ACTA Scientiarum Polonorum Acta Sci. Pol., Technol. Aliment. 7(2) 2008, 21-34

THE EFFECT OF PRE-TREATMENT, TEMPERATURE AND LENGTH OF FROZEN STORAGE ON THE RETENTION OF CHLOROPHYLLS IN FROZEN BRASSICAS*

Waldemar Kmiecik, Zofia Lisiewska, Jacek Słupski, Piotr Gębczyński

University of Agriculture in Krakow

Abstract. The investigation covered broccoli, green cauliflower and Brussels sprouts. The evaluation concerned the raw material; the material after blanching; the material after cooking; and frozen products from blanched (traditional method) and cooked (modified method) material, stored at –20°C and –30°C then prepared for consumption using water-cooking in the traditional method and thawing by microwave in the modified method after 0, 4, 8 and 12 months of frozen storage. Depending on the investigated sample, the vegetables prepared for consumption after 12 months of frozen storage retained total chlorophylls as follows: broccoli, 45-66% of the content in the raw material; green cauliflower, 30-45%; and Brussels sprouts, 66-78%. In comparison with the traditional method, the mean content of chlorophylls in Brussels sprouts obtained using the modified method was 16% higher; however, in broccoli the content was lower by 23% and in cauliflower by 21% on average. Lower storage temperature resulted in higher content of chlorophylls in all investigated vegetables.

Key words: brassicas, chlorophylls content, blanching, cooking, frozen storage, preparing for consumption

INTRODUCTION

Green brassicas such as broccoli, green cauliflower and Brussels sprouts are widely grown and consumed. In temperate climates their supply is limited to a few months of the year. In order to provide year-round availability, various storage conditions improv-

* This work was supported from means on science in years 2006-2008 as a scientific project N31203831/2450.

Corresponding author – Adres do korespondencji: Prof. dr hab. Zofia Lisiewska, Department of Raw Materials and Processing of Fruit and Vegetables of University of Agriculture in Krakow, Balicka 122, 30-149 Cracow, Poland, e-mail: rrlisiew@cyf-kr.edu.pl

ing shelf-life are used [Pogson and Morris 1997, Yamauchi and Watada 1998]. They are also processed, the best available method being freezing [Dong et al. 2004, Gębczyński 2003, Gębczyński and Kmiecik 2006, Gębczyński and Lisiewska 2006]. The low temperatures commonly prescribed for frozen foods (–18ºC) can maintain initial quality and nutritive value practically unchanged, so that frozen and fresh vegetable products differ only in texture [Canet 1989]. The freezing of vegetables immediately post-harvest guarantees consumers higher vitamin C content than could be attained by any other form of preservation and distribution. Furthermore, if properly handled before freezing and during distribution, there is no possibility of growth of microbial contaminants between freezing and thawing [Kennedy 2000].

The colour of green vegetables depends on the level of chlorophyll a and chlorophyll b and on their ratio. The content of chlorophylls depends not only on the species and cultivar of the vegetable and its edible part [Murcia et al. 2000], but also on the developmental stage; possible signs of senescence [Suda et al. 1994]; and conditions of storage after harvest [Yamauchi and Watada 1998]. In the product prepared for consumption the level of chlorophyll is also affected by pre-treatment before processing. The significant parameters are the temperature, the length of thermal processing and the pH of the medium in which the pre-treatment is carried out [Canet et al. 2005, Murcia et al. 2000, Tijskens et al. 2001]. Freezing and frozen storage also are important factors in retaining the attractive bright green colour [Gębczyński 1999, Lisiewska et al. 2004].

In recent years the consumption of frozen vegetables has rapidly increased, prompting efforts to improve their quality and shelf-life. Appearance is taken as an indication of freshness, palatability and nutritional value [López et al. 1998]. Particular attention is paid to food products of the "do-it-for-me" or "ready-to-eat" type. Frozen vegetable products of this type are obtained when, in the pre-treatment stage, blanching is replaced by cooking to consumption consistency. After frozen storage, such products only require thawing and heating to consumption temperature. Thus the product is prepared for consumption using a microwave oven instead of cooking in water, which always increases the leaching of nutritional compounds.

Modifications to processes should be the outcome of basic research into the physical, chemical, and biological features of phytosystems at low temperatures aimed at improving our understanding of the behavioural processes that take place during freezing. The development of new generations of frozen vegetables products with higher added values and competitive pricing will depend on the creative efforts and technological development arising out of cooperation between scientists and manufacturers.

The first aim of the present work was to study and compare the effect of two pretreatments before freezing (traditional and new modified technology) on the chlorophyll content of frozen broccoli, cauliflower and Brussels sprouts. The second was to ascertain, in each studied frozen vegetable, the effect on the cited content of chlorophylls of the temperature and length of frozen storage prior to both the final culinary methods (boiling and microwaving). This paper follows on from a series of papers describing the content of minerals, heavy metals and antioxidative compounds in brassicas [Gębczyński and Lisiewska 2006, Kmiecik et al. 2007, Lisiewska et al. 2007].

