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COMPARATIVE STUDYON MILK COLD PASTEURIZATION USING HYDROGENPEROXIDE

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ABSTRACT
The study evaluates two methods of non-thermal milk pasteurization, namely cold pasteurization using hydrogen peroxide and hydrogen peroxide followed by its decomposition under the action of added catalase extract. These two methods seem to be effective, far less expensive than conventional ones, much more simplistic and do not cause significant changes in the chemical composition of milk, thus preserving its nutritional value.

INTRODUCTION
Conventional pasteurization generates in addition to the process itself a change in the chemical composition of milk in proportions that vary depending on the time / temperature regime applied. Also, the process involves high production costs due to the needed equipment, the consumed utilities, the space used and the workforce (Banu et al., 2000).

In the hot season and in the situation when the refrigeration equipment is not available to store the milk for a certain period of time until it is transported to the processing stations or more seriously, it is not possible to ensure a refrigerated transport and taking into account that the bacteriostatic period of milk is short and strictly dependent on temperature, cold pasteurization can be applied, using as a preservative the hydrogen peroxide. Hydrogen peroxideis the only antibacterial agent composed only of water and oxygen like ozone, hydrogen peroxide destroys disease-causing organisms through oxidation and is considered the safest and most effective natural disinfectant in the world (http://ampress.ro/sanatate/16-beneficii-si-utilizari-uimitoare-ale-apei-oxigenate). Also, a study on mice and one on hamsters has yielded satisfactory results with regard to the toxicological activity of hydrogen peroxide, so it is not classified as potentially carcinogenic for the human body (International Agency for Research on Cancer, 1999).

There are a number of studies on the use of hydrogenated water in milk pasteurization. In this regard, Lück (1962) makes a synthesisand *claims that only highly concentrated hydrogen peroxide solutions can lead to the oxidation of aldehydes, ketones and acids and that the addition of 0.04% hydrogen peroxide improves the curd obtained from cold pasteurized milk by the use of rennet. Also, electrophoresis analyzes showed that there are no differences between the casein micelles of the milk treated with hydrogenated water (in the proportions of 0.1% and 0.4%) and of the untreated one, but the coagulation time increases. Regarding amino acids, the addition of 0.03% or even 0.1% hydrogen peroxide does not affect their sulfhydryl groups even under the conditions of keeping the milk for 20 hours at room temperature. Considering milk chemical composition, some studies did not find that lactose, fat, total nitrogen content or pH would be affected if 0.04% hydrogen peroxide would be added but others claim that following the addition of 0.01-0.08% hydrogen peroxide, the lactose content would decrease, for example from 5.01% for untreated milk to 4.60% for the highest dose. Also, unsaturated fatty acids present in milk do not react with hydrogen peroxide and even under severe treatment conditions (addition* *of 0.3% hydrogen peroxide for 24 hours at 51 ° C), the ultraviolet absorption spectrum of dairy fat has not presented significant changes. As for vitamins, vitamin C is most affected, but it is also greatly affected by conventional pasteurization, and milk is not an important source of this type of vitamin; vitamin B complexes are very poorly affected by cold pasteurization and the liposoluble ones are also quite stable when treated with hydrogen peroxide being probably protected by fat.*

When adding hydrogen peroxide in normal proportions no changes in milk taste are observed and it seems that this method would prevent the appearance of the "light taste" defect in the treated milk, also delaying the appearance of its oxidized taste (Lück, 1962).

Also, Lück (1962) showed that a series of studies investigated the effect of cold pasteurization on milk microorganisms and deduced the following conclusions: Gram-negative bacteria (coliforms) are more sensitive to the action of hydrogen peroxide being inactivated, compared to Gram-positive (spore-generating), lactic bacteria being among the two types of bacteria in terms of their sensitivity to hydrogen peroxide; researches on the action of hydrogen peroxide on the total number of germs showed a massive decrease in the milk treated with 0.05% H_2O_2, up to 98.64%; also, cold pasteurization destroys most pathogenic microorganisms possibly present in milk, the most resistant being Mycobacterium tuberculosis, which can withstand 0.08% H_2O_2 concentrations up to 25 hours.

