APPLICATION**NOTE**

Food – DSC / Kinetics Neo Software

Kinetics Neo: Prediction of Protein Denaturation Due to Pasteurization

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Pasteurization

Pasteurization is a controlled, non-sterilizing preservation process primarily designed to reduce microbial load and enzymatic activity on food, hence minimizing the risk of foodborne illnesses and extending the shelf life of food products. Pasteurization can be done using non-thermal pasteurization techniques, such as High-Pressure Processing (HPP) and Pulsed Electric Field (PEF). These techniques have been developed more recently to address the increasing demand for fresher, minimally processed food [1].

Traditional pasteurization methods, however, involve the application of mild heat to the food for a certain period of time. The applied heat has to be sufficient to inactivate pathogenic microorganisms and spoilage agents, while retaining most of the organoleptic, nutritional and functional properties of the products. Classic thermal pasteurization methods include [2]:

1. Batch (Vat) or Low-Temperature, Long-Time (LTLT): Heating at 65°C for 30 minutes.
2. High-Temperature, Short-Time (HTST): Heating at 72°C for 15 seconds.
3. Ultra pasteurization: Heating at 89 to 100°C for 1 second.
4. Ultra-High pasteurization: Heating at 138°C for 2 seconds.

Heat treatment can have deleterious effect on the food product, for example: alteration of color due to water evaporation or Maillard reaction[1], partial loss of the nutritional value, or protein denaturation. The last of these is extremely important if the pasteurized product has applications as a functional ingredient in a food product. Protein denaturation can affect solubility, emulsifying capacity, and gelation properties. The choice of pasteurization technique must therefore balance microbial safety with the desired sensory, nutritional, and functional quality of the food product.

Kinetics Neo is a software tool, specialized for the kinetic analysis of temperature-dependent chemical processes. These processes may involve changes in mass, enthalpy, decomposition, and crystallization, among other phenomena. The software supports both model-free and model-based kinetic analyses.

In the model-based approach, Kinetics Neo enables detailed characterization of individual reaction steps, providing critical kinetic parameters such as activation energy, reaction order, and the quantitative contribution of each step to the overall process. This comprehensive analysis facilitates accurate predictions of reaction behavior under unmeasured or experimentally inaccessible temperature profiles. It includes the prediction of the degree of protein denaturation, here named as conversion, due to a certain time of exposure to different temperatures as discussed in the following.

[1]The Maillard reaction is a non-enzymatic browning reaction in which free amino groups react with reducing compounds such as sugars. The Maillard reaction is responsible for browning and flavor development in various cooking processes.
https://flexikon.doccheck.com/de/Maillard-Reaktion#:~:text=The%20Maillard%2Dreaction%20describes%20a,flavours%20during%20

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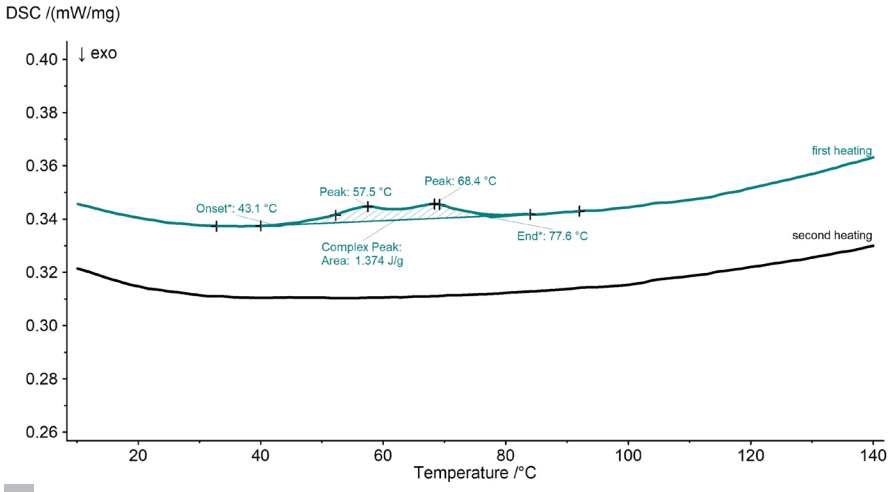
Predicting Protein Denaturation

