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Effect of Hydroxyapatite Nanoparticles on Kinetics of Enzymatic Gelation in Milk

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We report a result of the research on the effect of hydroxyapatite nanoparticles (nHAP) on the processes of enzymatic gelation in milk. There has been made a research on the nHAP effect on duration of the gelation and its modulus of elasticity. There has been revealed the existence of the process's bifurcation point, from which it can develop by two different scenarios. There is shown the nHAP's behavior to involve serum proteins into the process of gelation.

Keywords: Nanoparticles, Enzymatic Gelation, Hydroxyapatite.

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1. INTRODUCTION

In milk, more than 80 percent of proteins are in the form of casein micelles, having a supramolecular structure consisting mainly of four types of caseins and colloidal calcium phosphate (CCP). In the micelle the casein molecules are connected with each other by the hydrophobic interaction, disulfide bonds and CCP nanoparticles with matrix $Ca_9(PO4)_6$ and sizes of 3-15 nm [1]. A micelle usually consists of several thousands of protein molecules and several hundreds of CCP nanoparticles at the ratio of about 10 : 1. The aim of our work is to define the nature of the effect of hydroxyapatite nanoparticles (nHAP) which are CCP dummy analogues, on the process of the gelation in milk.

2. RESULTS AND DISCUSSION

In the research we have used the HAP nanoparticles provided by the laboratory of heterogeneous processes, Faculty of Chemistry, Moscow State University. The gelation has been carried out with the help of enzymatic agent with the activity of 100000 in a concentration of 36 mcg/g at an initial temperature of 34 °C. In the first part of the study we have examined the nHAP effect on the kinetics of the enzymatic gelation (Fig. 1), with the use of specialized computer information and measuring system. The addition of a small amount of nHAP into a sample has caused some elongation of the induction period of gelation, as compared to the check one, and a decrease of its loss modulus. Probably, these changes have been caused by the nHAP blocking of the agent's effect on the kappa-casein. The increase of the nHAP amount in the second sample has caused an abrupt change in the process of gelation. The attitude of a gel point has shifted in the direction of a significant time decrease of gelation, at the same time there has rather decreased the storage modulus value of the sample and the process of syneresis has accelerated. The posterior increase of nHAP concentration has caused the time increase of gelation up to values individual for the check sample. At the same time the system tendency to syneresis remained significant. The decrease of the maximum values of the gel storage modulus in the second and third samples can be explained by the fact that the initial gelation and syneresis start on the nHAP surface and in a system gelation has two-phase character. This assumption is indirectly confirmed by the organoleptic evaluation of the gel structure which is significantly different from typical homogeneous one and is characterized by frank soft fine-grains. In the third experiment the induction period of gelation has abruptly changed and got two explicitly frank sections. The first section of the induction period is close to the previous samples by its character and duration, and the second new section conforms to the latent processes of structure formation with HAP nanoparticles. On the basis of the general character of the obtained results we can assume that the moment of the transition from one section to the other is a bifurcation point (indicated with an arrow) from which the process of gelation is able to develop in a traditional or a new way.

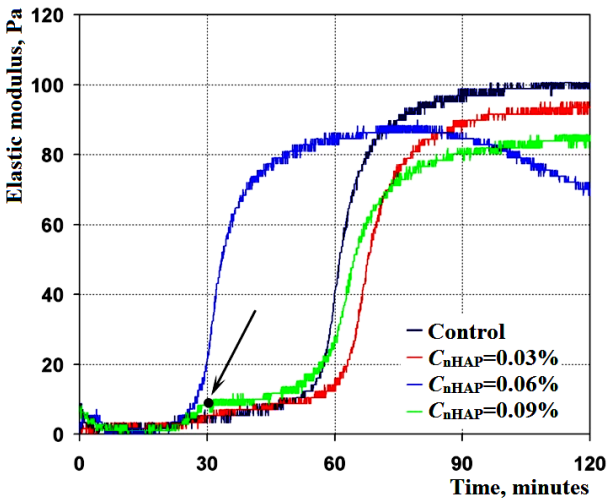

Fig. 1 – Effect of nHAP concentration on kinetics of gelation

The analysis of the experimental results has disclosed that there is a nonlinear connection between the

