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Effects of freezing and storing of frozen products on the content of nitrates, nitrites, and oxalates in dill (*Anethum graveolens* L.)

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Abstract

The aim of the work was to compare the contents of nitrates, nitrites, and oxalates in the leafy part of dill and in whole plants (leaves with petioles and stems) of dill harvested at the 25 cm stage of growth. Changes in the levels of these compounds in the technological processes of freezing and refrigerated storage were also determined. The investigation concerned two kinds of the raw material (leafy parts and whole plants), different treatments before freezing (blanching or non-blanching of the raw material), differentiated temperature of storing frozen products (−20 and −30 °C), and a storage time of 12 months. Analyses of the frozen products were conducted every three months. In relation to whole plants (leaves, petioles, and stems) the leaves alone of fresh dill were characterized by a much lower content of nitrates (54%), a higher content (though below 1 mg/1000 g) of nitrites, and also a higher one of oxalates (26%). The blanching induced a considerable reduction of the contents of nitrates, nitrites, and oxalates. This concerned whole plants to a greater degree than leaves, oxalates being an exception. Freezing did not affect the levels of the analysed compounds in frozen products with the exception of non-blanched whole plants in which the level of nitrites significantly rose in relation to the raw material. Irrespective of the applied temperatures during refrigerated storage, the level of analysed compounds was slightly changed in relation to that recorded in the material directly after freezing.

Keywords: Dill; Freezing; Nitrates; Nitrites; Oxalates

1. Introduction

Leafy vegetables are an excellent source of vitamins, minerals and biologically active compounds (Favell, 1998; Kidmose, Knuthsen, Edelenbos, Justesen, & Hegelund, 2001). Moreover, dill, which is classed in this group, is characterized by an attractive flavour (Bauermann, Ehrich, & Thomann, 1994). These traits of dill are decisive in its wide utilization in the human diet. This vegetable is also used for medical and cosmetics purposes. (Huopalahti & Linko, 1983; Pszczola, 2001; Yang, Huang, Peng, & Li, 1996). However, apart from its desirable properties, dill, like other leafy vegetables, contains compounds known as unfavourable for human nutrition. In this group of compounds, above all, are nitrates, nitrites, and oxalates (Gupta & Wagle, 1988). At harvest time, young dill plants are at the stage of intensive growth and maintain their consumption values for a short time. In order to prolong the durability of dill and to cover the demand for this crop outside the vegetative period, various methods for its conservation can be used. Kmiecik, Lisiewska, and Jaworska (2001b) suggested the possibility of using not only leaves but also whole young plants of dill in the dehydration industry. After grinding, dried dill can be used as a component of seasoning concentrates. The present authors also attempted to use whole dill plants for freezing, frozen products being above all used in the preparation of soups and sauces.

The aim of the work was to determine the effects of blanching, freezing, and refrigerated storage on the levels of nitrates, nitrites, and oxalates in frozen leaves and whole plants of the dill.

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2. Material and methods

2.1. Raw material

The raw material, dill cv. Amat, was harvested in the experimental field of the Department carrying out the present investigation. The dill was grown on brown soil developed from loess formations of the mechanical composition of silt loam. The production was carried out in the second year after stable manure fertilization with pod plants as the fore-crop. The soil in a good horticultural culture showed a reaction approximating neutral (pH in H_2O – 6.5), an average content of humus (1.55%), and high contents of phosphorus (83 mg/dm^3), potassium (160 mg/dm^3), and calcium (1320 mg/dm^3). In mineral fertilization, applied before sowing, the contents of different macro-components and the requirements of the species were taken into consideration. The following fertilizers were used: nitrogen, 30 kg N ha^{-1} in the form of ammonium nitrate, phosphorus, 15 kg $P_2O_5^{-1}$ in the form of triple superphosphate, and potassium, 30 kg K_2O ha^{-1} in the form of 60% potassium chloride.