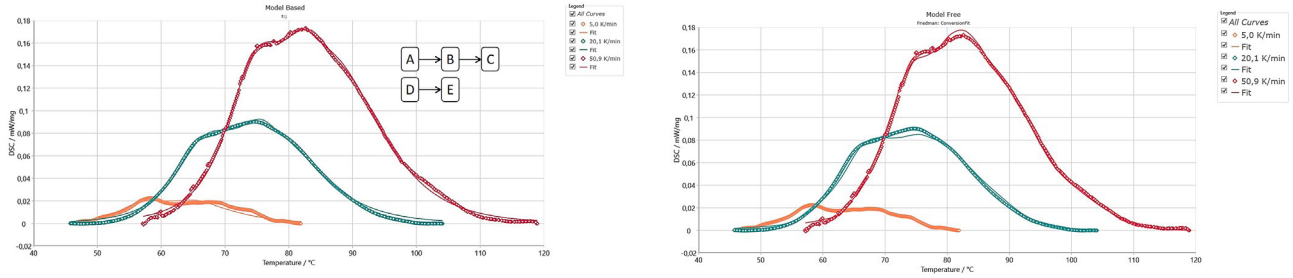
The effect of pasteurization on yeast protein extraction was investigated using a DSC 300 *Caliris*® and the NETZSCH Kinetics Neo software.

The yeast protein was dispersed in distilled water at a final concentration of 15% (w/v)[2]. A sample mass of 25 mg of dispersion, corresponding to 3.75 mg protein, was analyzed in low-pressure[3] aluminum crucibles under a nitrogen gas atmosphere at a heating rate of 5 K/min between 0°C and 140°C. The yeast protein denaturation occurs in the range of 44°C and 78°C, as shown in the first heating curve (green) in figure 1. The endothermic effect is broad and shows two maxima, indicating that the sample has a mixture of proteins, as expected for an extraction of protein. The second heating curve (black) shows the absence of thermal effects, which indicates that the denaturation is irreversible.

The dependence of the denaturation on the heating rate allows for evaluation of the process with the help of the NETZSCH Kinetics Neo software. To this end, DSC curves were acquired at different heating rates, 5 K/min, 20 K/min, and 50 K/min. Several different kinetic and models were tried in order to find the best fit. The two best results were the Friedman analysis and the three-step kinetic model, with correlation coefficients of 0.9988 and 0.9989, respectively; see figure 2.



1 DSC curve of yeast protein denaturation; heating rate of 5 K/min.



2 Kinetic evaluation of the denaturation of yeast protein. Left: model-based analysis – 3-step kinetic model, R^2=0.9988. Right plot: model-free analysis – Friedman Analysis, R^2=0.9989. Dotted lines: measured curves; solid lines: calculated curves. Heating rates of measured curves: 5 K/min (orange), 20 K/min (green), and 50 K/min (red).

[2]weight per volume
[3]Low-pressure crucible consist of aluminum, withstanding a slight overpressure that might occur during the measurement.

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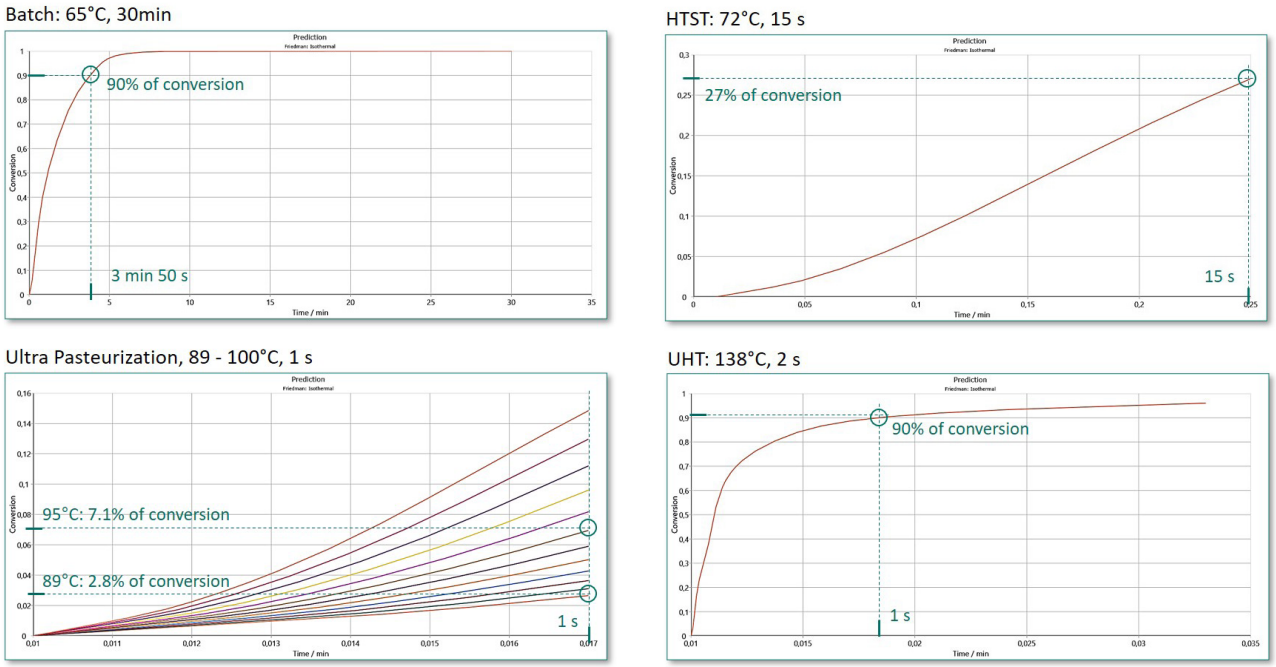
The DSC results obtained were used for prediction of the protein denaturation under four different pasteurization temperature schemes described in literature [2]. According to the prediction, Friedman analysis, not shown, and the three-step kinetic model, shown in figure 2 below, three of the four pasteurization methods tested will not be applicable for this product; see figure 3.