Other studies (FAO, 1954; Grindrod et al., 1967; Venden Berg, 1985; Food Laboratory News Letter 1987) consider acceptable to use the H_2O_2 preservative to improve the quality of the milk and its products, and any hydrogen peroxide residues may be destroyed in a subsequent conventional or nonconventional pasteurization process.

Also, a study conducted by Rokhsana et al. (2007), recommends cold pasteurization because the milk treated with 0.05% hydrogen peroxide kept its validity for 20 days at 8 ° C compared to 5 days in the raw milk case, and almost no coliform bacteria developed on the traditional products obtained from it after 20 days of storage.

Any hydrogenated water left in the milk can be decomposed for food safety by means of catalase, passing into water and oxygen, process also called budeisation (Guzun et al., 2001). In the enzymes class of catalases are found three different types of enzymes: monofunctional catalases, bifunctional catalases-peroxidases, and manganese catalases, all being independently evolved in two different protein families: the heme-containing catalases and the non-hemecatalases (Grigoraș, 2017).

Considering the budeisation operation, important results were obtained by the addition of 0.03% hydrogen peroxide when products with double or even higher diacetyl content were obtained (Pack et al., 1968).

Therefore, it is justified to initiate a research on replacing classic pasteurization with 2 types of cold pasteurization: pasteurisation using hydrogen peroxide (because in the experimental part, hydrogen peroxide could not be detected with $K_2Cr_2O_7$ in milk after an average period of 7 minutes) and pasteurization using hydrogen peroxide and decomposing it in water and oxygen under the action of catalase, also known as budeisation (for safety reasons).

MATERIALSAND METHODS

The main working materials used in the experiment were: raw milk purchased from the local market of Baia Mare, hydrogen peroxidepurchased from Chemical Company and the catalase extract which was obtained from potato by milling 10 g of potato with 5 g of calcium carbonate, then it was placed in a 100 ml volumetric flask, left for 24 hours after which it was centrifuged,resulting the clear extract.

Two methods of cold pasteurization were investigated:

- *pasteurisation using hydrogen peroxide*; in this case, 0.06% hydrogen peroxide was added in milk and after determining its presence,using$K_2Cr_2O_7$,during several time intervals it was concluded that H_2O_2 was decomposed after an average of 7 minutes due to the action of peroxidase, normally present in milk.

- *budeisation*; though initially H_2O_2 was decomposed after an average of 7 minutes, for safety reasons, subsequent one minute from its addition, catalase extract was added in a proportion of 2‰.

Milk treated by those two methods of pasteurization was analyzed in order to determine: dry substance (by the drying method at 103°C); acidity (by Thörner method); fat content (by Gerber method); protein (by the titer analysis); lactose (the potassium ferrocyanide method); freshness of dairy fat (Kreiss reaction); vitamin C (by titration with 2,6-diclorfenolindofenol).

RESULTS AND DISCUSSIONS

Dry substance determination

The dry substance content of the hydrogen peroxide-treated milk was greatly affected by this type of pasteurization, recording an increase from 12.4256% to 14.0934%, probably due to the fact that the sample ultimately suffers a concentration phenomenon and oxygen a elimination phenomenon (Fig 1).

The dry matter content of the milk treated with hydrogen peroxide and catalase decreased from 12.4256% to 12.0333% due to the sample dilution phenomenon achieved by:
- addition of hydrogen peroxide solution (the hydrogen peroxide being allowed to act for only one minute, during which the evaporation phenomenon was very low),
- adding catalase extract (2 ml extract to 1000 ml milk) (Fig.1).