Seeds were sown on August 10 in 2000. The sowing date was adjusted so as to ensure the harvest times on the turn of summer and autumn, this permitting shortening of the storage period of frozen dill to the new harvest and reducing the costs of refrigerated storage. Prior to sowing, the seeds were treated with a mixture of Funaben I and Marshal 250 DS preparations. On the plantation, the cultivation measures included mechanical weeding and two sprayings with preparations controlling aphids and fungal diseases. The sprinkling irrigation (on September 8 with 15 mm of water) was used once the moisture in the soil decreased to an excessive degree. Favourable weather conditions prevailed throughout the growth of dill. The total temperature was 704 °C, the mean pentad temperature ranging from 13.7 to 25.1 °C, this with the fairly low precipitation beneficially affecting the sanitary condition of the plantation and the quality of the obtained yields.

The harvest carried out after 37 days, when the plants had reached about 25 cm in height, consisted of cutting plant tops about 5 cm above the soil. The harvested plants were therefore 20 cm in height. They were then surveyed for removal of individuals of discoloured or unhealthy appearance. It should be mentioned that the plants were healthy, traces of yellowing appearing only on single stunted leaves at the plant base. The dill plants were harvested in the morning and the time from cutting to the beginning of analyses and technological processing of the raw material did not exceed 2 h. The first measure was to separate the leaves from the remaining parts of the plants. It was determined that the leaves constituted 51% of weight of whole plants.

The investigation concerned: (1) two kinds of the raw material: leaves alone and whole dill plants, i.e. leaves with petioles and stems, (2) differentiated treatment before freezing, i.e. blanched and non-blanched samples, (3) differentiated temperatures of refrigerated storage, i.e. at −20 and −30 °C, (4) time of refrigerated storage throughout the year, frozen products being analysed at 3-month intervals.

2.2. Preparation of the material for analyses and freezing

Non-blanched leaves were cut into 5–7 mm sections. A sample, representative of whole non-blanched plants, was prepared by mixing leaves (previously cut into 5–7 mm sections) with the stem and petioles strained through a sieve of 2 mm sieve mesh. The preparation of blanched samples consisted of blanching in water at 94–96 °C, the proportion of water to the blanched material being 1:5. The time of blanching was adjusted to that necessary for decreasing the activity of peroxidase to at least 95%. The planned decrease in activity was attained after 30 s in leaves and after 3 min in stems with petioles. After cooling in water and removing the remaining water by centrifugation to the weight equal to that before blanching, the leaves were cut into sections of 5–7 mm while stems with petioles were granulated as in the case of the non-blanched dill. In blanched and non-blanched samples the same proportion as in the raw material was maintained between the leaves and the stems with petioles.

2.3. Freezing and storage of frozen dill

Packing the dill in polythene bags, 0.08 mm thick, preceded its freezing. The content of a bag was 650 g of the material. The bags were pressed tightly to remove as much air as possible, then welded closely. Directly after closing, the product was frozen at −40 °C in a 3626-51 Feutron blast freezer with forced air circulation to a temperature of −20 °C and to a temperature of −30 °C. After freezing, the bags were placed in storage chambers at −20 and −30 °C, respectively, and kept there until the time of evaluation.

Depending on the type of the material and on the applied pre-treatment, bags of the same weight had different volumes. In the calculation, per 1 kg of weight, the volume of leaves was about 3.5 dm^3 and of whole plants 2.0 dm^3 in non-blanched samples and in blanched ones about 1 dm^3 of both leaves and whole plants.

2.4. Evaluation of the levels of components

In chemical analyses, all the samples cited in "Material and methods" were taken into consideration. In each object investigated, the average evaluated sample contained 650 g of the material and the manner of sampling ensured its being representative of the entire lot of the dill. Analyses of the raw material were begun

within 2 h of the harvest and of frozen products after the storage period appropriate to the method of the investigation. The samples for analysis were defrosted at 2–4 °C during 17–18 h. An average laboratory sample, representing a given combination of the experiment, was granulated with distilled water in a laboratory food mixer in a weight ratio of 1 part dill to 1 part water.

Analyses of the components were carried out in four replications, according to standard methods: dry matter (AOAC, 1984) nitrates and nitrites (ISO/6635, 1984), total oxalates (AOAC, 1984).

