Impact of $SCCO_2$ on flour characteristics and lipase activity in different flour types

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1. Introduction

Supercritical carbon dioxide ($SCCO_2$) has been widely used for the inactivation of various microorganisms and bioactive compounds. Methods of food sterilization and inactivation of enzymes, which catalyze adverse reactions, especially in fruit and vegetable juices, are also known, while inactivation of enzymes in flour is still a relatively new approach. $SCCO_2$ has emerged as an innovative and promising technology for processing food ingredients and products. It is generally recognized as safe (GRAS), environmentally friendly, inexpensive, readily available in high purity, and easy to remove. Most importantly, the supercritical state of CO_2 can be achieved at a relatively low temperature and pressure[1].

Certain enzymes, which are found in flour are amylases, hemicellulases, xylanases, lipases, glucose oxidases and proteases. Numerous enzymes are important from the standpoint of baking, some already at storage. However, various enzymes that are present in flour influence desired and undesired reactions. Adverse reactions include flour browning and oxidation, which impairs the quality and shelf life. Lipases cause hydrolytic rancidity by hydrolysis of triglycerides into free fatty acids (FFA). Therefore, lipases reduce the shelf life of flour due to the fast oxidation of FFA[2].

This study focused on applying high pressure and carbon dioxide (CO_2) processing conditions on different flour types to inactivate enzyme lipase and to acquire the optimal processing conditions under which the highest level of inactivation was achieved. Also, the rate of CO_2 release from the system is an important factor that affects the final activity of the enzyme. Finally, physicochemical properties of untreated and $SCCO_2$-treated flour were determined.

2. Materials and Methods

White wheat flour, semi-white wheat flour, rye flour, rye wholemeal flour, graham flour and spelt flour were used in the study. First, the activity of the enzyme lipase was determined in a particular type of flour. The activity of lipase was determined based on P-nitrophenol (pNP) reaction, which was monitored at 400 nm for 5 min and 37 ˚C using p-nitrophenyl butyrate (PNPB) as the substrate. Furthermore, different types of flour were exposed to $SCCO_2$ conditions at 300 bar for 3 h and 35 °C in a high-pressure batch reactor (Figure 1). After exposure to certain conditions, the degree of lipase inactivation in individual flour types was determined. It was ascertained in which type of flour the highest lipase inactivation occurred. The physicochemical properties of the flour were followed by SEM analysis, where the surface morphology and particle sizes of the flour at rapid and slow exposure were examined, and FTIR analysis of the samples was performed to observe the possible change in the functional groups. Also, laser granulometry

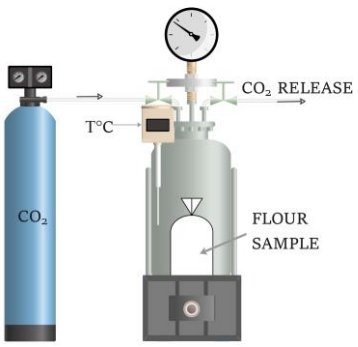

Figure 1. $SCCO_2$ reaction system.

was used to detect the size of the flour particles before and after $SCCO_2$ treatment depending on the rate of CO_2 expansion.

3. Results and discussion

The objective of this phase of the study was to evaluate the differences in lipase activity within different flour types. The activity of lipase varied depending on flour type, where the highest lipase activity was presented at rye flour and the lowest in spelt flour (Figure 2). Afterwards, the influence of $SCCO_2$ on the residual activity of lipase was investigated after 3 h of treatment time at 300 bar and 35˚C. As can be seen from Figure 3, the highest inactivation of lipase occurred in white wheat flour, where the residual activity of lipase was 43%. In comparison, the residual activity of lipase in graham flour was 65%. The lowest effect of $SCCO_2$ treatment on lipase activity was found in rye and spelt flour, where the residual activity was 93% and 99%, respectively.

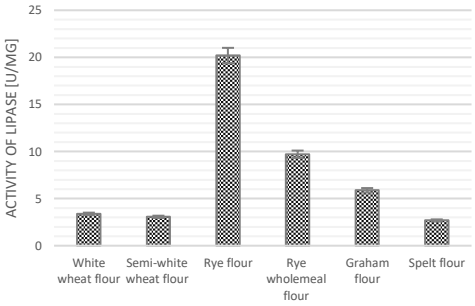

Figure 2. The activity of lipase in different flour types.

Furthermore, the particle size distribution was determined according to laser diffraction analysis, where the white wheat flour particles were distributed within the size of 56 μm for untreated flour and for untreated rye flour of 87 μm. After exposure to $SCCO_2$, we found that the particle size of flour in both types is significantly reduced in the rapid expansion process to 53 μm in rye flour, while in white wheat flour, the particle size of flour was slightly increased to 60 μm. The mean particle size in white wheat flour after slow expansion of CO_2 was 66 μm. Moreover, the granular morphology of $SCCO_2$-treated white wheat flour observed by SEM was presented in Figure 4. Wheat flour showed a spherical shape with a size of 64 μm, which was in accordance with laser granulometry and the report by Ma et al [3].

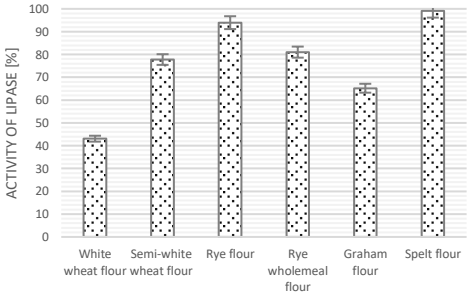

Figure 3. Lipase inactivation after $SCCO_2$ treatment of flours.

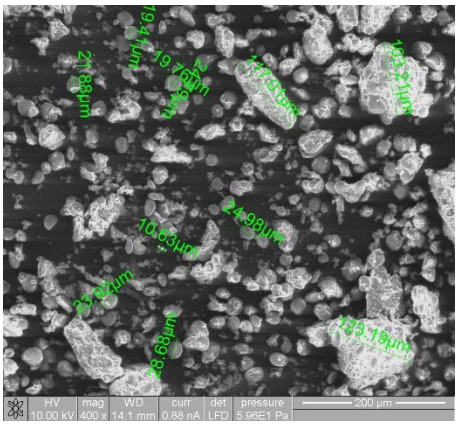

Figure 4. Micrograph of white wheat flour sample by scanning electron microscopy (SEM) (magnitude ×400).

4. Conclusions

Inactivation of the enzyme lipase by $SCCO_2$ depends on the type of flour, where the composition of the flour is an important factor, as bran is also included in wholemeal flours, where access to the active site of the enzyme is more demanding. Therefore, the rate of enzyme inactivation in wholemeal flours is lower. Moreover, from these results, we can conclude that rapid expansion has a more significant impact on flour particle size. Most importantly, FTIR analysis further detected that the structure of flour particles remains the same, as no changes were observed in functional groups. To conclude, $SCCO_2$ has proven to be a promising technology for the inactivation of enzymes in flour, however $SCCO_2$ does not affect the quality of flour, which can be further used for the preparation of bakery products.

References

1. Wang W, Rao L, Wu X, Wang Y, Zhao L, Liao X. *Food Eng Rev*. **2021**, *13*, 570-591.

2. Zhou N, Haynes L, Slade L, Levine H, *AACCI 91st Annual Meeting*, **2006**.

3. Ma Y, Xu D, Sang S, Jin Y, Xu X, Cui B. *Food Hydrocolloids*, **2021**, *112*, 106362.