MATERIAL AND METHODS

Plant materials

The investigated material consisted of three species of brassicas recommended for freezing: broccoli cultivar Lord F1, green cauliflower cultivar Trevi F1 and Brussels sprouts cultivar Lunet F1. Analytical procedures were carried out by analysing the raw, blanched and cooked vegetables and frozen vegetables obtained using the two different pre-treatments (blanching and cooking) and prepared for consumption using two domestic culinary treatments (cooking and microwaving). The products were evaluated directly after freezing (0 months) and after 4, 8, and 12 months of frozen storage at –20°C and –30°C.

The raw material was harvested in the experimental field of the Agricultural University of Krakow in 2005. The field lies in southern Poland, on the western outskirts of Krakow. The soil was of good horticultural structure, with neutral pH and high levels of potassium, phosphorus and calcium. The application of mineral fertilizers was determined by the fertility of soil and the nutritional requirements of the investigated species. The doses of mineral fertilizers were of 120 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1} for the Brussels sprouts, and 150 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1} for the broccoli and the cauliflower. The cultivation included mechanical weed control, sprinkler irrigation, and protection against diseases and pests as necessary. The harvest of broccoli and cauliflower was carried out in the first 10 days of October and of Brussels sprouts in mid-October.

A sample representing the whole batch of a given cultivars was taken for the analysis of the raw material and the preparation of frozen products. Heads of broccoli and cauliflower were trimmed of leaves and divided into florets about 5 cm in diameter, the stalks being cut to a depth of 2 cm below the lowest ramification. Heads of Brussels sprouts 25-30 mm in diameter were cleaned of stipules and their stalks were shortened.

Pre-treatment before freezing

Two different pre-treatments were used in preparing the fresh vegetables for freezing. Using the traditional technology (pre-treatment I) the fresh vegetables were blanched and, after freezing and frozen storage, the frozen product required traditional water cooking. With the modified technology (pre-treatment II) the fresh vegetables were cooked before freezing to an approximately consumption consistency; giving a ready-to-eat products which merely requires defrosting and heating in a microwave oven after freezing and frozen storage.

In pre-treatment I the fresh material was blanched in a stainless steel vessel in water, the proportion of water to the fresh material being 5:1 and the blanching temperature 95-98°C. The blanching time (Table 1) used for the different vegetables was determined in preliminary experiments. These blanching conditions permitted a decrease in the activity of catalase and peroxidase to a level below 5% of the initial value. The total absence of peroxidase activity indicates overblanching and there is a substantial body of evidence suggesting that the quality of products frozen after blanching is superior if a certain level of peroxidase activity remains at the end of the blanching process. As Canet (1989) showed, the residual activity of peroxidase in blanched material of:

Table 1. Time of blanching and cooking before freezing and time of preparing for consumption after frozen storage
Tabela 1. Czas blanszowania i gotowania przed mrożeniem oraz czas przygotowania do spożycia po składowaniu zamrażalniczym

Vegetable Warzywo	Before freezing Przed mrożeniem		After frozen storage Po składowaniu zamrażalniczym		
	blanching blanszowanie	cooking gotowanie	cooking* gotowanie*	thawing and heating in microwave oven, goods stored at: rozmrażanie i podgrzewanie w kuchence mikrofalowej produktów przechowywanych w:	
				–20°C	–30°C
Broccoli Brokuł	3 min	6 min	5 min	7 min 45 s	8 min 15 s
Green cauliflower Kalafior zielony	3 min 15 s	6 min	5 min	7 min 45 s	8 min 15 s
Brussels sprouts Kapusta brukselska	5 min	15 min	9 min	7 min 45 s	8 min 15 s

* Time was the same for both storage temperature (–20°C and –30°C).
* Czas gotowania był taki sam dla obydwu temperatur składowania (–20°C i –30°C).

2.0-6.3% in peas; 0.7-3.3% in green beans; 2.9-8.2% in cauliflower; and 7.5-11.5% in Brussels sprouts did not reduce the quality of frozen products. During frozen storage no regeneration of these enzymes was recorded. After blanching the material was immediately cooled in cold water and left for 30 min on sieves to drain the water remaining on the surface.

In the pre-treatment II the vegetables were cooked in a stainless steel vessel in water with 2% added salt (NaCl), the proportion of the weight of the raw material to brine being 1:1. The vegetables were placed in boiling water. The cooking time measured from the moment when the medium began boiling again is given in Table 1. After cooking to consumption consistency, the material was left on sieves and cooled in a stream of cold air.

Freezing process and frozen storage

The materials from blanched and cooked samples were placed on trays and frozen at t = –40°C in a Feutron blast freezer type 3626-51. The time required for the inside of the product to reach the storage temperature of –20°C was 90 min and of –30°C was 120 min. The frozen vegetables were then packed in 500 g polyethylene bags and stored at –20°C and –30°C respectively for 0, 4, 8, and up to 12 months.

