

The amino acid composition of kale (*Brassica oleracea* L. var. *acephala*), fresh and after culinary and technological processing

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Abstract

The aim of the investigation was to evaluate the level of amino acids and the quality of protein in fresh and cooked leaves of kale and in two types of frozen product prepared for consumption after 12-months storage at $-20\text{ }^{\circ}\text{C}$. Kale blanched before freezing (the traditional method) was cooked after refrigerated storage, while that cooked before freezing (the modified method) was defrosted and heated in a microwave oven. Both fresh and processed leaves of kale were a good source of amino acids. In all the samples, glutamic acid, proline and aspartic acid were the dominant, while lysine and leucine were the limiting amino acids. Cooked leaves contained 78% of the total amino acid content found in fresh leaves, while the traditional and modified frozen products contained 76% and 78%, respectively. The proportion of essential amino acids in total amino acids was 44% and 43%, respectively for fresh and cooked leaves and 46% for the frozen products. The lowest EAA index was found for the traditional frozen product (99); it was higher for the remaining samples, which were broadly similar to each other (105–106).

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1. Introduction

Amino acids play an important role in human nutrition and also affect the sensory traits of products (Belitz & Grosch, 1999). During the processing of foods, protein sources are treated with heat, oxidizing agents, organic solvents, alkalis and acids for a variety of reasons (Friedman et al., 1984; Schwass & Finley, 1984). Such treatments may cause modification of the nutritional value of proteins, decreasing the amino acid content through desulfuration, deamination or isomerization; reactions with lysine, methionine, cystine and tryptophan are the most susceptible to damage (Cheftel, Cuq, & Lorient, 1989). The Maillard reaction between proteins and reducing sugars is primarily responsible for the loss in nutritional value of food proteins during heat processing (Sohn & Ho, 1995). A few amino

acid derivatives which are formed during food processing, may cause undesirable metabolic or even toxic events in the body (Finot, 1997; Halasz, Barath, Simon-Sarkadi, & Holzappel, 1994).

Vegetables of the *Brassica* group are the most commonly grown and consumed on a global scale. Belonging to this group, kale is consumed in the form of soups and its fresh and processed leaves are also served as part of the main course. The high nutritive and dietetic value of kale derives from its rich chemical composition (Rosa & Heaney, 1996). It is particularly rich in vitamins, minerals, dietary fibre and antioxidative compounds (Gębczyński & Korus, 2007; Kurilich et al., 1999). Moreover, nitrogen compounds, in which amino acids predominate, constitute about one third of the dry matter of kale.

The availability of fresh kale is seasonal. However, the period of its consumption can be extended throughout the year by processing. According to Cano (1996), freezing is a suitable method of processing seasonally harvested vegetables. This is reflected in the steady rise in frozen

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vegetable consumption over recent years (Martins & Silva, 2003). It is worth noting that consumers are mainly interested in products which can be prepared for consumption by heating only, for instance in a microwave oven. The food industry meets these requirements by the supply of products of the “ready-to-eat” type. The results of investigations show that such vegetables as carrot, green asparagus, broccoli or cauliflower are good raw materials for this kind of frozen product (Gębczyński, 2006, 2007; Gębczyński & Kmiecik, 2007; Gębczyński & Lisiewska, 2006). However, the production of ready-to-eat frozen vegetables involves obtaining consumption consistency before freezing; hence, cooking in salted water (for reasons of taste) replaces blanching in the pre-freezing processing.

As earlier studies by Gębczyński and Korus (in press) showed, compared with the traditional frozen product, frozen kale obtained using the modified method had similar sensory quality and contained more carotenoids and polyphenols with a similar level of vitamin C and antioxidant activity.

The aim of the present work was to evaluate the level of amino acids and protein quality in raw and cooked fresh kale leaves, and in two types of frozen products stored at $-20\text{ }^{\circ}\text{C}$ for 12 months and then prepared for consumption.

2. Materials and methods

2.1. Materials

The investigated material consisted of leaves of kale cv Winterbor F₁. The evaluation concerned fresh leaves, leaves cooked to consumption consistency and frozen kale leaves prepared for consumption after 12 months of refrigerated storage at $-20\text{ }^{\circ}\text{C}$.

The kale was grown in the experimental field of the research unit, where technological experiments were conducted. The harvest and processing were carried out in mid-october. Yellow and damaged leaves were discarded. From the remaining leaves the main rib was removed and the leaf blades were cut in crosswise stripes 2–3 cm in width. A mean sample representing the whole batch was taken for evaluation. The remaining material was divided into two parts, each being processed using different methods of preparation for freezing and consumption.

2.2. Freezing of kale

Two variants of processing the raw material before freezing were used. In variant I the traditional technology of freezing blanched material was applied. After processing and storage, the obtained frozen product must be cooked before consumption. In variant II the raw material was cooked to consumption consistency. This produced a “ready-to-eat” product, which only had to be defrosted and heated before consumption.

In variant I kale was blanched in a stainless steel vessel for 3 min at 96–98 $^{\circ}\text{C}$, the proportion of water to the raw

material being 5:1 by weight. The blanching was regulated so as to reduce the activity of catalase and peroxidase to a level not exceeding 5% of the initial activity. After blanching the material was cooled in cold water (14–16 $^{\circ}\text{C}$) and left to drip on sieves for 30 min.