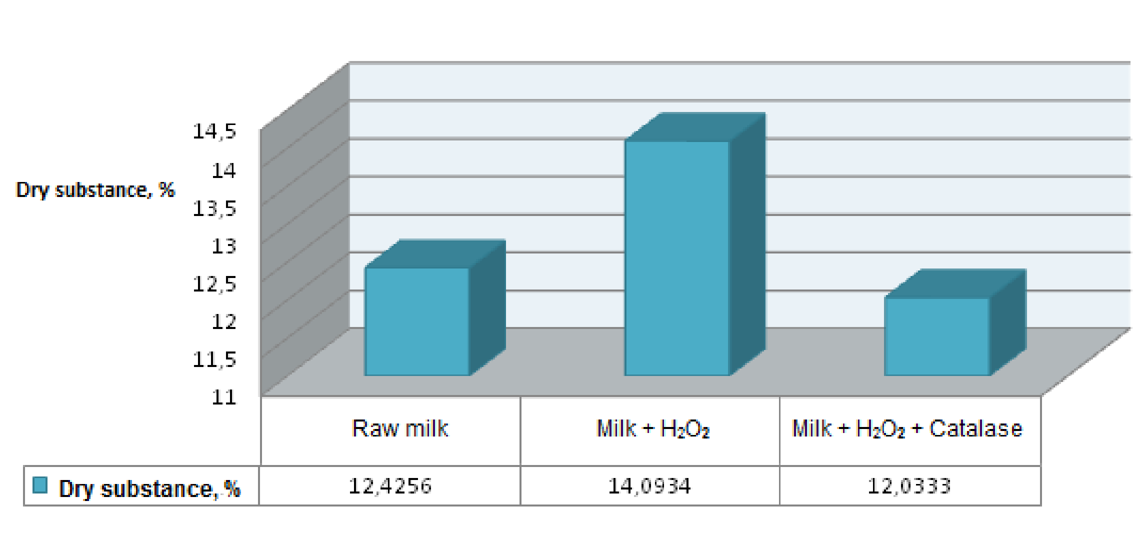

Fig.1. Cold pasteurization methods influence on dry substance content of milk

Acidity determination

The acidity value of the milk pasteurized by the two methods shows an increase compared to raw milk value from 18 ° T to 20 ° T, due to the addition of hydrogen peroxide, which is known to be acidic (Fig. 2).

