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The Effect of Lipid-Degrading Enzyme Activities on Quality of Blanched and Unblanched Frozen Stored Cauliflower Estimated by Sensory and Instrumental Analysis

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ABSTRACT

Three varieties of cauliflower were blanched as florets, frozen and stored at −20°C for 8 months. Unblanched cauliflower florets were used as a reference. Blanching completely inactivated peroxidase, palmitoyl-CoA hydrolase and α-oxidation, while residual activity was found for acylhydrolase, lipoxygenase and catalase. Protein content was reduced with increased blanching time. Unblanched cauliflower was inedible and sensorially unacceptable after 4 weeks of storage at −20°C. The heat treated samples also developed off-flavours and off-odours, but to a lesser extent. Significant correlations between sensory and colour analysis were obtained.

INTRODUCTION

Unblanched vegetables will, in most cases, develop off-flavours and off-odours during storage at −20°C (Baardseth, 1978; Adams, 1983). Blanching of the vegetables before freezing, however, should not be too severe. With extensive heat treatment nutrients will be lost, sensory quality will decrease and energy requirements during processing will increase (Steinbuch, 1983). As a blanching indicator, the heat-stable enzyme peroxidase has been used,

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while less heat-stable enzymes such as acylhydrolase (Duden *et al.*, 1977), palmitoyl-CoA hydrolase (Baardseth & Slinde, 1983*a*) and lipoxygenase (Williams *et al.*, 1986) have been suggested. These enzymes take part in the development of off-flavours and off-odours, while peroxidase is only empirically related to off-flavour (Burnette, 1977).

The aim of this study was to investigate the effect of blanching on the activity of some enzymes which can cause lipid degradation in cauliflower and relate this to changes in sensory and instrumental quality which occur in the frozen product.

MATERIALS AND METHODS

Materials

Three different cultivars (White Rock, White Top and Celestra) of cauliflower (*Brassica oleracea* var. *botrytis*) were purchased from the Rygge area, and processed at Stabburet A/S, Fredrikstad, Norway. Cauliflower florets (maximum stem thickness, 1·0–1·5 cm) were blanched at 89°C for 2 and 3 min in a trunnion type blancher (1150 litres water; 1100 kg cauliflower h^{-1} (Bøg Jørgensen, Denmark), frozen at −40°C (Frigo Scandia Flo Freeze model 44M, Sweden), packed in plastic bags (nylon (PA), polyethylene (PE)) and stored in the dark at −20°C.

All chemicals were of analytical grade. The [1-^{14}C] palmitoyl-CoA (specific activity 57 μCi mg^{-1}) and the [1-^{14}C] palmitic acid (specific activity 213 μCi mg^{-1}) were purchased from Amersham International, Amersham, England.

Methods

The frozen cauliflowers were cut in a vertical cutter and 50 g were homogenized with an Ultra-Turrax TP 18/10 tissue macerator in 50 ml buffer (15 mmol HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethansulphonic acid per litre, pH 6·3) at 4°C. After filtration through cheese-cloth, the resulting homogenate was centrifuged (42 ml, 14 000 rpm, 20 min, $R_{av} = 8{\cdot}3$ cm, 4°C) and the supernatant was analysed for activities of palmitoyl-CoA hydrolase, peroxidase, catalase, acylhydrolase and lipoxygenase.

Palmitoyl-CoA hydrolase activity (EC 3.1.2.2) was assayed by the release of [1-^{14}C] labelled fatty acid from CoA ester (Baardseth & Slinde, 1983*b*). Peroxidase activity (EC 1.11.1.7) was determined at 420 nm using guaiacol and hydrogen peroxide as substrate (Lu & Whitaker, 1974). Catalase (EC 1.11.1.6) activity was measured at 230 nm using hydrogen peroxide as substrate, having an absorbance at 230 nm of 1·0 (Bergmeyer *et al.*, 1974).

