ratio of the oxidized/reduced forms of NAD/NADH+H <sup>+</sup> at A <sub>270</sub> /A <sub>340</sub>	The differences (±) from the witness variant Mt
V8	2,2576	-0,3144
V13	2,660	+0,088
V14	3,160	0,588
V15	2,964	0,392
Mt	2,570	0

As can be seen from Table 2 and Figure 2, the recorded values at V<sub>13</sub> are the closest to the basic variant, Mt. In addition, in the case of reduced forms of NADH + H<sup>+</sup>, the V<sub>13</sub> proved the smallest difference from the Mt (a difference of negative insignificant -0003).

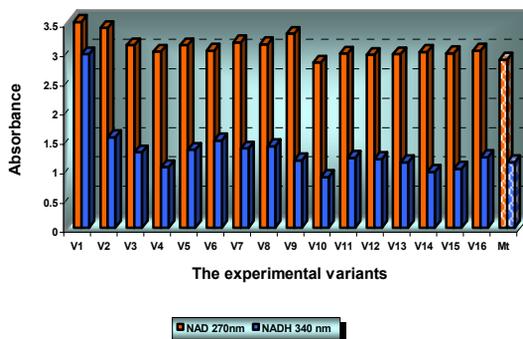


Figure 2 - Changes of absorbance for the oxidized and reduced forms of NAD to the experimental variants

Following the analysis of experimental variants [3] could be easily observed that ascorbic acid (from V<sub>13</sub>) prove the best protection for the redox systems in which A vitamin is involved (ensuring in particular the reduced forms of A vitamin can therefore protect these redox systems of milk and any oxidation). Although variant V<sub>15</sub>-using E vitamin like as protective agent in to case of hydrogen peroxide attack prove the very small differences from those of similar witness (+0.001 units) at the oxidized form of A vitamin, the reduced form recorded significant negative differences against witness (-0.128).

## CONCLUSIONS

The molecular absorption spectra of the experimental variants taken study in the 2 ranges of wavelength fall more in the near UV and less in to visible, the absorption is different depending on the type of introduced antioxidant compared with the witness.

There are significant differences between absorption of samples containing only the antioxidants and the samples containing antioxidants and hydrogen peroxide like as conservation agent. Thus, V<sub>1</sub> (with A vitamin) has a molecular absorption spectrum much different from simile of the witness,

but after adding the peroxide is very close to that of witness.

In case of V<sub>8</sub> (milk fat 3.5% + A Vitamin + hydrogen peroxide) the A vitamin are oxidized and act on redox systems from milk and can increased the concentrations of reduced forms, the difference on the concentrations of oxidized / reduced forms at witness to being the largest.

The molecular absorption spectra of the samples did not vary significantly according to the order to introducing the antioxidant and hydrogen peroxide in case of A vitamin, selenium and Q10 coenzyme, but shows greater variation in ascorbic acid and E vitamin.

Form analyze of concentrations for the oxidized and reduced forms of oxidoreductases of milk (xanthine oxidase, superoxide dismutase, lactate peroxidase, a total Lactate Dehydrogenase cyt-P450 reductase), a flavoproteins, cytochrome a, b, c, c<sub>1</sub> of coenzymes FMN, NAD and riboflavins [9] shows that V<sub>13</sub> is the best experimental variant. This variant used as antioxidant ascorbic acid 5%, solution put before the conservation agent (hydrogen peroxide). In this variant of the oxidized form of FMN and reduced form of NADH + H<sup>+</sup> proved the concentrations were closely similar to those of the witness. The

differences in values recorded at the maximum absorption of each enzyme similar to those of untreated witness are smaller, the experimental version is better, being closer to natural redox systems in milk.

From the study of  $V_{13}$  result that the milk with 3.5% ascorbic acid solution 5% prove the best protection for the redox system consisting of reduced and oxidized forms of coenzymes. Thus, the variant has been the closest values of the concentrations of oxidized and reduced forms with simile of reference.

Of tests [7] result that ascorbic acid can protect highly the redox system which is involved in A vitamin and A provitamins ( $\beta$ -carotene, lycopene) in milk. And in this case, the ascorbic acid acting as a reducing power, it oxidizes priority and can protect the work environment to oxidation;

The study of redox processes of the cow's milk can lead to improved technology classical processing milk, both by extending the duration of preservation of milk and by eliminating the risk due to toxicity of hydrogen peroxide. Although  $V_{15}$  has very close to those of the witness-rate on degradation of hydrogen peroxide, the  $V_{13}$  remains the best experimental variant, managing to keep almost all redox systems at the nearest level of natural variant, like as the witness.

Therefore recommend the application of ascorbic acid-according  $V_{13}$ , for efficient the technology of milk, by extending the duration of storage until processing of milk.

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