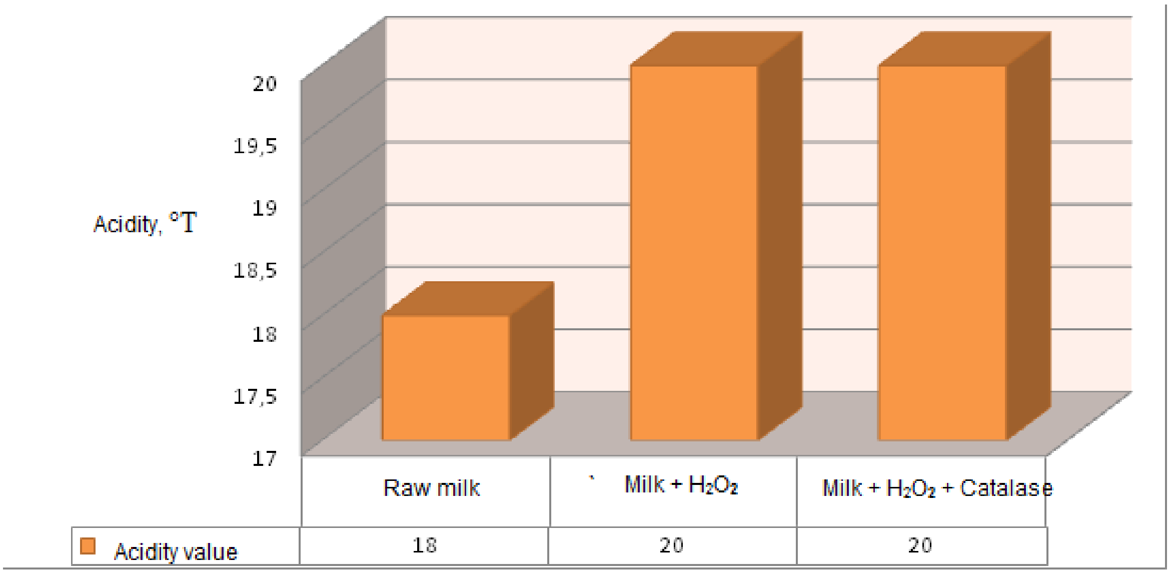

Fig.2. Cold pasteurization methods influence on milk acidity

Fat content determination

A very low drop in the fat content can be observed from 3.4% in the case of raw milk to 3.3% in the case of the other types of milk, the most affected being the saturated fatty acids, as according to Lück et al. (1958), unsaturated fatty acids do not react with hydrogen peroxide even under severe treatment conditions (addition of 0.3% hydrogen peroxide). Also, Lück (1962) indicates that when 0.04% of hydrogen peroxide was added, dairy fat was not affected(Fig. 3).

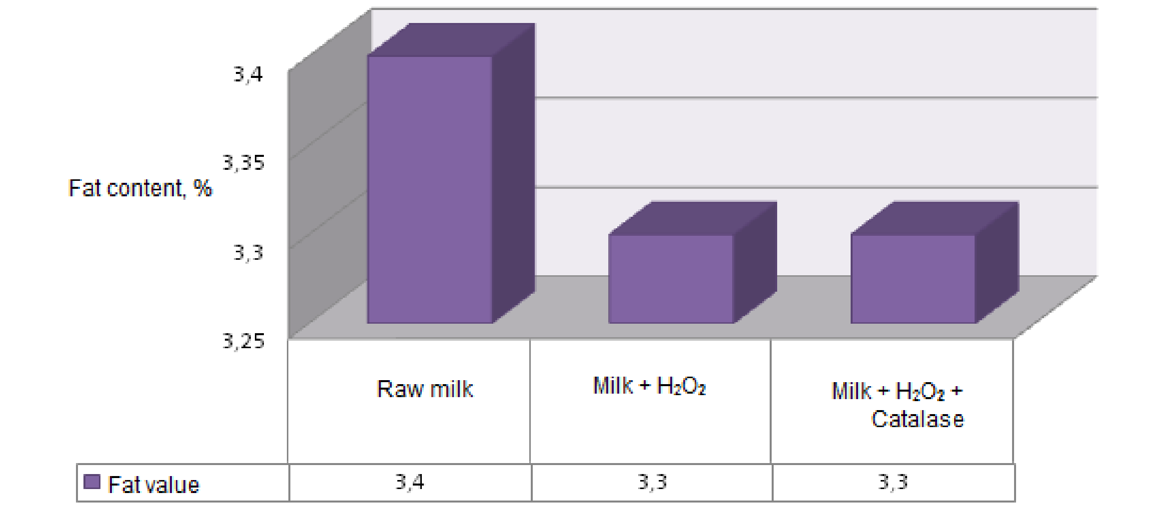

Fig.3. Cold pasteurization methods influence on fat content of milk

Protein determination

Following the determination of protein titer, it can be observed that the total protein and casein content was not affected by the two types of cold pasteurization, which is also confirmed by the literature. Thus, Luck et al. (1955 a, b) demonstrated by electrophoresis analysis that there is no difference between 0.1-0.4% oxygen treated and untreated milk.

Also, Lück (1962) claims that the addition of 0.04% hydrogen peroxide improves the curd obtained from cold pasteurized milk (Fig. 4).

