Acta Alimentaria, Vol. 29 (2), pp. 181–186 (2000)

POTENTIAL INDICATOR ENZYMES AT BROCCOLI BLANCHING TECHNOLOGY

M. NAGY-GASZTONYI, Á. KARDOS-NEUMANN and P. Á. BIACS

Central Food Research Institute, H-1022, Budapest, Herman Ottó u. 15. Hungary

(Received: 14 January 1999; revision received: 1 November 1999; accepted: 24 November 1999)

Blanching of vegetables before freezing has some advantages as well as a number of disadvantages. Process optimization involves measuring the rate of enzyme destruction, such that the blanching time is just long enough to destroy the indicator enzyme. Eventually, peroxidases were almost universally the enzymes of choice, as they are usually the most heat-stable enzymes found in vegetables and fruits, so by the time they are inactivated no other enzymes or micro-organisms remain. But there is no evidence that peroxidases are involved in deteriorative reactions in the food.

The aim of this work was to improve blanching technology in Hungarian frozen food industry with special emphasis on broccoli treatment. Instead of peroxidases, lipoxygenases were chosen to determine the adequate blanching parameters. Usually, lipoxygenases accompany lipases, so lipase activity is measured, too.

On the basis of model blanching experiments, the conclusion is that lipoxygenase could be used as indicator enzyme. Being less heat stable than peroxidase, this enzyme requires shorter heat treatment, hence its inactivation should result in minimum quality deterioration and economic loss.

Keywords: broccoli, indicator enzyme, blanching technology, freezing industry, quality deterioration

New uses of enzymes are described in the food industry to change the properties of proteins and lipids, to eliminate off-flavour in beers and sterilized milk, and to monitor adequate blanching of fruits and vegetables. Blanching of vegetables prior to freezing has some advantages as well as a number of disadvantages. Process optimization involves measuring the rate of enzyme destruction, such that the blanching time is just long enough to destroy the indicator enzyme. There are some problems with the use of peroxidase as the universal indicator of adequate blanching for vegetables and fruits (WILLIAMS et al., 1986). BÖTTCHER (1975) concluded that the complete absence of peroxidase activity indicated overblanching. Better quality products, with lower energy utilization, may be possible with less heat treatment by

0139-3006/2000/$ 5.00

using the enzyme(s) responsible for quality deterioration as the detector for adequate blanching: lipoxygenase was identified as the key enzyme in English green peas and green beans (VELASCO et al., 1989). Research studies have indicated that lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.12.11.12 or LPO) should be considered as an enzyme indicator alternative to peroxidase.

The aim of this work was to improve the blanching technology of broccoli and to get a product with higher quality. Lipoxygenase and lipase were chosen as indicator enzymes. The loss of enzyme activity was measured during the heat treatment in order to determine the adequate blanching parameters (temperature, time). Pigments such as chlorophylls, carotenoids, anthocyanins and betalains in fruits and vegetables and myoglobin in meats make a major contribution to perceive colour and their degradation by enzymes such as lipoxygenase can reduce colour quality significantly.

1. Materials and methods

1.1. Crude enzyme preparation

Broccoli was purchased on the retail market. Ten grams of fresh broccoli was homogenized with quartz sand at low temperature (in crushed ice) in the presence of 100 ml 0.1 mol^{-1} phosphate-buffer pH 5; 6; 7; 8; 9 in a mortar. The mixture was strained through a double layer cheese cloth and it was centrifuged at 12 000 r.p.m. for 20 min at 2 °C. The supernatant was separated and used as crude extract for lipoxygenase and lipase measurements. The accompanying components of the crude broccoli extracts were not studied in this experiment.

1.2. Model blanching experiments

Blanching experiments were performed under the following conditions: temperature was tuned to 75 °C, 78 °C, 82 °C, 85 °C and 90 °C with a UNIPAN water bath, type 356 (Poland). Samples were taken after 5, 10 and 15 min of blanching time. The crude extract of blanched samples was prepared in the same way as it was done in the case of the raw samples.

1.3. Enzyme assay

Lipoxygenase catalyzes the hydroperoxidation of polyunsaturated fatty acids and esters containing a cis,cis-1,4-pentadiene system. The standard laboratory assay for LPO is the spectrophotometric method wherein an increase in absorbance at 234 nm due to conjugated diene formation is monitored. Pure linoleic acid was applied as a substrate

(OBAIDY, 1981) for the activity determinations. 0.1 ml of substrate solution, prepared with Na-tetraborate, pH 9.0, (2.1 mmol l^{-1}) was added to 2.9 ml of 0.1 mol l^{-1} phosphate buffer (pH 5), vigorously shaken and allowed to stand for one minute. The reaction was initiated by adding 0.1 ml of enzyme extract and the increase in absorbance at 234 nm was measured. The blank was prepared without enzyme solution. One unit of enzyme activity was defined as the amount which produces 0.01 change in absorbance per min (abs g^{-1} min^{-1}).

Lipase activity was measured with the pH stat method in an automatic titrimeter, type OP-506 (Radelkis, Hungary). The reaction mixture contained 0.5 ml of tributyrin as substrate, 4.5 ml of 10% gum arabic and 1.0 ml of 15% sodium taurocholate, 1 ml of 0.075 mol l^{-1} Ca Cl_2. These components were emulsified with Ultra-Turrax T25 mixer at 10 000 r.p.m. for 5 min and 10 ml of distilled water was added to reaction mixture. The pH was adjusted by adding 0.01 mol l^{-1} NaOH to the experimental value, which was 8. The temperature was adjusted to 35 °C, 3 ml of crude broccoli extract (in blank 3 ml of distilled water) was added for the measurement. The lipase activity which was expressed in micromoles of fatty acid(s) liberated per minute, were titrated with 0.01 mol l^{-1} NaOH (free fatty acid, FFA μmol g^{-1} min^{-1}).

