

ENZYME DEACTIVATION USING HIGH PRESSURE CARBON DIOXIDE TECHNOLOGY

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Abstract For some foods, low enzyme activity is desirable in order to stabilize the quality of the product. High enzyme activity adversely affects food quality, causing enzymatic browning in vegetables and fruits and degradation of starch in grains, and enzymatic reactions cause redness, occlusion and oxidation processes in flour. During storage of whole wheat flour, lipids can influence the quality of the flour through enzymatic hydrolysis, which is catalyzed by lipases (Budisa et al., 2014). Our research focused on the use of scCO₂, which has attracted particular attention in research and technology because of its “green” (i.e., sustainable) properties with the possibility of reusability of CO₂ while pursuing a circular economy approach (Li et al., 2016). Enzyme deactivation by scCO₂ treatment at different pressures, temperatures and exposure times of wheat flour was performed.

Keywords:

Supercritical technology, supercritical carbon dioxide, enzyme deactivation, high pressure, reusability of CO₂.

1 Introduction

Flour, a product obtained in the process of grain milling, is one of the most important raw materials in the food industry. Flour is indispensable for the production of a wide variety of staple products such as bread, pasta, cakes, and biscuits. Flour must be of satisfactory quality for processing to obtain acceptable end products (Sujka *et al.*, 2017). Many of the bioactive compounds present in food products, such as enzymes, anthocyanins and vitamin C, are thermo-sensitive and are destroyed by heat treatment. High temperature also affects the fresh flavor of fruits and other products. Therefore, it is necessary to use efficient non-thermal preservation techniques in food production.

Because of the development of undesirable chemical changes in flour during storage, deactivation of enzyme activities may be an appropriate strategy to extend shelf life and maintain the functional properties of whole wheat flour. Previous studies have used different thermal processing methods, including steaming, microwave heating, and passing through infrared and gamma radiation to decrease enzymatic activities (Poudel *et al.*, 2018).

For instance, deactivation using scCO₂ is a promising non-thermal method to significantly decrease enzyme activity through the combined effect of high pressure of up to 65 MPa with mild heating of up to 50 °C. Enzyme deactivation using scCO₂ processing is usually connected with lowering the products' pH combined with physical disruption of the enzymes (Marszałek *et al.*, 2019).

2 Experiments

The chemicals and reagents used in this study were distilled water, white wheat flour type 500, carbon dioxide (99.5 % purity, Messer, Ruše). Ethanol, phosphoric acid, sodium chloride, Coomassie-Brilliant Blue G250 and acetonitrile were supplied by Merck, while chicken egg albumin, sodium acetate, acetic acid and p-nitrophenyl butyrate were supplied by Sigma. α -amylase enzymes from *Aspergillus oryzae* were obtained from Sigma and lipase from *Aspergillus niger* was obtained from BioChemics. All other chemicals used in the laboratory were of analytical grade.

The following experimental methods were used in the study:

- **Time optimization of enzyme extraction**, where the optimal conditions for the extraction of enzymes from wheat flour were determined. The optimal shaking time and method of centrifugation of the sample were experimentally determined in order to obtain the highest concentration of proteins present in the supernatant after centrifugation.
- **Determination of protein content in flour** was estimated using the Bradford dye binding method with bovine serum albumin as the standard, measured on a UV-spectrophotometer at a wavelength of 595 nm.
- **Determination of enzyme activity**: White wheat flour was selected for this study since it is most commonly used in bakery products. After the examination of previously published scientific studies, we decided to determine the activity of the desired and undesired enzymes which are most often present in different types of flour. In the study, we investigated the activity of α -amilase and lipase enzymes. The activity of individual enzymes was determined based on enzymatic activity assays using a UV-spectrophotometer method at different wavelengths.
- **Deactivation of enzyme using high-pressure reactor and determination of residual enzyme activity after scCO₂ treatment**. In order to determine the effect of scCO₂ on the activity of enzymes in white wheat flour, free enzymes (α -amilase and lipase) were exposed to different conditions in a high-pressure batch reactor (200 bar and 300 bar at 35°C). Then, white wheat flour was exposed under the same conditions in a high-pressure batch reactor. Later, enzymes were extracted from scCO₂ treated wheat flour, where the supernatant was used to determine the enzyme activity. For comparison, the enzyme activity of untreated flour was determined. Residual activities of treated wheat flour were correlated to the native enzyme activity, taken as 100 %.

