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AMINO ACID PROFILES AND PROTEIN QUALITY OF FRESH AND FROZEN DILL DEPENDING ON USABLE PART OF RAW MATERIAL, PRE-TREATMENT BEFORE FREEZING, AND STORAGE TEMPERATURE OF FROZEN PRODUCTS

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ABSTRACT

The aim of the work was to determine the level of dry matter, total nitrogen, and amino acids in two kinds of green dill parts (leaves or whole plants 25 cm in height) subjected to different pre-treatment before freezing (blanching or non-blanching) and stored after freezing for 12 months at temperatures of -20°C and -30°C. The leaves of dill contained more dry matter and nitrogen compounds than whole plants. Blanching reduced the content of dry matter and total nitrogen. Refrigerated storage (-20°C and –30°C) for 12 months did not change the content of dry matter and nitrogen compounds. No differences were observed between the leaves and whole plants in the content of total amino acids and sum of essential amino acids while significant differences appeared in the content of some amino acids. In comparison with the FAO/WHO standard of 1991 the level of essential amino acids was very high, lysine being a limiting amino acid. Blanching effected a significant decrease in the content of some amino acids. Depending on the pre-treatment of the raw material and the temperature the 12-month refrigerated storage effected small, though in some cases significant changes in the level of amino acids.

Key words: dill, pre-treatment, freezing, storage, amino acids

INTRODUCTION

In the zone of temperate climate the period of fresh dill harvest is fairly long (from June to October) but supply throughout the year can be ensured only by its preservation. The basic method is drying, however, a considerable part of aromatic compounds being lost in this process. Freezing should be regarded as a more favourable measure of conservation, while the problem is whether the raw vegetable should be blanched prior to freezing or this pre-treatment could be omitted [14,15]. As the unpublished results of investigations carried out by the present authors show, blanching before freezing is recommended for a better preservation of important sensorial traits of frozen dill products for a period longer than 9 months.

Green parts of dill plants are in some cases regarded as a spice and sometimes as a leafy vegetable. It can be accepted that both determinations are appropriate and depend on the use of these parts. The use of dill in domestic cooking and gastronomy increases owing to the growing production of ready-to-eat dishes, spiced cheeses, and fish in different brines. In all these products the dill is used for spicing. It can also be used as the main raw material in preparing soups and sauces.

Neither in source materials nor in tables concerning the nutritional value of food products [11,27] was any information found on the amino acids composition of green dill. Therefore the objective of the present authors was to investigate the content of these compounds so significant in the human diet.

The aim of the work was to determine the content of total nitrogen and amino acids in two kinds of green dill parts (leaves alone and whole plants), subjected to varied processing (blanching or non-blanching) before freezing and after refrigerated stored at -20°C and -30°C for 12 months.

MATERIALS AND METHODS

The material of the experiment consisted of fresh and frozen dill cultivar Amat harvested in the experimental plot of the Department, which conducted the investigation. The sowing time (August 10) was adapted to the desired harvest time at the turn of summer, this making it possible to shorten the period of storing frozen products up to new harvest, and to reduce the costs of the refrigeration storage.

Harvesting was carried out within 38 days of sowing, when the dill reached 25 cm in height. The plant tops were cut about 5 cm above the ground, the length of harvested plants being 20 cm. Harvesting was carried out in the forenoon hours, the time from cutting plants to analyses of the raw material and technological processing not exceeding 2 h. First the leaves were separated from the remaining parts of plants. It was determined that the leafy part constituted 51% of the 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) different treatment before freezing: non-blanched and blanched samples; (3) varied temperatures of refrigerated storage: -20°C and -30°C; (4) the time of refrigerated storage of 1 year with analyses of the content of dry matter and total nitrogen in the frozen product conducted at 3-month intervals, and the level of amino acids determined after the whole storage period.

Non-blanched leaves were cut in sectors 5-7 mm in length. A sample representing whole non-blanched plants was prepared by mixing leaves cut into 5-7 mm sectors with stems and petioles rubbed through a 2 mm mesh sieve. The preparation of blanched samples consisted of blanching in water at 94-96°C, the ratio of plant mass to water mass being 1:5. The blanching time was adapted to a desired decrease in peroxidase activity of at least 95% in relation to the raw material. This was attained in leaves after 30 sec and in stems with petioles after 3 min. After cooling in water and removing the water by rotation to the weight before blanching, the leaves were cut into 5-7 mm sectors and the stems and petioles rubbed as in the case of non-blanched dill samples. The same ratio as in the raw material was maintained between leaves and stems with petioles in non-blanched samples and in blanched samples containing whole plants.

