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EVOLUTION OF MILK CATALASE ACTIVITY IN PRESENCE OF INHIBITOR AND ACTIVATOR SUBSTANCES

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ABSTRACT

Milk catalase activity were carried out to determine the effect of different activators and inhibitors on enzyme activity. The experiments where conducted to estimate the catalase activity from fresh milk, fresh milk in addition with trichloroacetic acid, fresh milk in addition with tannin, fresh milk during the pasteurization process, fresh milk in addition with iron, fresh milk in addition with vitamins. Iron, vitamins, high temperature influence positive milk catalase activity; tannin, trichloroacetic acid and pasteurization temperature influence negative milk catalase activity.

INTRODUCTION

The enzymes are biocatalysts efficiently employed in biotechnology. The class of the catalases covers three different types of enzymes: monofunctional catalases, bifunctional catalases-peroxidases, and manganese catalases.

All these metalloenzymes independently evolved in two different protein families: the heme-containing (type I) catalases possessing an iron-porphyrin cofactor, and the non-heme (type II) catalases (known as “pseudocata-lases”) having a dinuclear manganese active site. (Grigoraș, 2017). Catalase, SOD and GSH are enzymatic antioxidant found in humans and animals (Emmanuel et al., 2015).

The main reaction of catalases is to degrade two molecules of hydrogen peroxide to water and molecular oxygen. Two distinct stages can be differentiated in the catalytic reaction pathway in catalases and catalase– peroxidases.

Oxidation of the haem iron by hydrogen peroxide to form compound A occurs in first stage. During this reaction, the oxygen–oxygen bond in peroxides (R-O-OH) is cleaved heterolytically. As a result, one oxygen ion bonds with a water molecule while the second remains at the haem iron (Fita and Rossmann, 1985; Zamocky et al., 2008; Zhao and Chen, 2016). In milk catalases can be inactivate at 65^{O}C heat treatment for 30 minutes.

High quantity can be find in milk provide from seek animals and from animals during first lactation period (Banu, 2000).

In milk catalase catalyzes reaction leads to the formation molecular oxygen and water molecule

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + \tfrac{1}{2}O_2 \quad (1)$$

Catalases from milk have double origin. One part provides form microorganisms which contaminated the milk (*Staphiloccocus* spp., *Streptococcus* spp) and other part provides from animals. (Giurgiulescu, 2007).

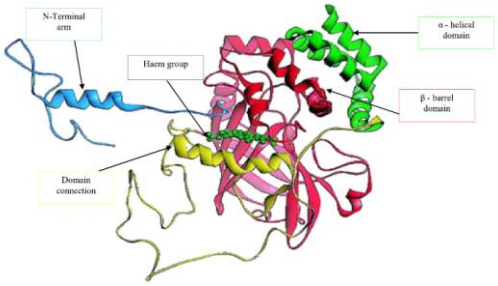

Fig. 1. Catalases structure provide from milk contamination microorganisms (Zamocky and Koller, 1999)

This study proposes to evaluate the catalase activity from fresh milk during the thermic treatment, addition of different substances, presence in milk of activators and inhibitors for enzymatic activity.

MATERIALS AND METHODS

Catalase bio-catalyzes reaction leads to the formation molecular oxygen and water molecule. Peroxide molecule undecomposed combine with one molecule of potassium iodate and releases ne molecule of iodine. Sodium thiosulfate react with iodine to produce tetrathionate sodium and sodium iodide.

$$2KI + H_2O_2 \rightarrow I_2 + 2KOH \quad (2)$$

$$2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI \quad (3)$$

In 2 flasks introduced 100 ml milk (control and analyses sample). In both flasks added 10 ml peroxide N/2. Both flasks left for 15 minutes for reaction. During the reaction peroxide transform in water and molecular oxygen by catalases. After reaction in both flasks added 10 ml of sulfuric acid to stop enzymatic reaction. From this step each sample was treat separately. Add 10 ml potassium iodate and 1-2 ml ammonium molybdate to increase react speed to release iodine from chemical compounds. Each sample was agitated and titrated with sodium thiosulfate in presence of starch as indicator.

Catalase activity was determinate as difference between ml sodium thiosulphate used in titration for sample and control.

For trichloroacetic acid where used 4 samples: first control – 100 ml milk; second 100 ml milk and 1g trichloroacetic acid, third 100 ml milk and 2 g trichloroacetic acid, forth 100 ml milk and 3 g trichloroacetic acid.

