CAMURE-11 11th International Symposium on Catalysis in Multiphase Reactors
ISMR-10 10th International Symposium on Multifunctional Reactors
MILANO, ITALY - 21-24 March, 2021

The impact of high-pressure technology on wheat flour enzyme

Maja Leitgeb[1,2]*, Željko Knez[1,2], Gordana Hojnik Podrepšek[1]

1 University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory for Separation Processes and Product Design, Smetanova ul. 17, 2000 Maribor, Slovenia.

2 University of Maribor, Faculty of Medicine, Taborska ulica 8, 2000 Maribor, Slovenia;

**Corresponding author E-Mail: maja.leitgeb@um.si*

1. Introduction

Wheat is one of the most important crops worldwide. Its functional properties are important criteria for food processing and quality [1].

The purpose of this study was to determine the impact of high-pressure technology on wheat flour enzyme. The process is based on the inactivation of enzymes present in food products through the application of high pressures and green media, such as CO_2 [2]. There is no need to use very high pressure, such as 600 MPa. By avoiding exposure of the food products to high temperatures, their natural qualities are better preserved [3]. Additionally, this technology is being applied to food and raw material processing for obtaining innovative sensorial and functional properties. The main advantage of this method is related to the low thermal treatment conditions that the temperature of the food does not exceed more than 40 °C. Therefore it is categorized as a non-thermal technology [4]. Consequently, the stability of covalent bonds to high pressure brings the better preservation of the nutritional values (like vitamins and pigments), sensorial and quality aspects of the food products [4,5].

The effect of treatment time and temperature on the activity of peroxidase enzyme in food is presented. Peroxidases are a class of oxidoreductase enzymes. They use hydrogen peroxide or organic hydroperoxides as oxidants. Peroxidase also plays a significant role in carotenoid bleaching during dough mixing and may be responsible for undesirable browning of flour [6]. Due to the undesirable effects of enzyme peroxidase, we performed a series of experiments to achieve inactivation of this enzyme in wheat flour.

2. Methods

Reagents. All reagents were of analytical grade and commercially available. White wheat flour type 500 was kindly donated from bakery Hlebček (Pragersko), while carbon dioxide (99,5% purity) was purchased from Messer, Ruše. Ethanol, phosphoric acid, sodium chloride, Coomassie-Brilliant Blue G250, acetonitrile were supplied from Merck, while chicken egg albumin, sodium acetate, acetic acid, p-nitrophenyl butyrate were supplied from Sigma. Enzyme peroxidase from *Horseradish* was obtained from Biozyme laboratories.

Methods. Optimal conditions for the extraction of enzymes from wheat flour were determined. Determination of protein content in flour was estimated by the Bradford dye binding method using BSA as the standard. Peroxidase activity was measured using phenol, 4-aminoantipyrine (AAP), and hydrogen peroxide as substrates. The determination of the effect of $scCO_2$ on the activity of native peroxidase and peroxidase in white wheat flour under different conditions in a high-pressure batch reactor (200 bar and 300 bar at 35˚C) was done. A temperature of 35 °C was chosen to avoid thermal inactivation.

3. Results and discussion

The influence of $scCO_2$ on the residual activity of peroxidase was investigated after 3 and 24 hours of treatment time at 200 bar and 300 bar at 35˚C. The experiments were performed in a high-pressure reactor (Figure 1).

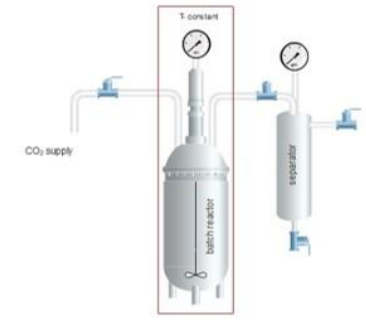

Figure 1. Schematic diagram of a high-pressure batch reactor.

The activity of native peroxidase after 3 hours of $scCO_2$ treatment at 200 bar increased, while after 24 hours of $scCO_2$ treatment its residual activity decreased (17%). As it can be seen from Tab. 1, that deactivation of peroxidase at 300 bar is further increased. Similarly, an increase in peroxidase activity of 105% was observed when wheat flour was $scCO_2$ treated for 3 hours at 200 bar. In common, the residual activity of peroxidase in wheat flour after $scCO_2$ treatment remained high at different treatment conditions with $scCO_2$.

Table 1. Residual activity of peroxidase after high pressure treatment of native enzyme and enzyme in wheat flour.

Enyzme	Residual activity [%]			
	200 bar, 3 hours	200 bar, 24 hours	300 bar, 3 hours	300 bar, 24 hours
Native peroxidase	113	17	98	4
Peroxidase in wheat flour	105	103	101	95

4. Conclusions

In conclusion, we found that treatment of native peroxidase with $scCO_2$ induced reduction in residual activity. However, under certain conditions an excessive decrease in peroxidase activity in the flour sample was not detected. Considering the high impact of $scCO_2$ on inactivation of native enzyme observed in this study, further study is necessary to determine the conditions under which the peroxidase in wheat flour will be more efficiently inactivated.

References

[1] C. Vogel, K.A. Scherf, P. Koehler, Effects of thermal and mechanical treatments on the physicochemical properties of wheat flour, Eur. Food Res. Technol., 244 (2018) 1367–1379.
[2] G. Hojnik Podrepšek, Ž. Knez, M. Leitgeb, Activation of cellulase cross-linked enzyme aggregates (CLEAs) in $scCO_2$, J. Supercrit. Fluids, 154 (2019) 104629.
[3] T. Fornari, R.P. Stateva, High Pressure Fluid Technology for Green Food Processing, Springer, 2014.
[4] J. Welti-Chanes, C.E. Ochoa-Velasco, J.Á. Guerrero-Beltrán, High-pressure homogenization of orange juice to inactivate pectinmethylesterase, Innov. Food Sci. Emerg. Technol., 10 (2009) 457–462.
[5] A.C. Polydera, E. Galanou, N. Stoforos, P. Taoukis, Inactivation kinetics of pectin methylesterase of greek Navel orange juice as a function of high hydrostatic pressure and temperature process conditions, J. Food Eng., 62 (2004) 291–298.
[6] D.W. Hatcher, W. Barker, A Rapid Microassay for Determination of Peroxidase in Wheat and Flour, Cereal Chem., 82 (2005) 233–237.