The dill was frozen in polyethylene bags 0.08 mm in thickness, the stock in one bag being 650 g of the vegetable. The bags were pressed to remove as much air as possible, then welded closely. Directly after sealing the product was frozen at -40°C in a Feutron 3626-51 blast freezer with forced circulation to -20°C and -30°C. After freezing, the bags were placed in storage chambers at -20°C and -30°C, respectively, and stored in these conditions to the estimation time.

The level of dry matter and content of total nitrogen was determined using the method given in AOAC [22], the content of amino acids (apart from tryptophan) using ion-exchange column chromatography [17,28]. The separation and determination of amino acids was carried out in an automatic AAA Carlo Erba amino acid analyser, model 3A 27. A sample of freeze-dried material was hydrolysed in 6M HCL for 22 h at 110°C. Before hydrolysis a sample for the determination of sulphuric amino acids was subjected to preliminary oxidation with performic acid to cysteine acid and methionine sulphone, their content being converted to cysteine and methionine. The literature data express the level of amino acids in g/100 g fresh matter or in g/16 g N [12,27,30]. The present authors decided in favour of the latter way of expressing the results. The index for the limiting amino acid (CS) using the Mitchell and Block [16] method and the integrated index of essential amino acids (EAA) using the Oser [23] method were calculated on the basis of the amino acid composition. In calculating the above indices the protein standard FAO/WHO [24] was used.

The determination of the investigated constituents was carried out in four replications. The mean values were compared using the least significant differences in order to determine which factor significantly affected the level of the components compared [26].

RESULTS AND DISCUSSION

A fairly differentiated level of total nitrogen is found in leafy vegetables. The interval was 0.34-0.94 g in 100 g fresh matter for 22 species [5] and 0.42-0.54 g for other 4 species [30]. In the investigations of Kmiecik et al. [10] and Varo et al. [29] dill contained 0.63-0.94 g nitrogen in 100 g fresh matter, depending on the usable part (leaves only or leaves with petioles) and conditions of growth, thus the amount of nitrogen in dill was found in the upper limit of the values quoted above. In the discussed experiment the leaves of dill contained 0.69 g of total nitrogen in 100 g fresh matter (Table 1). The amounts determined corresponded to 4.31 g crude protein with the assumption that the 6.25 factor of converting nitrogen to protein was used, i.e., that applied by other authors with respect to leafy vegetables [4,6,21]. Whole plants contained less total nitrogen than leaves by 36%. However, this difference was much smaller in dry matter, amounting to 13%, since the dry matter of leaves was 12.9 g and of whole plants only 9.5 g in 100 g of dill.

Table l. Content of total nitrogen in raw and frozen dill, g·100 g^{-1} fresh matter

Item	Months of storage	Kind of raw material				LSD p=0.01
		leaves		whole plants		
		non-blanched	blanched	non-blanched	blanched	
Before freezing	-	0.69	0.64	0.44	0.42	for factor: I = 0.018 II = ns I x II = ns
After storage at -20°C	0	0.68	0.65	0.45	0.42	
	3	0.68	0.64	0.44	0.43	
	6	0.70	0.65	0.42	0.43	
	9	0.69	0.64	0.44	0.42	
	12	0.69	0.65	0.44	0.44	
After storage at -30°C	0	0.69	0.64	0.43	0.42	
	3	0.70	0.63	0.44	0.41	
	6	0.69	0.64	0.43	0.42	
	9	0.69	0.63	0.43	0.42	
	12	0.68	0.65	0.44	0.43	

ns - not significant; factor I: kind of raw material and pre-treatment; factor II: time and temperature of storage

The treatment of blanching reduced the content of dry matter in leaves by 18% and in whole plants by 10%. Blanching also effected a significant decrease in the content of total nitrogen (Table 1).

Neither the temperature nor the period of refrigerated storage affected the level of dry matter or nitrogen compounds in frozen dill. In comparison with the level determined in the raw material, greater decreases in the content of total protein in frozen spinach were recorded by Murcia et al. [18]. The cited authors claimed that this result was due to the denaturation and solubilisation of nitrogen compounds during blanching.

