Reduction of milk protein allergen by Physical and Enzymatic methods

Eszter Lubica Blaskó

Food Science and Technology Engineering, Master, Full time training

Institute of Food Science and Technology/ Department of Food Process Engineering

Insider subject leader: András Koris PhD, professor, Arijit Nath PhD, postdoctoral researcher, Krisztina Zita Albert PhD, senior lecturer

Cow's milk proteins (CMPs) are recognised as a high-quality protein because they contain all the essential amino acids needed for human nutrition. Nevertheless, cow's milk protein is included in the list of the eight major allergens due to the presence of linear and conformational epitopes in the protein structure. CMPs are hydrolysed by various plant-based proteases such as papain and bromelain, which are cysteine protease enzymes, thus reducing allergenic activity. Enzymatic hydrolysis of milk proteins can be controlled by selection of the specific enzyme, reaction conditions and operating parameters. The food industry currently uses animal-based trypsin and chymotrypsin to hydrolyse milk proteins, but the use of plant-based enzymes is not widespread.

The enzymatic hydrolysis was carried out in a well-equipped bioreactior, which was operated in a batch mode under isothermal conditions. The allergenic activity was measured by sandwich-type immonusorbent assay (ELISA). The use of papain is considered to be the most suitable for reducing the activity of cow's milk proteins. To optimize the hydrolysis, papain was used in the following contentrations: 0.08 g/L, 0.016 g/L and 0.032 g/L. The hydrolysis was carried out in time intervals of 10-20 minutes and the temperatures between 40-60 °C. A 3^P factor design was used to determine the optimal operating conditions for the enzymatic hydrolysis reaction. Statistica 11 was used to develop a factorial design and reaction surface diagram.

Further measurements were carried out with the values found after optimisation, which were 0.012 g/L for concentrartion, 50 °C for temperature and 15 min for time. There are several methods for reducing allergenic activity, but many of their drawbacks have been desribed in previous studies. In second round, enzymatic hydrolisis was combined with different physical treatment methods such as ohmic heating and radiofrequency heating. Then the performances

of the three different treatments (normal enzymatic hyrdolisis -EH, EH+radiofrequency, EH+Ohmic heating) were compered. Changes in the structure of milk proteins were detected when enzymatic hydrolysis was combined with ohmic and radiofrequency heating in the examined cases. During the Ohmic heating, fat and protein precipitates were formed on the two electrodes, while in the case of the radiofrequency, a change in the whole structure of the milk was observed. To compare the three types of treatment, I tested the reduction of allergenic activity level with with ELISA method.

The decrease in allergenic activity was influenced by the concentration of papain rather than by the time and temperature of hydrolysis. A linear correlation was observed between the decrease in allergen activity and papain concentration. This means that fast heating methods (such as ohmix heating and radio-frequency heating) could be considered as alternative heating methods to carry on such processses, given their higher energy transfer efficency, withiut compromising the enzimatic activity of papain. Furthermore, the correlation of the decrease in allergenic activity with hydrolysis temperature shows a quadratic fitThe results of the present study can be used by the diary industry to open new opportunities for consumers allergic to cow’s milk.