For tannin experiment where used 4 samples: first control – 100 ml milk; second 100 ml milk and 1g tannin, third 100 ml milk and 2 g tannin, forth 100 ml milk and 3 g tannin.

For the influence of temperature treatment where used 4 samples: first control – 100 ml milk; second 100 ml milk 60°C at 30 min, third 100 ml milk 75°C at 15 min, forth 100 ml milk 85°C at 10 min.

Influence of Fe over the milk catalase activity where used 4 samples and Venofer vial 5ml contain 100 mg Fe: first control – 100 ml milk; second 100 ml milk and 1ml Venofer, third 100 ml milk and 2 ml Venofer, forth 100 ml milk and 3 ml Venofer.

Influence of vitamins add in milk over milk catalase activity, where used 4 samples and 2 types of vitamins B1 Vitamin flask 1ml/0.025g thiamin and C Vitamin flask 2ml/0.20g ascorbic acid: first control – 100 ml milk; second 100 ml milk and 1 ml Vitamins (0.5 ml thiamin + 0.5ml ascorbic acid), third 100 ml milk and 2 ml Vitamins (1 ml thiamin + 1 ml ascorbic acid), forth 100 ml milk and 4 ml Vitamins (2 ml thiamin + 2 ml ascorbic acid). The experiment was repeated twice.

RESULTS AND DISCUSSIONS

Influence of trichloroacetic acid over milk catalase activity

Trichloroacetic acid present a strong inhibitory activity. Inhibitory activity increase with quantity of trichloroacetic acid added in milk. Compare with control, fresh milk, catalase activity decreases with 8.5 E.U. by addition 1g trichloroacetic acid, 9.2 E.U. addition 2g trichloroacetic acid and 9.9 E.U. addition 3g trichloroacetic acid. (Fig.1).

Fig.1. Influence of trichloroacetic acid over milk catalase activity

Influence of tannin over milk catalase activity

Another inhibitor over milk catalase activity was tannin. Inhibitor activity increase with tannin doses add in milk. 1g tannin add in milk decrease the catalase activity with 2 E.U., 2 g tannin add in milk decrease the catalase activity with 4 E.U. The high influence over milk catalase activity record on sample with 3g tannin added in milk. (Fig. 2)

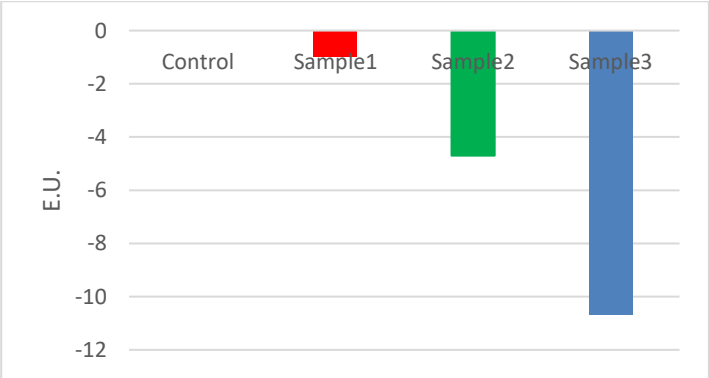

Fig.2. Influence of tannin over milk catalase activity

Influence of temperature treatment over milk catalase activity

Temperature have an inhibitory activity over the milk catalase activity. Start with milk treatment at 60^0C and 30 minutes, the catalase enzyme transform by protein coagulation process and it will be inactivated. Increase the temperature and shortening the exposure time determine decrease milk catalase activity. (Figure 3).

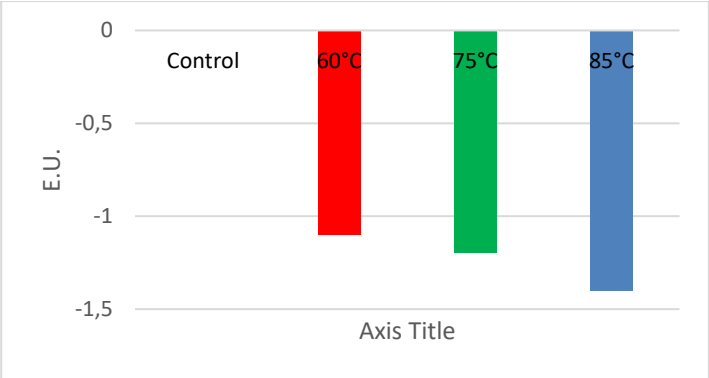

Fig.3. Influence of the milk temperature treatment over milk catalase activity

Influence of Fe added in milk over milk catalase activity

Depends the quantity of Fe added in milk, the catalase activity can be stimulated or can be blocked. If add 1ml of Venofer (5 ml flask contains 100mg Fe) the catalase activity increase with 2.1. E.U., if add 2 ml of Venofer in 100 ml milk the catalase activity decreases at 2 E.U. If add 3 ml of Venofer the catalase activity decreases and at the final catalase was inactivated.