2.5. Elaboration of results

Determination of the investigated components was carried out in four replications, average results of the determinations being calculated per 100 g fresh matter. To determine the significance of differentiation in the contents of the investigated components between the investigated combinations, statistical calculations were carried out, using one-factor analysis for the material before freezing and two-factor analysis for the results including the whole experiment. Factor I was the usable part, pre-treatment, and the temperature of storage, and factor II the period of storing frozen dill. Statistical analysis was carried out according to the Excel 5.0 program, using the Snedecor F test and the Student t test. The least statistical difference (LSD) was calculated for the probability level $p = 0.01$.

3. Results and discussion

The distribution of chemical components in various parts of the plant is differentiated. Dry matter content in dill leaves was 12.89 g/100 g fresh matter and significantly lower in whole plants −9.49 g (Table 1). A slightly higher level of dry matter, depending on the analysed part of the plant and the growing time, was found by Kmiecik, Lisiewska, and Jaworska (2002). The content found by those authors varied over a range of 11.68–17.86 g/100 g dill leaves and 8.96–16.15 g in leaves with petioles, though without stems. Blanching significantly reduced the level of dry matter, by 18% in leaves and by 10% in whole plants. According to Kmiecik and Lisiewska (1999), a 22% decrease was determined in dry matter content of blanched chive. Lisiewska and Kmiecik (1997) found a 16% decrease in dry matter content after blanching the leafy-type and Hamburg parsley. The freezing of dill did not change the level of this index. Frozen storage had a negligible effect on the content of dry matter and after 12 months, in all the objects of the experiment, this content increased by 1% to 3% in relation to the material directly after freezing.

The differentiation in nitrate concentration can be explained by the variable intensity of metabolic processes in the different organs of plants (Kovacik, 1994). According to Gębczyński, Korus, and Słupski (2001) the content of nitrates was three times lower in leaves than in the stem part of the dill. In the present work, leaves had twice less nitrates than had the whole plants. The leaf blades of other species of leafy vegetables also contained fewer nitrates than other parts of the plant (Biemond, Vos, & Struik, 1996; Jaworska, 2003; Kovacik, 1994; Santamaria, Elia, Serio, & Todaro, 1999). Extreme values of nitrates in dill given in the literature range from 2 to 12,320 mg $NaNO_3$ in 1 kg fresh matter. However, the most frequently quoted values are found in the range of 1200–2500 mg (Kmiecik, Gębczyński, & Korus, 2001a; Oztekin, Nutku, & Erim, 2002). It is hard

Table 1
Content of dry matter in raw and frozen dill (g in 100 g of fresh matter)

Usable part	Method of pre-treatment	Before freezing	Storage temperature	After storage time in months					Mean
				0	3	6	9	12	
Leaves	Non-blanched	12.89 ± 0.17	−20 °C	12.90 ± 0.32	12.89 ± 0.29	12.99 ± 0.37	13.00 ± 0.17	13.08 ± 0.17	12.96
		12.89 ± 0.17	−30 °C	12.85 ± 0.23	12.84 ± 0.24	12.95 ± 0.27	12.92 ± 0.14	13.01 ± 0.17	12.91
	Blanched	10.51 ± 0.23	−20 °C	10.54 ± 0.14	10.62 ± 0.25	10.71 ± 0.22	10.77 ± 0.13	10.77 ± 0.10	10.65
		10.51 ± 0.23	−30 °C	10.52 ± 0.21	10.45 ± 0.15	10.52 ± 0.20	10.60 ± 0.10	10.65 ± 0.10	10.54
Whole plant	Non-blanched	9.49 ± 0.19	−20 °C	9.44 ± 0.16	9.57 ± 0.18	9.63 ± 0.19	9.70 ± 0.11	9.73 ± 0.14	9.59
		9.49 ± 0.19	−30 °C	9.53 ± 0.13	9.49 ± 0.19	9.42 ± 0.18	9.59 ± 0.12	9.65 ± 0.15	9.53
	Blanched	8.56 ± 0.15	−20 °C	8.54 ± 0.16	8.59 ± 0.17	8.67 ± 0.21	8.71 ± 0.12	8.74 ± 0.12	8.63
		8.56 ± 0.15	−30 °C	8.53 ± 0.19	8.42 ± 0.15	8.55 ± 0.17	8.61 ± 0.10	8.64 ± 0.12	8.55
	Mean	10.36		10.36	10.36	10.43	10.49	10.53	
LSD ($p = 0.01$) for material before freezing: 0.403				For whole experiment:					
				factor (I)					0.139
				factor (II)					0.120
				interaction (I × II)					ns