Preparation of the frozen product for evaluation

Frozen samples of the vegetables blanched before freezing (subjected to the pre-treatment I) were cooked in 2% brine, the proportion in weight of brine to processed products being 1:1. As was the case when cooking fresh vegetables, the frozen product was placed in boiling water. The cooking time measured from the moment when the medium began boiling again is given in Table 1. After cooking, the water was immediately drained and the products were cooled to 20°C for analyses. Frozen samples of the vegetables cooked before freezing (subjected to the pre-treatment II) were thawed and heated in a Panasonic NN-F621 microwave oven. In this case a 500 g portion was placed in a covered heatproof vessel. The time required for thawing and heating to consumption temperature [Codex Alimentarius 1993] is given in Table 1. The samples were then cooled to 20°C and analysed. The changes in the weight of vegetables resulting from all the technological processing and culinary treatments applied are shown in Table 2. Because these changes were relatively small and similar for both types of products, their effect on the content of chlorophylls was not taken into consideration in the discussion section.

Table 2. Weight* of vegetables after freezing and preparing for consumption (weight of raw material = 100%)
Tabela 2. Masa warzyw* po procesie technologicznym zamrażania i przygotowaniu do spożycia (masa surowca = 100%)

Material Materiał	Broccoli Brokuł	Green cauliflower Kalafior zielony	Brussels sprouts Kapusta brukselska
Blanched before freezing and cooked Blanszowany przed mrożeniem i ugotowany	95-98	95-98	98-100
Cooked before freezing and prepared in microwave oven Gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	95-97	94-96	95-98

* Range of values for all stages and temperatures.
* Zakres wartości dla wszystkich terminów i temperatur.

The table salt used in the experiment was always taken from the same batch; the pH of the tap water varied within 7.3-7.7.

Chemical analyses

Dry matter content was determined by gravimetric as the mass loss of the sample at 96-98°C [AOAC 1984]. The content of chlorophylls a and b was determined using the Lichtenthaler and Buschmann [2001 a, b] method. Chlorophylls were extracted with pure acetone until all colour was removed from the sample. After suitable dilutions were obtained, colorimetric measurements were carried out. The measurement of absorbance was conducted in a Shimadzu UV-160A spectrophotometer at 661.6 and 644.8 nm wavelengths, i.e. at the absorbance maxima for chlorophylls a and b. The pigment concentrations were calculated on the basis of the absorbance coefficients at the above wavelengths.

The content of chlorophylls in the raw material, in the partly processed material and in frozen products prepared for consumption was calculated per 100 g fresh matter. The level of dry matter given in Tables 3-5 allows the reader to apply the presented results to calculate the content of dry matter.

Table 3. Content of chlorophyll in broccoli prepared for consumption depending on pre-treatment before freezing and temperature of frozen storage, in fresh matter
Tabela 3. Zawartość chlorofilu w brokułach przygotowanych do konsumpcji w zależności od obróbki przed mrożeniem i temperatury składowania, w świeżej masie

Stage of estimation Etap badań	Material Materiał	Storage temperature °C Temperatura składowania °C	Dry matter g·100 g^{-1}* Sucha masa g·100 g^{-1}*	Chlorophyll, mg·100 g^{-1}* Chlorofile, mg·100 g^{-1}*		
				a	b	a + b
Before freezing Przed mrożeniem	raw – surowy	–	10.34 ±0.129	10.3 ±0.26	5.1 ±0.22	15.4 ±0.50
	after blanching – blanszowany	–	8.37 ±0.108	8.5 ±0.25	4.2 ±0.05	12.7 ±0.30
	after cooking – gotowany	–	10.74 ±0.120	7.3 ±0.28	3.6 ±0.13	10.9 ±0.35
After storage (months) and preparing for consumption Po przechowywaniu (miesiące) i przygotowaniu do spożycia: 0	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.19 ±0.075	8.1 ±0.13	4.0 ±0.26	12.1 ±0.39
		–30	9.23 ±0.079	7.7 ±0.24	4.2 ±0.12	11.9 ±0.34
	cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.02 ±0.091	6.8 ±0.14	3.4 ±0.19	10.2 ±0.32
		–30	11.13 ±0.103	6.9 ±0.13	3.5 ±0.15	10.4 ±0.27
4	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.17 ±0.080	7.2 ±0.29	3.9 ±0.06	11.1 ±0.46
		–30	9.22 ±0.076	7.6 ±0.24	4.2 ±0.14	11.8 ±0.33
	cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.18 ±0.071	6.1 ±0.17	3.0 ±0.13	9.1 ±0.29
		–30	11.22 ±0.074	6.2 ±0.24	3.2 ±0.21	9.4 ±0.34
8	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.05 ±0.081	6.7 ±0.28	3.4 ±0.10	10.1 ±0.33
		–30	9.09 ±0.069	7.4 ±0.10	3.6 ±0.15	11.0 ±0.21
	cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.22 ±0.102	5.5 ±0.14	2.8 ±0.08	8.3 ±0.25
		–30	11.24 ±0.084	5.9 ±0.10	3.0 ±0.10	8.9 ±0.29
12	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.09 ±0.075	6.2 ±0.19	3.1 ±0.10	9.3 ±0.29
		–30	9.02 ±0.083	6.9 ±0.20	3.3 ±0.15	10.2 ±0.33
	cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.28 ±0.092	4.6 ±0.19	2.4 ±0.08	7.0 ±0.26
		–30	11.21 ±0.099	5.4 ±0.08	2.7 ±0.10	8.1 ±0.17
	LSD, $\alpha < 0.01$ NIR, $\alpha < 0,01$		0.142	0.38	0.28	0.61

*Mean value of four samples and standard deviation.
*Średnia dla czterech oznaczeń i odchylenie standardowe.