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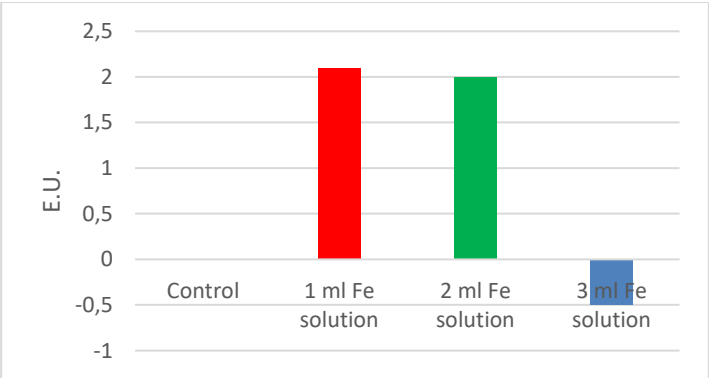

Fig.4. Influence of Fe add in milk over milk catalase activity

Influence of Vitamins (B_1 and C) added in milk over milk catalase activity

Vitamin B_1, thiamine is known in literature for the microorganisms survive role at the end of fermentation period. On the other hand, vitamin C introduce in milk an important

antioxidant activity. Both vitamins combined offer a protective role for catalase activity. At the beginning, if the quantity added is low, (1 ml/ 0.5 vitamin ml B_1 + 0.5 vitamin ml C) the effect is to decrease catalase activity. If the quantity added in milk increase (2 ml/ 1 ml vitamin B_1 + 1 ml vitamin C) the effect is to increase catalase activity. The same result was obtained with 4 ml vitamins mix. (Figure 5).

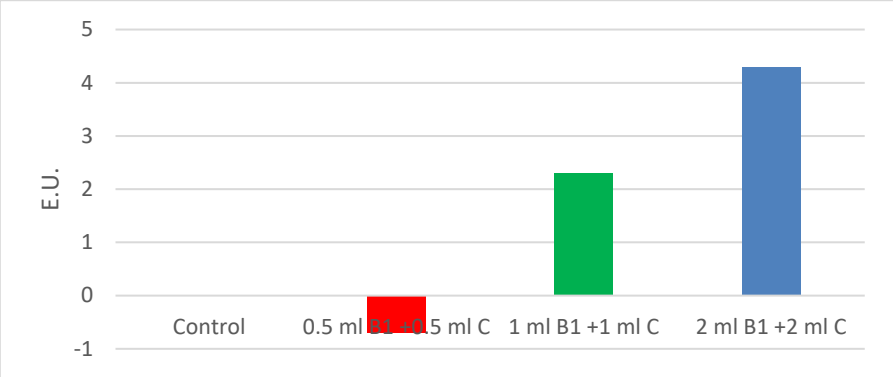

Fig.5. Influence of Fe add in milk over milk catalase activity

CONCLUSIONS

Trichloroacetic acid is a strong inhibitor of catalase activity. Enzyme activity decrease in milk function the trichloroacetic acid concentration. The same conclusion can be achieved for tannin activity.

Temperature treatment inactivate the catalase enzyme from milk. Milk treat at 65^0C for 30 minutes inactivate total catalases activity.

High temperature used in milk pasteurization decrease with 1.2 and 1.4 E.U. catalases activity.

Addition in milk Fe solution increase the catalase activity. High concentration of Fe in milk inactivate the catalase activity.

Finally, addition of vitamins: thiamine and ascorbic acid in milk in low quantity inactivate catalase enzyme. Increase the quantity of vitamins in milk determinate an increase of catalase activity.

This study can be used to decelerate the milk falsification by addition milk colostrum or milk mastitis. The catalase number can modify by addition in milk activator or inhibitor substances.

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