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nHAP concentration and the time of gelation. This dependence is shown in Fig. 2 from which it's obvious that there exists the best value of the concentration of the added HAP nanoparticles which provide a minimum gelation time. Fig. 2 also shows the effect of the nHAP concentration on the residual mass concentration of proteins in serum. It follows from the figure that with the addition of nHAP a decrease of the residual protein can attain up to 20 % that is promising and economically and ecologically efficient as it allows involving of serum proteins straight in the processes of gelation and as the final result in the ready products. The character of the dependence of the residual protein content in serum from nHAP concentration correlates with the dependence of gelation time from concentration that confirms the assumption of the nHAP concentration best value existence.

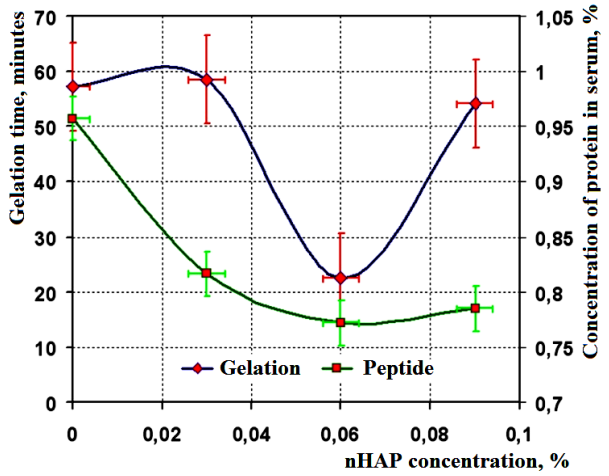

Fig. 2 – Effect of nHAP concentration on the attitude of a gel point and on the residual protein content in serum

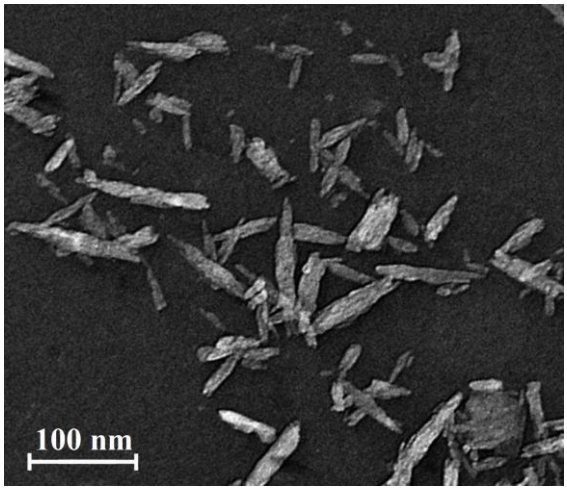

Fig. 3 – Hydroxyapatite nanoparticles

In the second part of the study we have estimated the localization and interaction of nHAP with the components of milk gel. Beforehand we received TEM images of a free preparation nHAP (Fig. 3).

The nHAP used had a very prolonged pointed form and appreciably differ in their size. The shortest nanoparticle of this sample is about 20 nm, and the largest one is about 160 nm while the breadth of nanoparticles varies from 10 nm to 30 nm.

Fig. 4 – Effect of nHAP on the milk gel

The analysis of the received images of the milk gel structural components has shown that interacting with casein micelles in the process of gelation the nHAP bulk becomes localized in protein aggregates appreciably changing their structure. In Fig. 4 of a test sample we can see that the interaction of nHAP with casein micelles has resulted in the destruction of protein globules and formation of protein and mineral agglomerates.

3. CONCLUSIONS

As a result of the work done it's shown that the addition of the artificially synthesized calcium phosphate nanoparticles in the form of hydroxyapatite strongly affects the processes of the enzymatic gelation in milk: in the process of gelation there appears a bifurcation point from which the process of structuring can develop by two different scenarios at small changes of the concentration of the added nanoparticles; nanoparticle concentration strongly and nonlinearly affects the gelation time and its modulus of elasticity so its best value exists; addition of HAP nanoparticles in an optimal dose decreases the residual protein in milk serum up to 20 % that allows involving serum proteins straight in the processes of gelation and as the final result in the ready food stuff. This provides dairy plants with economic and ecological benefits.

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