MATHEMATICAL MODELS OF COLLAGEN STRUCTURE DISORDERING BY CULTURED MILK COMPOSITIONS

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A lot of enterprises face nowadays the problem of utilizing protein-containing waste. In recent decades based on the results of research aimed to study connective tissue there has been offered a way to utilize protein waste, i.e. to produce on their basis products of collagen dissolution (PCD). The process of PCD manufacturing is a complex of chemical and technological processes based on breaking both alkali- and acid-labile bonds. To decrease protein losses in breaking acid-labile bonds it is recommended to use cultured milk compositions (CMC). CMC are a symbiosis of acid-tolerant microorganisms, as well as organic acid and enzymes produced by them. Objective of the study was to obtain the models of changing physical and chemical properties of PCD in reference with the conditions of the process of disordering its structure. The disordering the collagen structure is a complex multi-stage process. Mathematical modelling has been chosen as the basic method of solving the problem. The feature of the research is the use of cultured milk compositions as acid agent. Research data could make it possible to develop the process and increase the quality of products of collagen dissolution at the cost of preserving in the collagen structure a considerable amount of polypeptide groups.

Key words: collagen, mathematical model, cultured milk compositions

INTRODUCTION

In recent decades based on the results of deep research aimed to study such a multifunctional system as connective tissue there has been offered a way to utilize protein waste, i.e. to produce on their basis products of collagen dissolution (PCD).

The process of PCD manufacturing is a complex of chemical and technological processes based on breaking both alkali- and acid-labile bonds. To speed up intermolecular horizontal bonds breaking in the structure of mature collagen there have been traditionally applied gas limes or alkali-salt solutions. As a result, the number of hydrolysed peptide bonds in collagen can increase that leads to the decrease of molecular mass of polypeptide chains. Besides breaking alkali-labile bonds in PCD manufacturing it is necessary to provide the breaking of acid-labile ones, the latter is achieved by applying acid of different chemical nature. However, acid-labile bonds are broken mainly at increased temperatures that cause additional protein losses because of its transfer to the treating solution.

To decrease the protein losses some researchers offer to apply enzymes (Pat. 2094999 RF). However, in this case it is impossible to achieve a high degree of substrate hydrolysis because of enzymes inactivation. In this connection hydrolysis is done in the regime of partial enzyme input that increases the amount of the latter and, consequently, the cost of the final product, i.e. the efficiency of enzymatic process decreases considerably.

To decrease protein losses in breaking acid-labile bonds it is recommended to use cultured milk compositions (CMC) developed by the authors (Pat. 2399678 RF). CMC are a symbiosis of acid-tolerant microorganisms, as well as organic acid and enzymes produced by them. Acid-tolerant microorganisms in CMC provide acid-liable bonds breaking.

On the basis of the above it could be supposed that in case of breaking intermolecular acid-liable bonds of CMC it might be possible to get PCD with high molecular masses and good colloid and chemical properties at the cost of preserving a considerable amount of poly-peptide groups in PCD.

MATERIALS AND METHODS

PCD have been manufactured by raw materials treatment in CMC after preliminary alkali-salt treatment. Not standard leather raw materials (GOST – State standard 28425-90. “Leather raw materials. Technical conditions”) has been applied. After rehydration, fatting, ashing and deashing made according to the accepted technology of leather production by chroming the treatment has been conducted according to the scheme on Figure 1.

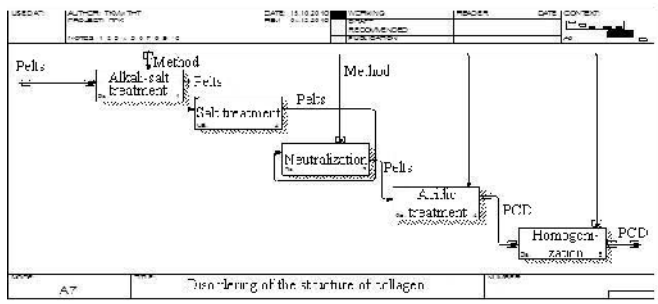

Figure 1. Raw material treatment to manufacture PCD

Acidic treatment of collagen-containing raw material has been made by CMC produced by cultivating symbiosis of kefir fungi in pasteurised quark whey (Pat. 2399678 RF).