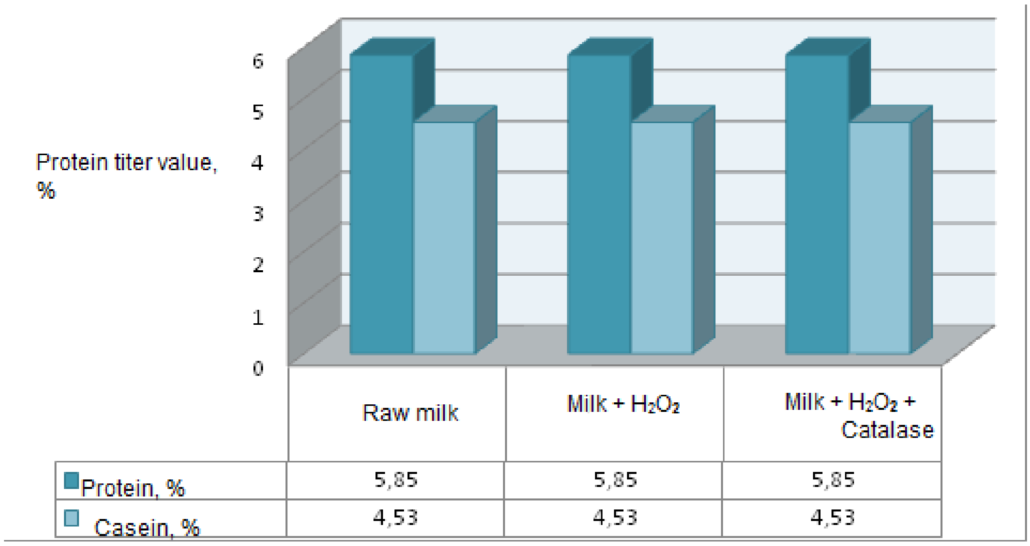

Fig.4. Cold pasteurization methods influence on protein titer of milk

Lactose determination

During the two types of pasteurization, lactose has undergone the same evolution as dry matter, (Figure 5).

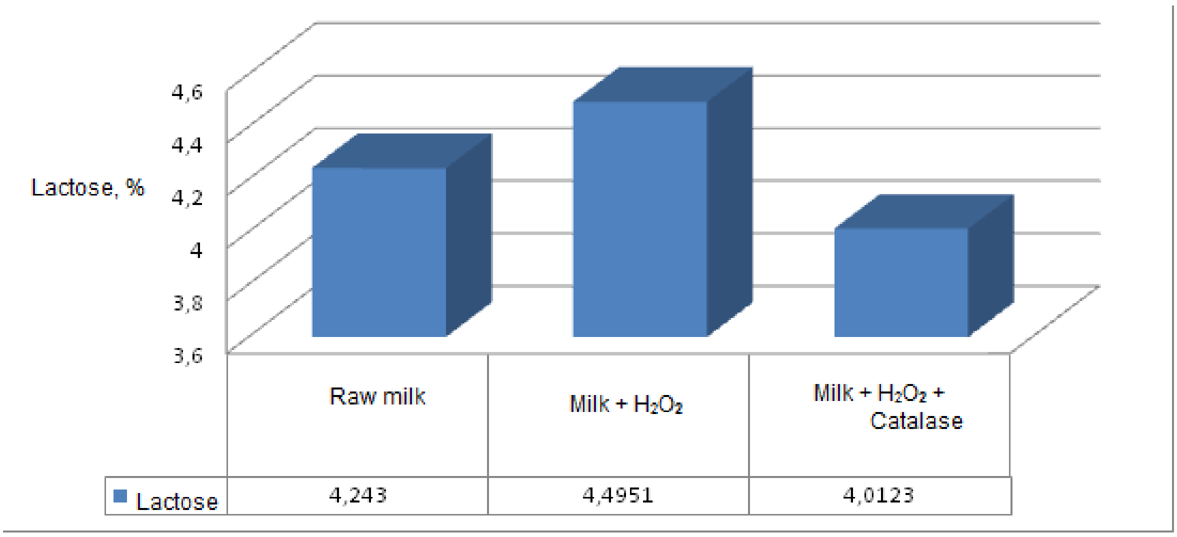

Fig.5. Cold pasteurization methods influence on lactose content of milk

Freshness of dairy fat determination

After 10 days of refrigeration of the three types of milk, under the conditions of the Kreisse method, it was found that:

- cold unpasteurized milk showed a beginning of a rancing processafter 5 days of storage, the samples developing a pale pink color and then advancing to an intense red coloration.