Acylhydrolase activity was measured at 400 nm using *p*-nitro-phenyl-palmitate as substrate (Galliard, 1971). The enzyme assays were carried out at 25°C using a Shimadzu UV-300 spectrophotometer (Shimadzu Seisakusho, Ltd., Japan) with 1 cm cell.

Lipoxygenase activity (EC 1.13.1.12) was measured as oxygen consumption, with a Clark oxygen electrode at 25°C (Yellow Spring Instr., Biological Oxygen Monitor) using linolenic acid as substrate (Galliard & Philips, 1971). Brij 58 was used to emulsify the linolenic acid. The substrate concentration used in the assay was 1 mmol per litre and a buffer of 15 mmol HEPES per litre (pH 6·3).

For α-oxidation activities, cauliflower florets were homogenized (1:1, w/w) with buffer containing 50 mmol HEPES and 1 mmol EDTA (ethylenediaminetetra-acetic acid) per litre, pH 7·2. After filtration through cheese-cloth, the resulting homogenate was centrifuged (3 000 rpm, 20 min, $R_{av} = 8{\cdot}3$ cm) and the α-oxidation activity was measured in the supernatant. To measure $^{14}CO_2$ production (α-oxidation) from ^{14}C-labelled fatty acid, the incubation technique described by Galliard & Matthew (1976) using 58 μmol [1-^{14}C] palmitic acid per litre was used. The reaction time was 60 min at 25°C. Protein content in cauliflower florets was determined by the Kjeldahl method (Kjeltec, Tecator, Sweden). All analyses were done in triplicate.

The colour parameters CIE (1976) L^*, a^*, b^* were obtained by means of Minolta Chroma Meters II CR 100 (Minolta Camera Co, Ltd., Osaka, Japan) with measuring area 8 mm diameter. The colour was measured on steamed cooked florets (flowers) after 1 and 3 months of storage at −20°C. Forty florets from each cultivar and treatment were measured.

The cauliflowers were evaluated by a trained sensory panel of 12 assessors after 1, 3 and 8 months of storage at −20°C. The cauliflower florets were steam-heated before being presented to the assessors. Three replicates from each of the nine samples were judged for the following sensory properties according to odour (1 = none, 9 = very strong), whiteness (1 = none, 9 = very strong), colour hue (1 = yellow, 9 = green), colour intensity (1 = none, 9 = very strong), fruity smell (1 = none, 9 = very strong), bitter taste (1 = none, 9 = very strong), sulphide smell (1 = none, 9 = very strong), off-flavours (1 = none, 9 = very strong), crispness (1 = none, 9 = very strong), woodiness (1 = none, 9 = very strong). The sensory data were tested by analysis of variance.

RESULTS AND DISCUSSION

Blanching of cauliflower florets at 89°C for 2 or 3 min inactivated the enzymes peroxidase, palmitoyl-CoA hydrolase and α-oxidation completely

TABLE 1

Enzyme Activities and Protein Content per 100 g Wet Weight in Three Varieties of Unblanched and Blanched Cauliflower after Two Weeks of Storage