Table 4. Content of chlorophyll in green cauliflower prepared for consumption depending on pre-treatment before freezing and temperature of frozen storage, in fresh matter
Tabela 4. Zawartość chlorofilu w kalafiorze zielonym przygotowanym do konsumpcji w zależności od obróbki przed mrożeniem i temperatury składowania, w świeżej masie

Stage of estimation Etap badań		Material Materiał	Storage temperature °C Temperatura składowania °C	Dry matter $g \cdot 100\ g^{-1}$* Sucha masa $g \cdot 100\ g^{-1}$*	Chlorophyll, $mg \cdot 100\ g^{-1}$* Chlorofile, $mg \cdot 100\ g^{-1}$*		
					a	b	a + b
Before freezing Przed mrożeniem		raw – surowy	–	9.30 ±0.057	8.6 ±0.36	3.8 ±0.13	12.4 ±0.45
		after blanching – blanszowany	–	8.08 ±0.059	6.4 ±0.10	3.2 ±0.14	9.6 ±0.21
		after cooking – gotowany	–	10.46 ±0.059	5.6 ±0.15	2.9 ±0.17	8.5 ±0.22
After storage (months) and preparing for consumption Po przechowywaniu (miesiące) i przygotowaniu do spożycia	0	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.22 ±0.052	5.5 ±0.05	2.7 ±0.10	8.2 ±0.14
			–30	9.24 ±0.053	5.6 ±0.05	2.7 ±0.13	8.3 ±0.16
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.19 ±0.065	5.0 ±0.06	2.4 ±0.13	7.4 ±0.24
			–30	11.28 ±0.064	5.0 ±0.06	2.4 ±0.13	7.4 ±0.18
	4	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.22 ±0.059	4.6 ±0.10	2.3 ±0.10	6.9 ±0.16
			–30	9.17 ±0.057	5.0 ±0.14	2.4 ±0.10	7.4 ±0.24
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.17 ±0.073	3.8 ±0.06	2.0 ±0.10	5.8 ±0.14
			–30	11.33 ±0.070	4.0 ±0.10	2.0 ±0.13	6.0 ±0.22
	8	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.24 ±0.056	3.9 ±0.13	1.9 ±0.19	5.8 ±0.19
			–30	9.22 ±0.050	4.4 ±0.10	2.2 ±0.15	6.6 ±0.23
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.15 ±0.067	3.1 ±0.08	1.5 ±0.13	4.6 ±0.17
			–30	11.26 ±0.074	3.5 ±0.13	1.6 ±0.13	5.1 ±0.24
	12	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	9.26 ±0.051	3.1 ±0.15	1.5 ±0.12	4.6 ±0.26
			–30	9.20 ±0.046	3.8 ±0.17	1.8 ±0.08	5.6 ±0.25
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	11.13 ±0.061	2.4 ±0.10	1.3 ±0.06	3.7 ±0.14
			–30	11.23 ±0.061	2.9 ±0.14	1.5 ±0.08	4.4 ±0.20
		LSD, $\alpha < 0.01$ NIR, $\alpha < 0,01$		0.109	0.25	0.23	0.42

*Mean value of four samples and standard deviation.
*Średnia dla czterech oznaczeń i odchylenie standardowe.

Table 5. Content of chlorophyll in Brussel sprouts prepared for consumption depending on pre-treatment before freezing and temperature of frozen storage, in fresh matter
Tabela 5. Zawartość chlorofilu w kapuście brukselskiej przygotowanej do konsumpcji w zależności od obróbki przed mrożeniem i temperatury składowania, w świeżej masie