2. Results

2.1. Lipoxygenase and lipase activities as a function of buffer solution

Lipoxygenase activity of crude broccoli extract was the highest when the 0.1 mol l^{-1} phosphate buffer solution (pH 7) was applied to prepare the crude vegetable extract.

Lipase activity of crude broccoli extract was the highest when the 0.1 mol l^{-1} phosphate buffer solution (pH 8) was used to prepare the crude vegetable extract. The results are illustrated in Fig. 1.

2.2. The results of model blanching experiments

Residual lipoxygenase (pH 7) and lipase (pH 8) activity values as a function of blanching temperature and time are summarized in Table 1. On the basis of the measurements we can make a conclusion that at the lowest blanching temperature (75 °C), after 15 min holding time, 25% of the original lipoxygenase activity (the activity of the crude enzyme extract without blanching treatment) was measured in the samples. At higher temperatures – both at 10 and 15 min the residual lipoxygenase activity was less than 10% in the broccoli samples.

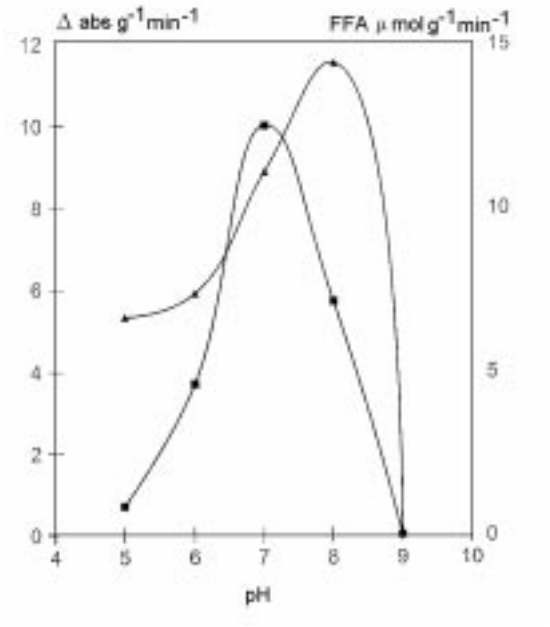

Fig. 1. Lipoxygenase and lipase activities of crude broccoli extracts as a function of pH. ■: lipoxygenase, ▲: lipase

In term of lipase at 75 °C after 15 min the residual activity was 35%. Increasing the blanching temperature – 78 °C, 82 °C, 85 °C and 90 °C – after 5 min about 40% of the original activity could be measured. After 10 min heat treatment at the same temperatures lipase was inactivated.

SD values of enzyme activities in term of all blanching experiments were 8–10%.

3. Conclusions

Nowadays peroxidase is applied to tune the blanching parameters in freezing industry, 96–97 °C for 5 min is the practice in the case of broccoli. On the basis of our results we suppose that lower temperature would be enough for broccoli blanching but further experiments are desired to get more evidence.

Many factors influence the blanching technology for example the blanching equipment (capacity, steam or water), the raw material (species, texture).

Table 1

Residual enzyme activities as a function of blanching parameters

Temperature (°C)	Time (min)	Residual lipoxygenase (%)	Residual lipase(%)
75	5	42	63
	10	39	45
	15	25	35
78	5	16	45
	10	14	–
	15	4	–
82	5	14	41
	10	9	–
	15	5	–
85	5	12	31
	10	6	–
	15	4	–
90	5	10	36
	10	7	–
	15	7	–

WILLIAMS and co-workers (1986) developed a rapid semi-quantitative assay for lipoxygenase (LPO) activity based on a potassium iodide-starch method. It required daily preparation of reagents and was applicable to many fruits and vegetables but limited in the case of commodities containing carotenoids.

ROMERO and BARRETT (1997) studied a rapid assay to determine LPO activity in sweet corn. Results indicated that the methylene blue bleaching method was suitable for both carotenoid-containing and non-carotenoid containing vegetables.

Further experiments should be done to apply a rapid assay to determine LPO activity in broccoli and in other vegetables, too.

*

Many thanks for the colleagues working at CAMPDEN and CHORLEYWOOD Food Research Association Budapest, Hungary, where the model experiments were carried out.

References

BÖTTCHER, H. (1975): Enzyme activity and quality of frozen vegetables. 1. Remaining residual activity of peroxidase. *Nahrung*, *19*, 173–177.

OBAIDY, H. M. & SIDDIQI, A. M. (1981): Properties of broad bean lipoxygenase. *J. Fd Sci.*, *46*, 622–625.

ROMERO, M. & BARRETT, D. M. (1997): Rapid methods for lipoxygenase assay in sweet corn. *J. Fd Sci.*, *62*, 696–700.

VELASCO, P. J., LIM, M. H., PANGBORN, R. M. & WHITAKER, J. R. (1989): Enzymes responsible for off-flavour and off-aroma in blanched and frozen – stored vegetables. *Biotechn. appl. Biochem.*, *11*, 118–127.

WILLIAMS, C., LIM, M. H., CHEN, A. O., PANGBORN, R. M. & WHITAKER, J. R. (1986): Blanching of vegetables for freezing - which indicator enzyme to choose. *Fd. Technol.*, *40*, 130–140.