Cauliflower cultivars	*Blanching time (min)*	*Palmitoyl-CoA hydrolase* ($nmol\,min^{-1}$)	*Peroxidase* ($\Delta A\,min^{-1}$)	*Catalase* ($\Delta A\,min^{-1}$)	*Acylhydrolase* ($\Delta A\,min^{-1}$)	*Lipoxygenase* (μ *atoms oxygen* min^{-1})	*α-oxidation* ($nmol\,min^{-1}$)	*Protein (g)*
White Rock	0	66·8 ± 12·3	57·0 ± 3·4	15·2 ± 2·3	4·6 ± 0·06	6·7 ± 0·4	1·6 ± 0·09	1·4 ± 0·03
White Rock	2	0·0	0·0	3·9 ± 0·4	2·3 ± 0·17	5·9 ± 1·0	0·0	1·3 ± 0·10
White Rock	3	0·0	0·0	3·7 ± 0·2	2·3 ± 0·01	5·0 ± 1·0	0·0	1·2 ± 0·00
White Top	0	43·7 ± 5·5	66·4 ± 1·1	12·9 ± 2·5	4·9 ± 0·42	9·0 ± 1·9	1·4 ± 0·07	1·3 ± 0·03
White Top	2	0·0	0·0	3·4 ± 0·5	2·6 ± 0·16	6·0 ± 0·7	0·0	1·3 ± 0·06
White Top	3	0·0	0·0	2·0 ± 0·4	2·6 ± 0·19	5·0 ± 0·5	0·0	1·2 ± 0·01
Celestra	0	58·0 ± 5·6	58·1 ± 3·6	17·4 ± 1·4	4·6 ± 0·11	7·8 ± 1·8	7·1 ± 0·85	1·5 ± 0·06
Celestra	2	0·0	0·0	4·3 ± 0·1	2·4 ± 0·01	3·4 ± 1·1	0·1 ± 0·01	1·5 ± 0·01
Celestra	3	0·0	0·0	1·8 ± 0·6	2·1 ± 0·17	3·3 ± 0·6	0·0	1·4 ± 0·04

(Table 1). The activity of lipoxygenase was about 40%, catalase about 24% and acylhydrolase about 50% compared to the activity in the unblanched samples. Protein content was reduced with increasing blanching time. After one month of storage at −20°C a significant positive effect of blanching on several sensory properties was found. The unblanched samples from the three varieties developed off-odour and off-flavours and lost the fruity smell during this period compared with the blanched samples (Fig. 1). The assessors described the unblanched samples as tasting of paint and soap. A similar development in flavour and odour was found in the heat-treated samples after 3 months of storage, but the effect was not as strong as in the unblanched samples (results not shown). After 8 months, the off-flavour was more pronounced in the blanched samples, especially cauliflower heat treated at 2 min (results not shown). The Celestra variety seemed to develop more off-flavour than the White Top and White Rock varieties. From the present results (Table 1) no differences in enzyme activities which could account for a difference in flavour stability among the three cauliflower varieties were found. Sulphide smell and bitter smell are less pronounced in the heat-treated samples, because the compounds responsible for these flavours are extracted into the blanching water. The fruity smell was found to be strongest in the White Top and White Rock varieties. This property seemed to be important in cauliflower acceptance (Fjeldsenden *et al.*, 1981). Analysis of variance showed significant difference ($p < 0{\cdot}01$) in flavour (odour, off-colour, fruity smell, bitter taste, sulphide smell and off-flavour) between the unblanched and blanched samples within the same varieties, but no significance between 2 and 3 min even after 8 months of storage. No

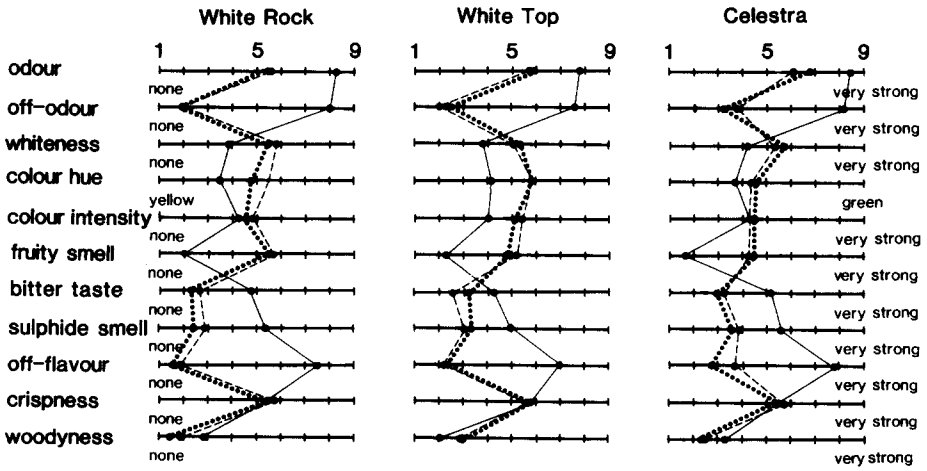

Fig. 1. Sensory properties of unblanched and blanched samples of the cauliflower varieties White Rock, White Top and Celestra after one month's storage at −20°C. —— unblanched; ----- 2 min at 89°C; ····· 3 min at 89°C.