- in the case of pasteurized milk by the two cold pasteurization methods, the beginning of the rancing process was noticeable after 10 days of storage, fact concluded by Rokhsana et al. (2007), but contrary to the resultsfound by Pop (2008) whosustains the sensitization of the milk fat treated with hydrogen peroxide which becomes more susceptible to the phenomenon of rancing.

Vitamin C determination

The results of the determination of ascorbic acid indicate that milk treated with hydrogen peroxide is affected from this point of view, recording a decrease from 1.8 mg% in the case of raw milk to 1.1 mg%, while milk treated with hydrogen peroxide and catalase suffers a more pronounced decrease from 1.8 mg% to 0.82 mg%.

This variation in vitamin C content is also confirmed by the specific literature, but ascorbic acid is also greatly affected by conventional pasteurization, and milk is not a very important source of vitamin C(Lück, 1962).

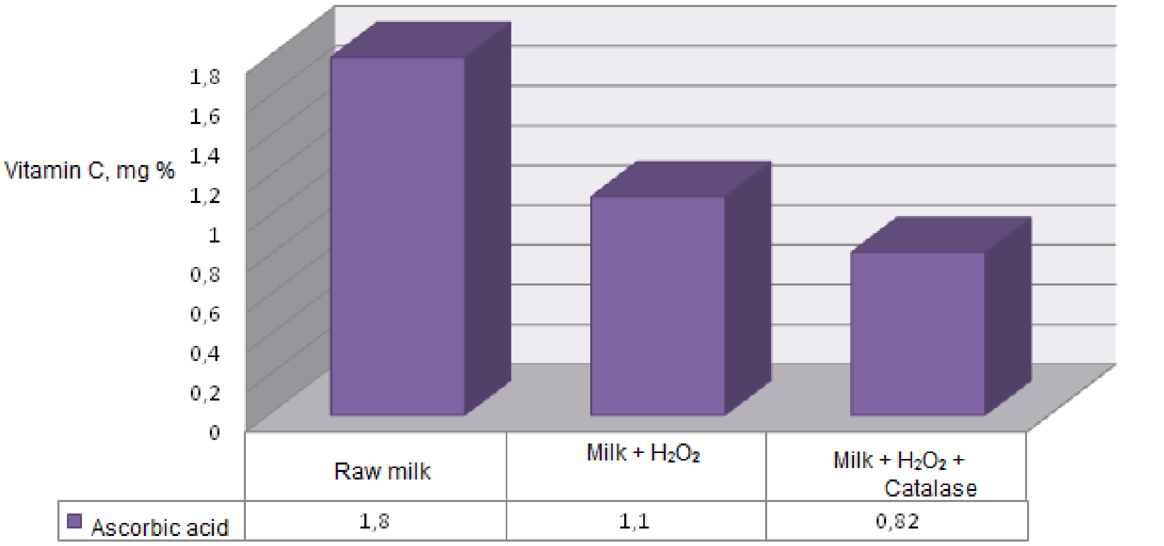

Fig.6. Cold pasteurization methods influence on vitamin C content of milk

CONCLUSIONS

It is already known that conventional pasteurization generates changes in the chemical composition of milk in a proportion that vary with the time / temperature regime applied, so a new approach of the pasteurization process is justified.

In this study, it was investigated the way in which milk is affected by a non-thermal pasteurization, the cold pasteurization using hydrogen peroxideas a preservative agent. Two methods of cold pasteurization were investigated:pasteurisation using hydrogen peroxideand the budeisation process, which uses in addition a 2‰ catalase extract.

The experimental activity did not show significant differences between raw milk and milk treated by the two cold pasteurization processes, as demonstrated also by other studies,fact that can be considered a real benefit of these methods, because it is known that pasteurization in the thermal regime affects quite a lot the chemical composition of the raw milk. Also, using these methods in the food industry, and more specifically in the dairy industry, would bring a number of benefits, primarily economic, in terms of production costs.

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