Most authors agree that leafy vegetables are a rich source of amino acids [1,6,30] and, according to Carlsson [2], the leaves of nine commonly consumed species had a more beneficial composition of amino acids than stems with leaves. In comparison with whole plants, the leaves of dill contained more total amino acids by 0.3% only and more essential amino acids by 1.3% (Table 2). However, in the level of individual amino acids a greater differentiation was observed between the investigated usable parts. The leaves contained significantly more cysteine, isoleucine, leucine, tyrosine, proline, and alanine, and significantly less methionine, phenylalanine, histidine, and serine than whole plants.

Table 2. Amino acid composition of raw and frozen dill, g·16 g^{-1} N

Amino acid		Before freezing	After 12-month storage at temperature					LSD p=0.01
		-20°C			-30°C			
		non-blanch.	blanch.	non-blanch.	blanch.	non-blanch.	blanch.	
Lisine	a	5.68	5.73	5.76	5.80	5.68	6.02	0.261
	b	5.71	5.57	5.72	5.57	5.45	5.92	
Threonine	a	5.47	5.64	5.20	5.84	5.49	6.07	0.271
	b	5.73	5.92	5.64	5.44	5.75	5.54	
Valine	a	5.92	5.78	5.38	5.48	5.56	5.66	0.282
	b	5.72	5.46	5.77	5.69	5.71	5.74	
Cystine	a	1.78	1.74	1.04	1.37	1.16	1.38	0.076
	b	1.58	1.81	1.21	1.32	1.32	1.34	
Methionine	a	3.47	3.61	4.26	4.13	4.39	4.16	0.180
	b	3.90	3.73	3.98	3.82	4.07	3.86	
Isoleucine	a	4.71	4.44	4.46	4.35	4.34	4.34	0.236
	b	4.34	4.37	4.40	4.73	4.42	4.84	
Leucine	a	10.10	10.13	10.03	10.39	10.00	10.15	0.288
	b	9.62	10.09	9.90	10.24	9.81	10.56	
Tyrosine	a	4.83	4.58	4.40	4.56	4.45	4.84	0.220
	b	4.15	4.09	3.62	3.68	3.73	3.70	
Phenylalanine	a	8.63	6.38	7.50	5.58	7.09	6.19	0.368
	b	9.20	6.14	8.12	6.69	8.56	6.38	
Histidine	a	2.57	2.70	2.70	2.27	2.70	2.04	0.149
	b	2.82	2.92	2.97	2.43	2.67	2.78	
Arginine	a	5.39	5.60	5.31	5.46	5.63	5.33	0.286
	b	5.30	5.51	5.21	5.53	4.91	5.66	
Aspartic acid	a	8.74	9.53	9.06	9.83	9.20	9.66	0.470
	b	9.09	9.92	8.53	9.46	8.61	9.50	
Serine	a	3.83	4.07	4.18	4.04	4.05	3.97	0.229
	b	4.11	4.36	4.39	4.37	4.12	4.20	
Glutamic acid	a	13.05	14.42	11.91	12.69	11.95	12.13	0.597
	b	13.32	14.87	12.13	13.01	11.98	13.07	
Proline	a	2.22	2.45	2.24	1.98	2.20	2.02	0.109
	b	2.04	2.27	1.97	2.10	1.90	2.06	
Glycine	a	5.60	5.50	5.01	4.79	4.99	4.80	0.253
	b	5.37	5.26	4.70	5.11	4.89	5.12	
Alanine	a	4.93	5.10	4.72	4.69	4.65	4.70	0.225
	b	4.62	4.82	4.68	4.68	4.70	4.72	

a – leaves; b – whole plants; non-blanch. – non blanched; blanch. – blanched

The level of limiting amino acids was very high in comparison with the FAO/WHO standard [24]. Depending on the usable part of the plant the highest value of the CS index was calculated for tyrosine with phenylalanine and for cysteine with methionine: 212-214 and 210-219, respectively. For the remaining amino acids the CS values varied from 146-169, except from lysine, which was the only limiting amino acid. The CS index for lysine was also fairly high, reaching 98 each for the two usable parts. Similarly, Ishida et al. [6] recorded the values of CS index exceeding 100 for all the amino acids except lysine in edible green parts of sweet potatoes. However, in leafy vegetables methionine with cysteine more frequently appeared as the limiting amino acid [3,7,20,25,30]. According to Dewanji [4], in five species of leafy crops the level of essential amino acids was comparable with the FAO/WHO standard [24].