Figure 1 shows that disordering the collagen structure to get a PCD is a complex multi-stage process; its results depend on a number of factors. Taking this into account as well as the peculiarities of the modelling object, mathematical modelling has been chosen as the basic method of solving the problem.

RESULTS AND DISCUSSION

The process of collagen structure disordering to produce PCD is a practical problem and it requires the application of simple mathematical means.

The treatment temperature and titratable acidity have been chosen as input variables. The treatment temperature is to be optimal as its decreasing could result in inhibiting metabolic processes in microorganisms while temperature increase leads to their death. The quality of the product produced depends on the degree of acid-liable bonds destruction due to organic acids and enzymes available in CMC. Decreasing the titratable acidity up to 200°T and less does not give positive results due to the insufficient amount of lactic acid in CMC. The treatment temperature influences upon the microorganisms life and productivity and, consequently, upon the degree of defibering. The degree of destruction of acid-liable bonds depends upon the value of titratable acidity.

The weight fractions of ash and fatty substances have been used as output variables. The weight fraction of ash is the index characterizing the PCD composition as well as the presence of additional admixtures that can decrease the quality of the final product. The weight fraction of substances being extracted by organic solutions can characterise the changes in the product composition during treatment as a result of extraction being caused by symbiosis action of microorganisms within temperature range. Initial availability of fatty substances in the leavens of pH chlorine-potassium extract characterises the presence of H^+-ions in the media under study; it is very important for acid treatment of collagen-containing raw material. Gelatine output is the criteria that characterises the degree of collagen structure disordering, emission of low-molecular proteins in the course of changing the condition of PCD production.

To begin with, there has been made an experiment to define the factor space. The experiment results have shown that dependence of such physical and chemical indexes as weight fraction of ash, fat substances, pH chlorine-potassium extract and gelatine output upon the process temperature and titratable acidity is not linear. Besides, there has been found out that output variables in case of input variables being limited can change linearly. With the account of that the plan of full factor experiment has been chosen to build the mathematical model. The number of experiments for two input variables ($k = 2$) equals $N = 2k = 4$.

The coefficients of the regression equation have been defined by the method of least squares according to the known technique. The expanded matrix of experiment planning in the coded (X_1, X_2) and natural (Z_1, Z_2) variables as well as the experiment results to define the weight fraction of mineral substances (Y_1), weight fraction of fat substances (Y_2), pH chlorine-potassium extract (Y_3), gelatine output (Y_4) are given in Table 1. All the research has been done in the laboratory.

There has been defined the succession of experiments with the help of the random numbers table (randomising) to compensate the experiment systematic errors. The mathematical model achieved looks like

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 \qquad (1)$$

The statistical analysis of the value of model's coefficients estimation and the accuracy control has been made according to the regression analysis formulas.

Regression equation coefficients has been considered significant in case $t_{calculated} > t_{tabled}$, where $t_{calculated}$ and t_{tabled} are calculated and tabled values of the Student criterion for the equation coefficients (defined on the significance level equal to 0,05, the number of freedom degree $f = 4$). If the calculated Student criterion is less than the tabled one, then the regression equations describe the experimental data adequately.

The models achieved are valid only for the chosen range of input variables changes, i.e. for the dissolution temperature 4 – 24°C and the titratable acidity of CMC 143 – 331°T.

To define the optimal parameters of the collagen dissolution process the coded variable equation was changed into the natural values equation (normalized model). There have been found equations describing the dependence of gelatine melting out, weight fraction of the substances being extracted by organic solutions and mineral substances as well as pH chlorine-potassium extract upon the process conditions.

While defining the gelatine output the regression equation is as follows:

$$Y = 79,6652 + 16,8063X_1 + 12,3547X_2 - 10,372X_1X_2 \qquad (2)$$

This equation has been used to make the parameters of the collagen dissolution process optimal. The equation analysis shows:

a) the coefficient b_1 has got the highest value (absolute value), so it has been the process temperature that has got the greatest influence upon the gelatine output in the range under research;

b) the coefficient b_{12} has proved to be significant, so in the range of two input variables under research the joint influence of the temperature and titratable acidity of the composition has a considerable influence on the output variable (though according to the absolute quantity the value is not large).