Stage of estimation Etap badań		Material Materiał	Storage temperature °C Temperatura składowania °C	Dry matter $g \cdot 100\ g^{-1}$* Sucha masa $g \cdot 100\ g^{-1}$*	Chlorophyll, $mg \cdot 100\ g^{-1}$* Chlorofile, $mg \cdot 100\ g^{-1}$*		
					a	b	a + b
Before freezing Przed mrożeniem		raw – surowy	–	18.11 ±0.132	4.0 ±0.05	1.8 ±0.19	5.8 ±0.24
		after blanching – blanszowany	–	16.14 ±0.097	3.6 ±0.21	1.6 ±0.10	5.2 ±0.28
		after cooking – gotowany	–	17.15 ±0.097	3.6 ±0.10	1.5 ±0.05	5.1 ±0.13
After storage (months) and preparing for consumption Po przechowywaniu (miesiące) i przygotowaniu do spożycia	0	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	16.54 ±0.107	3.5 ±0.10	1.5 ±0.14	5.0 ±0.22
			–30	16.75 ±0.128	3.5 ±0.10	1.5 ±0.13	5.0 ±0.22
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	18.48 ±0.116	3.5 ±0.05	1.6 ±0.17	5.1 ±0.21
			–30	18.56 ±0.108	3.5 ±0.05	1.6 ±0.14	5.1 ±0.17
	4	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	16.47 ±0.130	3.0 ±0.18	1.5 ±0.06	4.5 ±0.10
			–30	16.75 ±0.139	3.3 ±0.19	1.4 ±0.05	4.7 ±0.22
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	18.46 ±0.127	3.3 ±0.10	1.5 ±0.15	4.8 ±0.16
			–30	18.63 ±0.112	3.5 ±0.08	1.6 ±0.13	5.1 ±0.19
	8	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	16.33 ±0.093	2.6 ±0.10	1.3 ±0.10	3.9 ±0.16
			–30	16.70 ±0.096	3.0 ±0.21	1.3 ±0.06	4.3 ±0.26
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	18.59 ±0.109	3.2 ±0.13	1.4 ±0.13	4.6 ±0.26
			–30	18.74 ±0.120	3.3 ±0.10	1.5 ±0.15	4.8 ±0.24
	12	blanched before freezing and cooked blanszowany przed mrożeniem i ugotowany	–20	16.34 ±0.108	2.6 ±0.10	1.2 ±0.10	3.8 ±0.17
			–30	16.78 ±0.102	2.6 ±0.10	1.3 ±0.14	3.9 ±0.24
		cooked before freezing and prepared in microwave oven gotowany przed mrożeniem i przygotowany w kuchence mikrofalowej	–20	18.55 ±0.123	3.0 ±0.05	1.4 ±0.13	4.4 ±0.13
			–30	18.66 ±0.109	3.0 ±0.13	1.5 ±0.10	4.5 ±0.22
		LSD, $\alpha < 0.01$ NIR, $\alpha < 0{,}01$		0.207	0.23	0.23	0.39

*Mean value of four samples and standard deviation.
*Średnia dla czterech oznaczeń i odchylenie standardowe.

Statistical analysis

The differences in the content of chlorophyll a, chlorophyll b and total chlorophylls at the various stages of evaluation were established using single-factor analysis of variance (ANOVA) on the basis of the Snedecor F and Student's t tests, the least significant differences (LSD) being calculated at the probability level $\alpha < 0.01$. The Statistica 6.1 program was applied in the calculation. All determinations were carried out in two experimental replications each in two parallel samples.

RESULTS AND DISCUSSION

In 100 g of the raw material broccoli contained 15.4 mg total chlorophylls, while green cauliflower and Brussels sprouts contained 12.4 mg and 5.8 mg respectively (Tables 3-5). The chlorophyll a : chlorophyll b ratio in these vegetables was 1:0.50; 1:0.44 and 1:0.45 respectively.

After blanching the content of both chlorophyll a and chlorophyll b decreased significantly, the loss in total chlorophylls compared with the raw material being 18% for broccoli; 23% for green cauliflower; and 10% for Brussels sprouts. After this process the chlorophyll a : chlorophyll b ratio was only slightly changed in comparison with the raw material. After cooking the raw vegetables, the content of chlorophylls a and b was also statistically lower than that found in the raw material. The losses in total chlorophylls were 29% in broccoli; 31% in green cauliflower and 12% in Brussels sprouts. After cooking, the ratio of chlorophyll a to chlorophyll b was 1:0.49 in broccoli; 1:0.52 in green cauliflower; and 1:0.42 in Brussels sprouts, being no different from that found in the raw material, apart from the cauliflower. Decreases in chlorophyll content due to cooking compared with blanching were statistically significant in the case of broccoli and green cauliflower. However, no significant loss was found in Brussels sprouts despite the fact that cooking took three times as long as blanching (Table 1).

Dong et al. [2004], Heaton et al. [1996] and Canjura et al. [1999] stressed that the degradation of chlorophylls was brought about by the activity of enzymes and the low pH of the medium. In the presented experiment the activity of peroxidase, which is the most heat-resistant indicatory enzyme [Barrett and Theerakulkait 1995], was below 5% of the initial activity, and the pH of the water varied from 7.3 to 7.7. According to Gunawan and Barringer [2000] and Tijskens et al. [2001] a pH of 7-8 is in the optimum range for the retention of chlorophylls. According to the literature, the losses of chlorophylls brought about by thermal processing vary from 12-66% depending on the species, the usable part of the plant and the length and temperature of the treatment [Lisiewska et al. 2004, Negi and Roy 2000, Murcia et al. 2000]. In the presented experiment, with blanching and cooking, for all the vegetables, being carried out at the same temperature, the difference in the length of thermal processing was determined by the degree of enzyme inactivation in the case of blanching, and in the case of cooking by the obtaining consumption consistency. Kidmose and Hansen [1999] also stress that the loss of chlorophylls was greater in heads of broccoli which were stored before freezing, and increased as the storage temperature of the raw material rose. The time from harvesting to beginning the technological process of freezing was short, varying from 2 to 4 h depending on the species.