For the two usable parts of dill plants the ratio of essential amino acids to total amino acids was about 0.52 showing that essential amino acids constituted above half of their content both in leaves and whole dill plants. The ratio of essential to non-essential amino acids was 1.09 and 1.07, respectively for leaves and whole plants. Against the background of other edible leafy plants, the two calculated dependences should be regarded as high, since for spinach leaves the respective values are 0.46 and 0.84 [12] and for green parts of two sweet potato cultivars 0.45 and 0.82 on average [6]. For four non-common leafy vegetables, the values of the above discussed dependences between essential amino acids and total content of amino acids, and also between essential and non-essential ones, amount to 0.41-0.44 and 0.71-0.82, respectively [30].

The highest percentages in total amino acids were found for glutamic acid 14%, leucine 10%, and aspartic acid and phenylalanine 9% each. Jin et al. [30] in jujube leaves and Choi and Lee [3] in *Agastache rugosa* leaves also determined the highest content of glutamic and aspartic acids and of leucine.

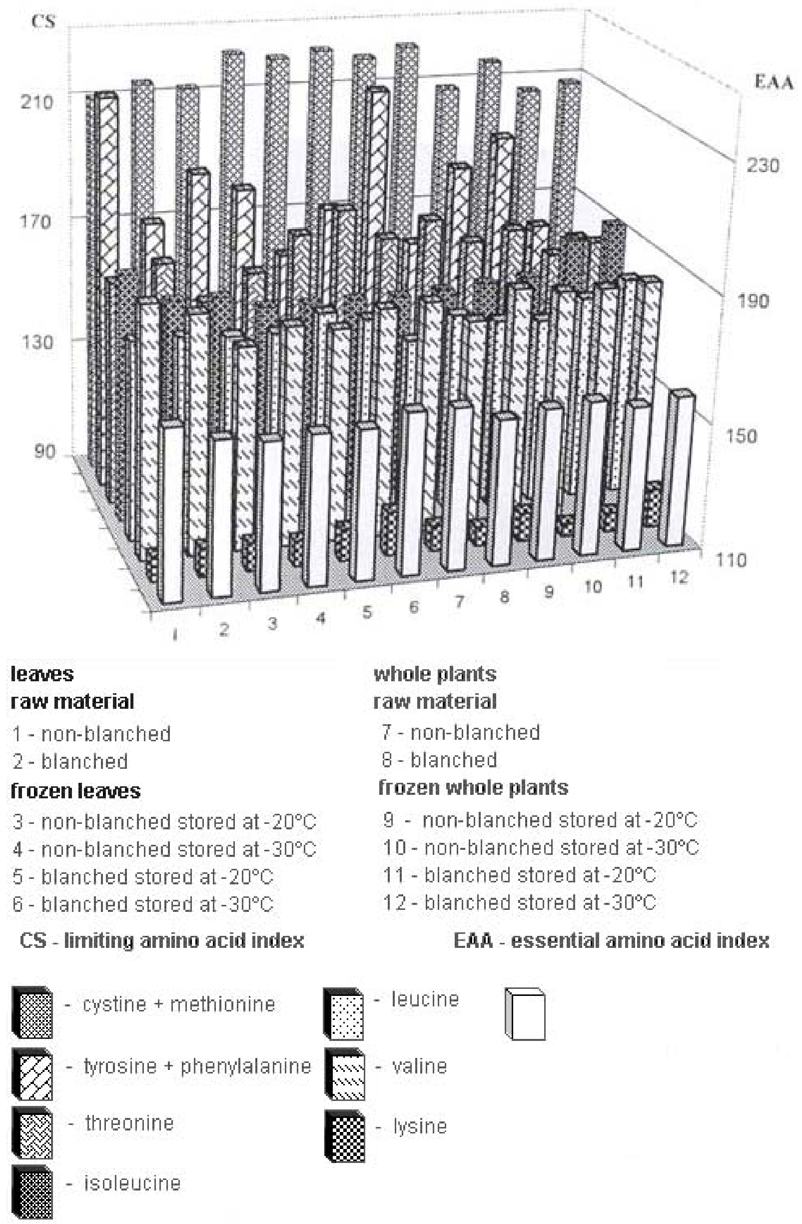
The treatment of blanching reduced the content of total amino acids in leaves by 6% and in whole plants by 3%. In total essential amino acids the losses were higher, amounting to 11% and 9% effecting a decrease in EAA index by five units. The ratio of essential amino acids to total amino acids and to non-essential ones was also reduced. A statistically significant decrease in the content of phenylalanine and an increase in aspartic and glutamine acids, and also in proline, were determined in the two usable parts. Moreover, after blanching, the content of isoleucine and tyrosine was smaller and that of histidine and serine greater in leaves, though in whole plants the content of cysteine and leucine increased. Changes in the content of essential amino acids affected the values of CS indices (Fig. 1).