Fig. 2

Colour Measurement of Unblanched and Heat-Treated (89°C) Cauliflower Florets ($n = 40$) after Storage at −20 C (Avg. std.: $L^* = 2{\cdot}6$, $a^* = 1{\cdot}0$, $b^* = 2{\cdot}5$)

Period of storage	*White Rock*			*White Top*			*Celestra*		
	Unblanched	*2 min*	*3 min*	*Unblanched*	*2 min*	*3 min*	*Unblanched*	*2 min*	*3 min*
One month									
Lightness (L^*)	75·8	78·0	77·5	75·0	74·1	73·6	75·9	75·8	76·5
Greenness (a^*)	−0·8	−5·3	−5·3	−3·4	−3·7	−4·1	−1·5	−2·1	−2·0
Yellowness (b^*)	11·3	14·7	14·7	13·7	12·0	13·1	12·0	8·9	8·8
Hue	85·9	70·2	70·2	76·1	72·9	72·9	82·9	76·7	77·2
Three months									
Lightness (L^*)	79·3	77·0	75·5	74·1	72·8	74·6	72·6	75·7	76·5
Greenness (a^*)	−0·8	−4·4	−2·1	−4·1	−4·6	−2·8	−1·4	−5·4	−2·9
Yellowness (b^*)	16·2	12·4	10·3	15·1	12·5	11·5	18·2	11·7	10·8
Hue	87·2	70·5	78·5	74·8	69·8	76·3	85·6	65·2	75·0

significant effect was detected in crispiness and woodiness of the cauliflower florets tested.

The presence of lipid-degrading enzymes could cause the development of off-flavour. In this experiment peroxidase seemed to be less heat-stable than lipoxygenase, catalase and acylhydrolase. Previous experiments indicated that a heat treatment of 4 min at 88°C for cauliflower florets was sufficient to achieve good storage stability (Baardseth, 1978). In the previous experiments, only peroxidase was measured. As in the present study, the enzyme was completely inactivated during blanching. The size of the florets is important when blanching conditions are to be recommended. Ramaswamy & Ranganna (1981) found optimal blanching conditions for floret stem thickness of 1·2–1·5 cm to be 2 min at 97°C and 4 min at 97°C for thicknesses of 1·5–2·0 cm. The cauliflowers were acceptable after 10 months at −18°C (peroxidase was inactivated).

Colour changes in the cauliflower florets were assessed both by sensory analysis and by instrumental colour analysis. The sensory analysis revealed blanched cauliflower to be significantly ($p < 0{\cdot}01$) more white and of more greenish hue than the unblanched samples, while colour intensity was not significantly affected by the heat treatment (Fig. 1). Also the instrumental colour analysis revealed significant effects of blanching upon colour of the cauliflower florets (Table 2). Blanched samples, especially from the White Rock variety, were more light, more green and more yellow in colour than the unblanched samples. The changes caused a more greenish hue in the blanched samples. During prolonged storage the unblanched samples increased in yellowness ($p < 0{\cdot}05$), while the blanched samples decreased or remained unchanged. In accordance with the results from sensory analysis, the blanched cauliflowers retained a more greenish hue compared with the unblanched, which had a more yellowish hue. The increase in yellowness during frozen storage of the unblanched cauliflower may possibly be due to the presence of polyphenol oxidase, which oxidizes polyphenols, thus causing brown colour development.

The presence of residual lipid-degrading activities from lipoxygenase and acylhydrolase can cause off-flavours and off-odours in stored blanched cauliflower. An increase in blanching time or blanching temperature might improve the quality of the frozen cauliflower.

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