The losses of chlorophylls brought about by blanching parsley [Lisiewska and Kmiecik 1997]; chive [Kmiecik and Lisiewska 1999]; and dill leaves [Lisiewska et al. 2004] were not significant. In a study by Gębczyński [2003] a significant 15% decrease in chlorophyll content was brought about by blanching leaf blades of beet-leaf. However, Canet et al. [2005] and Kmiecik and Budnik [1997] found an increase in chlorophyll content in French bean and broccoli after thermal processing. These authors explained that this was only an apparent increase due to considerable leaching of soluble constituents. According to Premavalli et al. [2001], in six out of seven species of green vegetables chlorophyll a was more stable than chlorophyll b, while in ground spinach Canjura et al. [1991] found a 2-6 times greater degradation of chlorophyll a compared with chlorophyll b. Teng and Chen [1999] reported similar results.

Irrespective of the processing methods applied to the investigated vegetables before freezing (blanching or cooking) and the temperature of frozen storage (–20°C or –30°C), a gradual decrease in the content of chlorophylls occurred during the storage period. Differences between the evaluation periods of 0, 4, 8 and 12 months were significant in most analogous samples (the same kind of pre-treatment and the same storage temperature) of broccoli and green cauliflower, while in Brussels sprouts only in certain samples. In the last species a statistically significant difference for all the samples was only found after 8 months of storage of frozen products. After 12 months of frozen storage, the decrease in total chlorophyll content was 14-31% in broccoli; 33-50% in green cauliflower; and 12-24% in Brussels sprouts in comparison with the material directly after freezing. A higher retention of the investigated compounds was found in samples stored at the lower temperature, with the exception of Brussels sprouts products blanched before frozen storage.

After 12 months of frozen storage, the various samples of vegetables prepared for consumption, whether cooked in water or heated in a microwave oven, retained 45-66% of total chlorophylls in broccoli; 30-45% in green cauliflower; and 66-78% in Brussels sprouts in comparison with the raw material. At this stage of evaluation the chlorophyll a : chlorophyll b ratio was 1:0.48-1:0.52 in broccoli; 1:0.47-1:0.54 in green cauliflower; and 1:0.46-1:0.50 in Brussels sprouts, being similar to that in the raw material in the case of broccoli but slightly lower in the remaining vegetables. According to Teng and Chen [1999], the ratio of chlorophyll a : chlorophyll b in spinach was subject to slight variation as the microwave cooking time varied; moreover, these authors cooked fresh spinach, rather than the frozen product.

As in the research discussed above, the retention of chlorophylls in the different vegetables species varied during frozen storage and, in general, the authors agree that the lower storage temperature limited the losses [Dong et al. 2004, Gębczyński 1999, Lisiewska and Kmiecik 1997, Lisiewska et al. 2004]. However, there is no agreement regarding the magnitude of losses due to the storage period or the distribution of losses over time. Oruna et al. [1997] reported a rapid decrease in chlorophyll content after the first month of storing frozen French bean products; thereafter this constituent was stable. According to Bahçeci et al. [2005], the frozen storage of French beans brought about considerable losses during the first three months; the losses were limited during the following three months but after that period their rate increased. However, Labib et al. [1997] reported that a three-month period of frozen storage did not affect the level of chlorophylls. The results obtained here cannot be compared with the results found in the literature since in only a few works were frozen products analysed after culinary processing [Canet et al. 2004].

After the maximum storage period, vegetables produced using the modified technology compared with products obtained using the traditional technology, contained 16% more chlorophylls on average in the case of Brussels sprouts, but respectively 23% and 21% less in the case of broccoli and green cauliflower; differences between all the samples were statistically significant. Compared with storage at –20°C, frozen products stored at –30° and prepared for consumption in general showed a higher retention of chlorophylls. In total chlorophylls average differences in favour of the lower temperature were: 12% in the case of broccoli; 20% in green cauliflower; and 2% in Brussels sprouts. The differences were statistically significant in broccoli and green cauliflower but not significant in Brussels sprouts.

CONCLUSION

In spite of their attractive green colour, raw broccoli, green cauliflower and Brussels sprouts contained relatively low amounts of chlorophylls: 15.4 mg; 12.4 mg; and 5.8 mg $\times$ 100 g^{-1} respectively.

Blanching and cooking fresh vegetables brought about 18% and 29% decreases respectively in the content of chlorophylls in broccoli; 23% and 31% in green cauliflower; and 10% and 12% in Brussels sprouts. In the course of frozen storage the content of chlorophylls gradually decreased, the decreases being greater in green cauliflower than in the remaining species. In all the samples 12-month storage of frozen products followed by preparation for consumption brought about the following decreases in the content of chlorophylls in relation to the raw material: 44% in broccoli; 63% in green cauliflower; and 28% in Brussels sprouts. At this stage of evaluation the content of chlorophylls was higher by 12% in broccoli; by 20% in green cauliflower; and by 2% in Brussels sprouts at the lower storage temperature. In comparison with the traditional method, the average content of chlorophylls was 16% higher in frozen Brussels sprouts produced using the modified method, while in the case of broccoli and green cauliflower it was lower by 23% and 21% respectively. The chlorophyll a to chlorophyll b ratio showed slight changes at the different stages of evaluation.