3 Results

Optimization of enzyme extraction from white wheat flour was performed during the research. Different times and methods for obtaining the supernatant had a significant influence on protein concentration in the sample. The protein concentration was determined by direct measurement of protein content in the remaining supernatant after extraction.

Optimal conditions and the highest protein concentration were achieved with a single centrifugation (step 3) at a shaking time of 90 minutes.

The influence of scCO₂ on the residual activity of enzymes was investigated after 3 hours of treatment time at 200 bar and 300 bar at 35°C. Deactivation of the α -amylase enzyme occurred after 3 hours at 200 bar. As can be seen from Tab. 1, deactivation of α -amylase is further increased at 300 bar. Lipase achieved half-life of the enzyme at treatment time after 3 hours and 300 bar.

Furthermore, the influence of scCO₂ on the residual activity of enzymes in white wheat flour was investigated after 3 hours of treatment time at 200 bar and 300 bar at 35°C. Comparing the results from Tab. 1, similar behavior of enzymatic activities of free enzyme and enzymes in white wheat flour under the same conditions and treatment with scCO₂ was observed. Residual activity of α -amylase decreased at 200 bar and further decreased at 300 bar. Also, inactivation of the lipase enzyme is less effective in white wheat flour than in the free enzyme, since it remains 75 % at 300 bar.

Table 1: Residual activity after deactivation of pure enzyme and enzyme in flour sample in scCO₂.

Enzyme	Residual activity [%]	
	200 bar, 35 °C, 3 hours	300 bar, 35 °C, 3 hours
pure α -amylase	11.7	6.8
pure lipase	125.8	53.4
α -amylase in sample	2.8	2.6
lipase in sample	97.1	75.8

A sample of white wheat flour was studied using FTIR spectroscopy, which offers qualitative and quantitative determination of food macro components such as proteins, lipids, saccharides, and water. FTIR spectroscopy, which relies on absorption of infrared radiation by oscillating molecules, is increasingly applied in food research (Sujka *et al.*, 2017). Several bands characteristic of proteins, fats, carbohydrates and water were observed in the flour spectrum. An intense band in the 3600–3200 cm^{-1} range is generated by the stretching vibration of O–H bonds. Bands in the 3000–2800 cm^{-1} range are caused by stretching vibrations of C–H bonds. Proteins were observed in the 1,600 cm^{-1} to 1,700 cm^{-1} range by bands caused by amide I bonds and 1,550 cm^{-1} to 1,570 cm^{-1} by amide II, respectively (Mohan Kumar *et al.*, 2019). The spectral region between 1500 and 900 cm^{-1} is called the fingerprint region because of the unique patterns characteristic of given samples. The assignment of spectral bands to vibrations generating these bands is presented in Fig. 1.

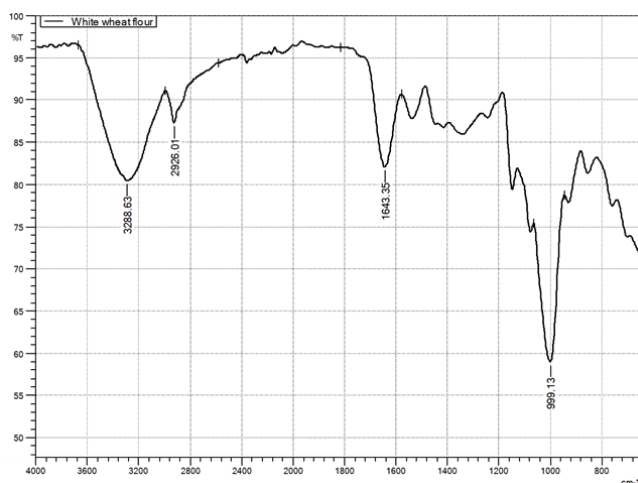


Figure 1: FTIR spectrum of white wheat flour.

4 Conclusions

Because of the negative effects of enzymes on flour quality, we decided to deactivate the α -amylase and lipase enzymes which are present in flour. The effect of process parameters (pressure and exposure time) on the activity of enzymes in flour was investigated. Protein concentration in flour was determined using the Bradford

method. scCO₂ affects the activity of enzymes, and the activity of certain enzymes in flour is significantly lower after exposure to certain pressures and temperatures, compared to the activity of enzymes in flour that was not exposed to scCO₂.

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