$x \pm$ SD – mean value of four samples and standard deviation.
ns – not significant.

to refer the values quoted above directly to the results of the present work, since no information was given about whether the analyses were carried out on whole plants of the dill or on leaf blades only. Moreover, neither the height of plants nor the harvest dates were given. It can be ascertained, however, that the contents of nitrates found in the present investigation, amounting in leaves and whole plants to 728 mg and 1571 mg in 1 kg fresh matter, respectively, were below the mean values given above (Table 2).

Changes of nitrates are accompanied by changes in the contents of toxic nitrites, being associated with the effects of nitrate reductase. However, only a part of the reduced form of nitrate remains in the form of nitrite while the remainder is subjected to further transformations to ammonia ion (Crawford & Glass, 1998; Yang, 1992). In the leaves and whole plants of dill the contents of nitrites were low, amounting to 0.5 mg NO_2^- and 0.3 mg NO_2^-/kg fresh matter, respectively (Table 3). Gębczyński et al. (2001) also found more nitrites in the leaves of dill than in whole plants, while in New Zealand spinach the relation was the reverse (Jaworska, 2003). In the dill grown for the investigation by Kmiecik et al. (2001a) and Gębczyński et al. (2001), the content of nitrites did not exceed 1 mg in 1 kg fresh matter. However, in samples taken from retail trade it reached

Table 2
Contents of nitrates in raw and frozen dill, in fresh matter (mg/kg)

Usable part	Method of pre-treatment	Before freezing	Storage temperature	After storage time in months					Mean
				0	3	6	9	12	
Leaves	Non-blanched	728 ± 32	−20 °C	719 ± 33	789 ± 28	780 ± 32	784 ± 16	724 ± 34	754
		728 ± 32	−30 °C	725 ± 31	782 ± 29	785 ± 31	791 ± 31	722 ± 28	756
	Blanched	432 ± 26	−20 °C	428 ± 30	475 ± 37	466 ± 30	473 ± 22	440 ± 33	452
		432 ± 26	−30 °C	435 ± 24	467 ± 37	460 ± 26	472 ± 31	435 ± 20	450
Whole plant	Non-blanched	1571 ± 31	−20 °C	1543 ± 40	1559 ± 35	1563 ± 35	1557 ± 32	1540 ± 43	1556
		1571 ± 31	−30 °C	1539 ± 24	1550 ± 36	1526 ± 40	1528 ± 32	1532 ± 55	1541
	Blanched	780 ± 34	−20 °C	782 ± 34	846 ± 32	818 ± 26	882 ± 39	894 ± 33	834
		780 ± 34	−30 °C	788 ± 29	796 ± 30	812 ± 34	852 ± 44	893 ± 37	820
	Mean	878		870	908	901	917	898	
LSD ($p = 0.01$) for material before freezing: 66.1				For whole experiment:					
				factor (I)					24.2
				factor (II)					21.0
				interaction (I × II)					59.3

$x \pm$ SD – mean value of four samples and standard deviation.