REFERENCES

AOAC, 1984. Official Methods of Analysis. Association of Official Analytical Chemists Arlington, USA.

Bahçeci K.S., Serpen A., Gökmen V., Acar J., 2005. Study of lipoxygenase and peroxidase as indicator enzymes in green beans: change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. J. Food Eng. 66, 187-192.

Barrett D.M., Theerakulkait C., 1995. Quality indicators in blanched, frozen, stored vegetables. Food Technol. 49, 62-65.

Canet W., Alvarez M.D., Luna P., Fernández C., 2004. Reprocessing effect on the quality of domestically cooked (boiled/stir-fried) frozen vegetables. Eur. Food Res. Technol. 219, 240--250.

Canet W., 1989. Quality and stability of frozen vegetables. In: Developments in food preservation. Ed. S. Thorne. Elsevier London, 1-50.

Canet W., Alvarez M.D., Luna P., Fernández C., Tortosa M.E., 2005. Blanching effects on chemistry, quality and structure of green beans (cv. *Moncayo*). Eur. Food Res. Technol. 220, 421-430.

Canjura F.L., Schwartz S.J., Nunes R.V., 1991. Degradation kinetics of chlorophylls and chlorophyllides. J. Food Sci. 56, 1639-1643.

Canjura F.L., Watkins R.H., Schwartz S.J., 1999. Color improvement and metallo-chlorophyll complexes in continuous flow aseptically processed peas. J. Food Sci. 64, 987-990.

Codex Alimentarius. 1993. Code of hygienic practice for precooked and cooked foods in mass catering. CAC/RCP 39 [http://www.codexalimentarius.net/ web/standard_list.jsp].

Dong H., Jiang Y., Wang Y., Liu R., Guan H., 2004. Effects of hot water immersion on storage quality of fresh broccoli heads. Food Technol. Biotechnol. 42, 135-139.

Gębczyński P., 1999. Ocena przydatności blaszek liściowych buraka liściowego (*Beta vulgaris v. cicla*) do mrożenia [Suitability of leaf blades of beet-leaf (*Beat vulgaris v. cicla*) for freezing]. Zesz. Nauk. AR Krak., Technol. Żywn. 11, 139-147 [in Polish].

Gębczyński P., 2003. Zmiany ilościowe wybranych składników chemicznych w procesie mrożenia i zamrażalniczego składowania głównych i bocznych róż brokuła [Quantitative changes of selected chemical components during freezing and storage of primary and secondary broccoli inflorescences]. Acta Sci. Pol. Ser. Technol. Alim. 2, 31-39 [in Polish].

Gębczyński P., Kmiecik W., 2006. Effect of traditional and modified technology in the production of frozen cauliflower on the content of selected antioxidative compounds. Food Chem. 101, 229-235.

Gębczyński P., Lisiewska Z., 2006. Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. Innov. Food Sci. Emerg. Technol. 7 (3), 239-245.

Gunawan M.I., Barringer S.A., 2000. Green color degradation of blanched broccoli (*Brassica oleracea*) due to acid and microbial growth. J. Food Proc. Preserv. 24, 253-263.

Heaton J.W., Lencki R.W., Marangoni A.G., 1996. Kinetic model for chlorophyll degradation in green tissue. J. Agric. Food Chem. 44, 399-402.

Kennedy C.J., 2000. Introduction. In: Managing frozen foods. Ed. C.J. Kennedy. Woodhead Publ. Cambridge, 1-5.

Kidmose U., Hansen M., 1999. The influence of postharvest storage, temperature and duration on quality of cooked broccoli florets. J. Food Qual. 22, 135-146.

Kmiecik W., Budnik A., 1997. Wpływ dwóch sposobów gotowania brokuła na poziom wybranych wskaźników fizykochemicznych [Effects of two methods of broccoli thermal treatment on some physical and chemical values of the product]. Bromat. Chem. Toksykol. 30, 303-309 [in Polish].

Kmiecik W., Lisiewska Z., 1999. Effect of pretreatment and conditions and period of storage on some quality indices of frozen chive (*Allium schoenoprasum* L.). Food Chem. 67, 61-66.

Kmiecik W, Lisiewska Z., Korus A., 2007. Retention of mineral constituents in frozen brassicas depending on the method of preliminary processing of the raw material and preparation of frozen products for consumption. Eur. Food Res. Technol. 224, 573-579.

Labib A.A.S., Latife Abd El S.A., Omran H., 1997. Quality indices of Jew's mallow and spinach during frozen storage. Plant Food. Hum. Nutr. 50, 333-347.

Lichtenthaler H.K., Buschmann C., 2001 a. Extraction of photosynthetic tissues: chlorophylls and carotenoids. In: Current protocols in food analytical chemistry. John Wiley New York, F4.2.1-F.4.2.6.

Lichtenthaler H.K., Buschmann C., 2001 b. Chlorophylls and carotenoids: measurement and characterization by UV-VIS spectroscopy. In: Current protocols in food analytical chemistry. John Wiley New York, F4.3.1-F.4.3.8.