Khalil and Monsour [8], Kmiecik et al. [9], and Lisiewska et al. [13] stress that, depending on their parameters, the technological processes differently affect changes in amino acid composition of protein. However, they usually effect decreases in the content of amino acids. According to Ziena et al. [33], the treatment with high temperature effects decreases in the content of almost all amino acids. Mutia and Uchida [19] claim that the prolonged treatment with high temperature effects changes in the content of lysine, cysteine, and methionine. On the other hand Youssef et al. [32] found a reverse reaction, i.e. an increase in the content of lysine after the application of a drastic dose of heat to the raw material. Wu et al. [31] present still another result and postulate the stability of amino acids lysine, valine, serine, and proline in the course of thermal treatments.

After the refrigerated storage the samples blanched before freezing contained more cysteine, leucine, aspartic acid, glutamine acid, and tyrosine and less methionine and phenylalanine than the non-blanched material. However, the differences were not always statistically proved and the lower storage temperature improved the preservation of threonine, valine, cysteine, methionine, and tyrosine, reducing the content of leucine and serine. With respect to the remaining amino acids the investigated factors had no effect or the effects were not quite explicit.

In comparison with the content before freezing, no significant changes were recorded in the content of lysine, arginine, aspartic acid, serine and proline, though only in samples which were non-blanched before freezing. Moreover, no changes were found in the level of valine, except for samples of non-blanched leaves, and of alanine, except for samples of blanched leaves, and also of leucine, except for whole plants, which were blanched before freezing and stored at -30°C. However, significant decreases were recorded in the level of cysteine and glutamine acid and in most samples of tyrosine, phenylalanine and glycine. The content of methionine was greater, though not always significantly, after the refrigerated storage than before, while no regular changes could have been determined in the changes of threonine, isoleucine, and histidine.

Figure 1. Amino acid indexes in raw and frozen dill according to FAO/WHO (1991)



In comparison with the content before freezing, frozen dill contained 96-97% of total amino acids after 12-month storage. Essential amino acids were preserved in 95-102% and the preservation of these groups of amino acids was better in samples blanched before freezing than in non-blanched ones and in those stored at -30°C than stored at -20°C.

In frozen dill lysine was still the limiting amino acid (Fig.1) however in samples blanched before freezing, except for frozen whole dill plants stored at -20°C, the value of CS index was 100 or higher. In comparison with the EAA index obtained for samples before freezing, its values were higher for blanched dill stored at -30°C, the same for frozen dill from blanched samples stored at -20°C, and then lower and lower for frozen products obtained from non-blanched samples stored at -30°C and -20°C. According to Ngudi et al. [20], the volume of water and time of heating might lead to losses of amino acids by diffusion and thermal degradation. Ziena et al. [33] postulate that the changes in the content of amino acids can be apparent, being affected by the decomposition of certain fractions of proteins whose content of particular amino acids is very large or rather poor.

CONCLUSIONS

The leaves of dill contained more dry matter and total nitrogen than whole plants. No differences were observed between the leaves and whole plants in the content of total amino acids and sum of essential amino acids while significant differences appeared in the content of some amino acids, lysine being a limiting amino acid. Blanching reduced the content of dry matter and total nitrogen and effected a significant decrease in the content of some amino acids. Depending on the pre-treatment of the raw material and the temperature the 12-month refrigerated storage (-20°C and -30°C) did not effected the content of dry matter and total nitrogen, and effected small, though in some cases significant changes in the level of amino acids.

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