Table 3
Contents of nitrites in raw and frozen dill, mg/1000 g fresh matter

Usable part	Method of pre-treatment	Before freezing	Storage temperature	After storage time in months					Mean
				0	3	6	9	12	
Leaves	Non-blanched	0.5 ± 0.1	−20 °C	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.5 ± 0.1	0.8
		0.5 ± 0.1	−30 °C	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.2 ± 0.1	0.7 ± 0.1	0.9
	Blanched	0.4 ± 0.1	−20 °C	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.0	0.5 ± 0.01	0.3 ± 0.1	0.4
		0.4 ± 0.1	−30 °C	0.3 ± 0.1	0.3 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4
Whole plant	Non-blanched	0.3 ± 0.1	−20 °C	4.0 ± 0.3	4.0 ± 0.3	3.8 ± 0.2	3.6 ± 0.1	1.8 ± 0.2	2.9
		0.3 ± 0.1	−30 °C	4.1 ± 0.2	4.2 ± 0.2	4.8 ± 0.3	4.9 ± 0.2	1.8 ± 0.2	3.4
	Blanched	0.2 ± 0.1	−20 °C	0.3 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.4 ± 0.1	0.5
		0.2 ± 0.1	−30 °C	0.2 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5
	Mean	0.4		1.4	1.5	1.7	1.6	0.8	
LSD ($p = 0.01$) for material before freezing: 0.17				For whole experiment:					
				factor (I)					0.10
				factor (II)					0.09
				interaction (I × II)					0.25

$x \pm$ SD – mean value of four samples and standard deviation.

Table 4
Contents of oxalates in raw and frozen dill (mg/100 g fresh matter)

Usable part	Method of pre-treatment	Before freezing	Storage temperature	After storage time in months					Mean
				0	3	6	9	12	
Leaves	Non-blanched	144 ± 7	−20 °C	154 ± 5	159 ± 7	151 ± 6	146 ± 6	156 ± 6	152
		144 ± 7	−30 °C	151 ± 6	152 ± 6	154 ± 6	148 ± 6	149 ± 6	150
	Blanched	95 ± 4	−20 °C	104 ± 5	103 ± 3	106 ± 4	101 ± 4	97 ± 4	101
		95 ± 4	−30 °C	102 ± 4	105 ± 5	107 ± 5	100 ± 4	95 ± 3	101
Whole plant	Non-blanched	114 ± 5	−20 °C	122 ± 6	130 ± 6	123 ± 4	132 ± 6	130 ± 5	125
		114 ± 5	−30 °C	124 ± 5	130 ± 5	128 ± 8	125 ± 5	127 ± 5	125
	Blanched	80 ± 3	−20 °C	91 ± 4	95 ± 3	93 ± 3	98 ± 6	96 ± 2	92
		80 ± 3	−30 °C	89 ± 3	90 ± 3	92 ± 2	90 ± 5	93 ± 4	89
	Mean	108		117	121	119	118	118	
nLSD ($p = 0.01$) for material before freezing: 10.6				For whole experiment:					
				factor (I)					3.6
				factor (II)					3.2
				interaction					8.9

$x \pm$ SD – mean value of four samples and standard deviation.

even 5.36 mg in 1 kg (Rostkowski, Borowska, Omieljaniuk, & Otłog, 1994).

Oxalates are also regarded as unwanted components of the human diet since they reduce the assimilability of calcium and, in its absence, also iron and magnesium (Kabasakalis, Tsitouridou, & Niarchos, 1995). The total content of oxalates in dill leaves amounted to 144 mg. In whole plants it was significantly lower, reaching 114 mg/100 g fresh matter (Table 4). Similarly, in the works by Gębczyński (1998, 1999), Jaworska (2003), and Savage, Vanhanen, Mason, and Ross (2000), a much higher content of oxalates was determined in the leaves of chard, New Zealand spinach, and spinach than in other parts of the plant.

A part of the nitrates, nitrites, and oxalates was significantly washed out from the plant tissue during blanching, this treatment usually preceding the freezing of vegetables. In referring the results to fresh matter, 41% of nitrates were washed out of leaves and 50% out of whole plants in the course of blanching. If the results were calculated per dry matter the respective losses were 27% and 45%. Gaiser, Rathjen, and Spiess (1997), Huarte-Mendicoa, Astiasaran, and Bello (1997), and Kmiecik and Lisiewska (1999) stressed that the blanching was also effective in decreasing the content of nitrates in spinach, broccoli and chive.