Lisiewska Z., Kmiecik W., 1997. Effect of freezing and storage on quality factors in Hamburg and leafy parsley. Food Chem. 60, 633-637.

Lisiewska Z., Kmiecik W., Słupski J., 2004. Contents of chlorophylls and carotenoids in frozen dill: effect of usable part and pre-treatment on the content of chlorophylls and carotenoids in frozen dill (*Anethum graveolens* L.), depending on the time and temperature of storage. Food Chem. 84, 511-518.

Lisiewska Z., Gębczyński P., Kmiecik W., Skoczeń-Słupska R., 2007. Effect of vegetable freezing and preparation of frozen products for consumption on the content of lead and cadmium. Pol. J. Environ. Stud. 16, 579-585.

López A.B., Murcia A.M., Garcia C.F., 1998. Lipid peroxidation and chlorophyll levels in spinach during refrigerated storage and after industrial processing. Food Chem. 61, 113-118.

Murcia M.A., López A.B., Martinez T.M., Garcia C.F., 2000. Effect of industrial processing on chlorophyll content of broccoli. J. Sci. Food Agric. 80, 1447-1451.

Negi P.S., Roy S.K., 2000. Effect of blanching and drying methods on β-carotene, ascorbic acid and chlorophyll retention of leafy vegetables. Lebensm. Wissensch. Technol. 33, 295-298.

Oruna C.M.J., González C.M.J., López H.J., Simal L.J., 1997. Effets of freezing on the pigment content in green beans and padrón peppers. Z. Lebensm. Unters. Forsch. A, 205, 148-152.

Pogson B.J., Morris S.C., 1997. Consequences of cool storage of broccoli on physiological and biochemical changes and subsequent senescence at 20°C. J. Amer. Soc. Hort. Sci. 122, 553--558.

Premavalli K.S., Majumdar T.K., Madhura C.V., 2001. Processing effect on colour and vitamins of green leafy vegetables. J. Food Sci. Technol. 38, 79-81.

Suda I., Furuta S., Nishiba Y., 1994. Fluorometric determination of a 1,3-diethyl-2-thiobarbituric acid-malondialdehyde adduct as an index of lipid peroxidation in plant materials. Biosci. Biotech. Biochem. 58, 14-17.

Teng S.S., Chen B.H., 1999. Formation of pyrochlorophylls and their derivatives in spinach leaves during heating. Food Chem. 65, 367-373.

Tijskens L.M.M., Barringer S.A., Biekman E.S.A., 2001. Modelling the effect of pH on the colour degradation of blanched broccoli. Innov. Food Sci. Emerg. Technol. 2, 315-322.

Yamauchi N., Watada A.E., 1998. Chlorophyll and xanthophyll changes in broccoli florets stored under elevated CO_2 or ethylene-containing atmosphere. HortSci. 33, 114-117.

ZACHOWANIE CHLOROFILI W PRZYGOTOWANYCH DO KONSUMPCJI MROŻONYCH WARZYWACH KAPUSTNYCH W ZALEŻNOŚCI OD OBRÓBKI WSTĘPNEJ PRZED MROŻENIEM

Streszczenie. Badaniami objęto brokuły, kalafior zielony i kapustę brukselską. Ocenie poddano surowiec, surowiec po blanszowaniu, surowiec po ugotowaniu oraz mrożonki przygotowane do spożycia po 0, 4, 8 i 12 miesiącach przechowywania zamrażalniczego. W badaniach uwzględniono mrożonki z surowca blanszowanego przed mrożeniem (technologia tradycyjna) i mrożonki z surowca ugotowanego przed mrożeniem (technologia zmodyfikowana) oraz dwie temperatury składowania –20°C i –30°C. W technologii tradycyjnej mrożonki przygotowano do spożycia przez ugotowanie w wodzie, a w technologii modyfikowanej przez rozmrożenie i podgrzanie w kuchni mikrofalowej. Warzywa przygotowane do spożycia, po 12 miesiącach przechowywania mrożonek, w zależności od próby badawczej, zachowały w stosunku do surowca sumy chlorofili: brokuły 45-66%, kalafiory zielone 30-45% i kapusta brukselska 66-78%. W porównaniu z tradycyjną metodą, zawartość chlorofili w brukselce otrzymanej metodą zmodyfikowaną była wyższa o 16%; jednak w brokułach zawartość była niższa o 23%, a w kalafiorze o 21%. Przechowywanie w niższej temperaturze wpłynęło na większe zachowanie chlorofilów we wszystkich badanych warzywach.

Słowa kluczowe: kapustne, zawartość chlorofili, blanszowanie, gotowanie, składowanie zamrażalnicze, przygotowanie do spożycia

Accepted for print – Zaakceptowano do druku: 11.04.2008

For citation – Do cytowania: Kmiecik W., Lisiewska Z., Słupski J., Gębczyński P., 2008. The effect of pre-treatment, temperature and length of frozen storage on the retention of chlorophylls in frozen brassicas. Acta Sci. Pol., Technol. Aliment. 7(2), 21-34.