While defining the gelatine output the normalised equation after transformations looks as follows:

$$Y = 4{,}2947Z_1 + 0{,}2957Z_2 - 0{,}0114Z_1Z_2 - 11{,}4995 \quad (3)$$

Figure 2 shows the model of the dependence of gelatine output weight fraction upon process parameters.

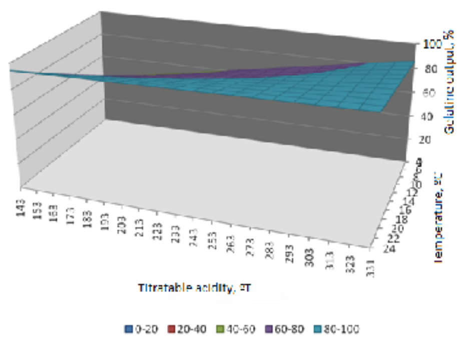

Figure 2. Model of dependence of gelatine output weight fraction upon process parameters

The gelatine output change is proportional to the temperature and titratable acidity increase. While choosing optimal parameters for collagen acidic dissolution besides gelatine melting out parameter an additional estimation criterion to define the quality of the product is necessary. Theoretical values of gelatine output dependence upon titratable acidity have been calculated according to the equation.

Analysing the model, it is necessary to point out that gelatine melting out grows considerably with the titratable acidity of the composition and temperature increase.

While defining the weight fraction of mineral substances the regression equation is as follows:

$$Y = 3{,}6864 - 0{,}6508X_1 + 0{,}6938X_2 \quad (4)$$

The equation analysis shows that:

a) the coefficient b2 has got the greatest value (according to the absolute quantity), so the titratable acidity of the cultured milk composition has a considerable influence on the weight fraction of the mineral substances in the range under research;

b) the coefficient b12 has turned out to be not significant, so in the range of two input variables under research the joint influence of the temperature and titratable acidity of the composition has not got a significant influence on the output variable. While defining the weight fraction of the mineral substances after transformation the normalized equation can be written as follows:

$$Y = 2,85 - 0,07Z_1 + 0,01Z_2 \quad (5)$$

The resulting equation was applied to make the parameters of collagen dissolution process optimal. Figure 3 demonstrates the model characterising the dependence of the weight fraction of mineral substances upon the parameters of the process.

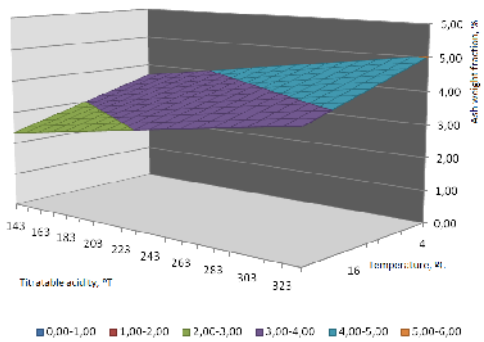

Figure 3. Model of dependence of weight fraction of mineral substances of PCD upon the process parameters

The change of the weight fraction of mineral substances is proportional to the temperature and titratable acidity increase. This might be connected with dependence of the metabolic activity of microorganisms and consequently the degree of collagen structure disordering upon the parameters mentioned above. The authors suppose that while choosing the optimal parameters of collagen acidic dissolution this very model should be applied with the account of the PCD properties which are to be achieved.

CONCLUSIONS

The analysis of models and their graphical interpretation has demonstrated that besides the gelatine output indexes, the ones of the weight fraction of substances being extracted by organic solvents as well as mineral substances pH chlorine-potassium extracts it is necessary to apply an addition estimation criterion to define the quality of the product achieved, for example, the microbiological analysis of CMC or a deeper research into the structure of PCD/PCD films which could help to produce high qualitative characteristics of the product.

There have been built according to the research results the models of changing physical and chemical properties of PCD in reference with the conditions of the process of disordering its structure. Thus, according to the authors the optimal process parameters to produce PCD is the temperature of 24±2°C, titratable acidity of 250 – 300°T and applying of CMC based on the jeaven of kefir fungi cultivated on pasteurised whey.

These data could make it possible to develop the process and increase the quality of products of collagen dissolution at the cost of preserving in the collagen structure a considerable amount of polypeptide groups.

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