The Batch (Vat) method would lead to 90% conversion after 3 minutes of heating, which is only 10% of the entire period of time recommended. The UHT method would also be too harsh; after 1 s at 138°C, the total content of native protein would be only 10%. The HTST method would still denature 27% of all protein content.

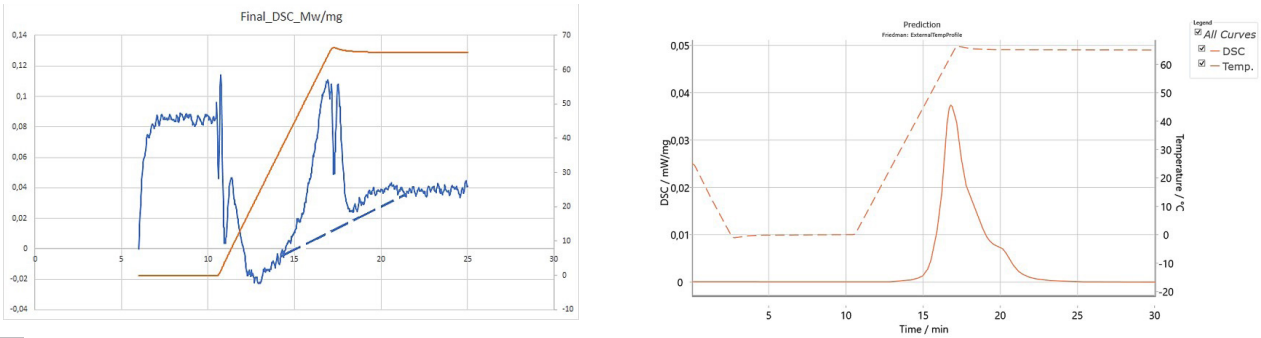
Only Ultra Pasteurization would yield acceptable conversion rates: 7% conversion after 1 s at 95°C.

Validation of the Results

In order to validate the kinetic model calculated by Kinetics Neo for prediction of the denaturation behavior under isothermal conditions, a yeast protein sample of 25 mg, 3.75 mg of protein, was heated to 65°C and then kept isothermal for 20 minutes. Figure 4 compares the endothermic effect determined via measurement to those determined via prediction (Kinetics Neo). The comparison shows the good agreement between the two curves and thus, the reliability of the calculation.



3 Model-based analysis – 3-step kinetic predictions of the denaturation of yeast protein under different pasteurization conditions. Upper left: Batch (Vat) method; upper right: High-Temperature, Short-Time (HTST) method; bottom left: Ultra Pasteurization method; bottom right: Ultra-High-Temperature (UHT) method.



4 Comparison of the measured and predicted endothermic effects related to denaturation of yeast protein during heating to 65°C and isothermal segment.

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Conclusion

Based on these results, a processing window was found for the pasteurization of protein products for the food industry. Kinetics Neo provides an opportunity to develop a mathematical model that accurately represents the experimental behavior of samples during thermal treatment. This approach simplifies the process of identifying the most promising temperature profile, eliminating the need for time-consuming trial-and-error methods.

References

[1] Fellows, P. J. (2022). Food Processing Technology: Principles and Practice. In Food Processing Technology: Principles and Practice. https://doi.org/10.1016/C2019-0-04416-0

[2] Deak, T. (2013). Thermal Treatment. In Food Safety Management: A Practical Guide for the Food Industry (pp. 423–442). Elsevier. https://doi.org/10.1016/B978-0-12-381504-0.00017-2

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