In the case of nitrites, the effect of blanching of chive was that their content increased three times Kmiecik and Lisiewska (1999), while in the leaves of parsley it decreased by almost half (Lisiewska & Kmiecik, 1997). In the investigated dill, nitrites were more easily washed out of the stem part and petioles than of leaves, as shown by the reduced content of nitrites by 8% dry matter in the leaves and by as much as 34% in the whole plants.

As in the case of nitrites, the effect of the thermal treatment in a water environment on the behaviour of oxalates is not uniform. Savage et al. (2000) showed that, in eight vegetable species, changes in the contents of oxalates ranged from +62% to −45% during cooking. Mosha, Gaga, Pace, Laswai, and Mtebe (1995) did not find any effect of blanching on the level of oxalates. According to Udosen and Ukpanah (1993), the level of oxalates fell during the preservation treatments. In both usable parts, the blanching significantly reduced the level of oxalates. The reduction amounted to 34% fresh matter in leaves and to 30% in whole plants, in dry matter these values being 19% and 27%, respectively (Table 4).

In the technological process of freezing the refrigeration was the next stage. In the works carried out by Gębczyński (2002) and Kmiecik and Lisiewska (1999), refrigeration did not change the contents of nitrates in Brussels sprouts or chive, but Lisiewska and Kmiecik (1997) found that the leaves of parsley contained 6–35% more nitrates after freezing. In the present experiment, refrigeration did not significantly affect the concentrations of nitrates, oxalates or nitrites in samples blanched before refrigeration. However in non-blanched samples, especially whole plants, the content of nitrites significantly increased. After the refrigeration of non-blanched parsley leaves Lisiewska and Kmiecik (1997) determined smaller amounts of nitrites than before freezing while in blanched leaves – as in the investigated dill – the contents of nitrites were similar before and after freezing.

During one-year of storage, the level of nitrates was fairly stable in the individual samples. As in the present work, the refrigerated storage induced limited changes in the concentrations of nitrates in parsley (Lisiewska & Kmiecik, 1997), Brussels sprouts (Gębczyński, 2002),

leafy beet (Gębczyński, 1999) and chive (Kmiecik & Lisiewska, 1999). However, Huarte-Mendicoa et al. (1997) claim that frozen broccoli contained two and a half times more nitrates than fresh. As in the case of frozen dill, the level of oxalates in frozen leafy beet (Gębczyński, 1998,1999) did not depend on the time and temperature of refrigerated storage. Unfortunately, this cannot be claimed in the case of nitrites. During storage, their contents varied and, no rule could be established in connection with any usable part, preliminary treatment before freezing, or temperature of storage. The only regularity determined was a distinctly lower level of nitrites in all the investigated objects after 12 month's storage than after 9 months. The results quoted in the literature concerning the effect of refrigerated storage on the level of nitrites are also varied. During the refrigerated 1-year storage of chive, a total disappearance of nitrites was recorded (Kmiecik & Lisiewska, 1999), but no change in stored broccoli (Huarte-Mendicoa et al., 1997). Changes in the contents of nitrites during refrigerated storage of parsley leaves depended on the temperature of storage. During a half-year storage at −20 °C an increasing tendency was observed and at −30 °C a decreasing one (Lisiewska & Kmiecik, 1997).

4. Conclusions

In relation to whole plants (leaves, petioles, and stems), the leaves alone of fresh dill were characterized by a much lower content of nitrates (54%), a higher content (though below 1 mg/1000 g) of nitrites, and also a higher one of oxalates (26%). The blanching induced considerable reductions of the contents of nitrates, nitrites, and oxalates. This concerned whole plants to a greater degree than leaves, oxalates being an exception. Freezing did not affect the level of the analysed compounds in frozen products, with the exception of non-blanched whole plants in which the level of nitrites significantly rose in relation to the raw material. Irrespective of the applied temperatures during refrigerated storage, the level of analysed compounds was slightly changed in relation to that recorded in the material directly after freezing.

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