In variant II kale was cooked in a stainless steel vessel in water with 1% added salt (NaCl), the proportion of water to material being 3:1 by weight. The cooking time, measured from the moment when the water came to the boil again, was 15 min. After cooking, the material was left on sieves and cooled in a stream of cold air (18 $^{\circ}\text{C}$).

The material from blanched and cooked samples was packed in 500 g batches in PE foil bags and frozen at $-40\text{ }^{\circ}\text{C}$ in a Feutron 3626-51 blast chamber for 90 min until the material reached a temperature of $-20\text{ }^{\circ}\text{C}$. The frozen products were placed in a chamber freezer at $-20\text{ }^{\circ}\text{C}$ until evaluation.

2.3. The preparation of frozen products for evaluation

Frozen products obtained using traditional technology (variant I) were cooked in water with 2% added salt, the proportion of the weight of water to weight of the raw material being 1:1. The frozen product was put in boiling water and the cooking time, measured from the moment when the water came to the boil again, was 12 min. After cooking the water was drained, the product was cooled to 20 $^{\circ}\text{C}$ and its chemical composition was established.

Frozen goods obtained from cooked material (variant II) were defrosted and heated in a heat-resisting vessel covered with a lid in a Panasonic microwave type NN-F621, to a temperature of 75 $^{\circ}\text{C}$ for 7 min 45 s. After heating the samples were cooled to 20 $^{\circ}\text{C}$ and their chemical composition was established.

2.4. Analytical procedures

The content of dry matter and total N were determined according to procedures described by the AOAC (1990). The content of amino acids (except for tryptophan) was determined using an AAA-400 amino acid analyzer (INGOS, the Czech Republic). The analytical procedure applied was in accordance with the recommendations of the producer. The freeze-dried material was hydrolyzed in 6 M HCl for 24 h at 110 $^{\circ}\text{C}$. After cooling, filtering and washing, the hydrolyte was evaporated in a vacuum evaporator at temperature below 50 $^{\circ}\text{C}$ for sulfur-containing amino acids and below 60 $^{\circ}\text{C}$ for others amino acids, the dry residue being dissolved in a buffer of pH 2.2. The prepared sample was analysed using the ninhydrine method. Buffers of pH 2.6; 3.0; 4.25; and 7.9 were applied. The ninhydrine solution was buffered at pH 5.5. A column 370 mm in length was filled with Ostion ANB INGOS ionex (the Czech Republic). The temperature of the column was 55–74 $^{\circ}\text{C}$; that of the reactor 120 $^{\circ}\text{C}$. The determination of the sulfur-containing amino acids, methionine and cysteine, was carried out by means of oxygenating hydrolysis,

using a mixture of formic acid and hydrogen peroxide (9:1) at 110 °C for 24 h. After cooling, the sample was processed as with acid hydrolysis. Buffers of pH 2.6 and 3.0 were used; the temperature of the column was 60 °C and that of the reactor 120 °C. The calculations were carried out according to the external standard.

All determinations were carried out in two replications for each experiment. Each experiment was carried out in three replications.

2.5. Expression of results

The level of amino acids was given in 100 g of edible parts of the products in order to compare the amino acid content in kale according to the culinary and technological processing applied.

The composition of amino acids was also expressed as grams per 16 g of N to estimate the quality of the protein in kale by comparing it with the FAO/WHO pattern (FAO/WHO, 1991; Institute of Medicine, 2002). On the basis of the amino acid composition, the CS index was calculated using the Mitchell and Block method (Osborne & Voogt, 1978), and the integrated EAA index using the Oser method (Oser, 1951).

2.6. Statistical analysis

Statistical analysis allowing a comparison of the content of amino acids in the fresh raw material, cooked raw material and frozen kale after preparation for consumption was carried out using single-factor analysis of variance (ANOVA) on the basis of the Snedecor F and Student's *t* tests, and the least significant difference (LSD) was calculated at the probability level $\alpha = 0.01$ and $\alpha = 0.05$ (Snedecor & Cochman, 1980). The Stastica 6.1 program was used.

3. Results and discussion

In fresh kale leaves with midribs removed, the dominant amino acids were glutamic acid, proline and aspartic acid; their proportion of the total amino acid content was 12%, 12% and 10%, respectively. The proportion of leucine, lysine, valine, arginine and alanine was in the range of 6–8%; that of tyrosine, phenylalanine, threonine, histidine, serine and glycine varied from 3–5%. The lowest proportions were in the sulphur containing amino acids, cystine (1.6%) and methionine (2%) (Table 1).

There is considerable variation in the proportions of individual amino acids in fresh kale leaves found by different authors (Ayaz et al., 2006; Eppendorfer, 1978; Eppendorfer & Bille, 1996; Krężel, Kołota, & Ściążko, 1998). However, in most cases glutamic acid had the highest proportion, similar to that found in the present study. Krężel et al. (1998), however, found the highest proportion in proline, as much as 19%. Aspartic acid came next, followed by proline, leucine or arginine, depending on the

source. All the studies showed that the sulphur containing amino acids were the limiting amino acids (Ayaz et al., 2006; Eppendorfer & Bille, 1996; Krężel et al., 1998). In the works cited above whole leaves of kale were analysed, while in the present investigation only the edible part of the leaf was taken into consideration, i.e. after removal of the midrib. As Ishida et al. (2000) demonstrated, leaf blades contain more amino acids than those parts involved in nutrient transportation, such as stalks and stems. Carls-son (1983) expressed a similar view, reporting that whole leaves of *Urtica dioica* and *Rumex acetosa* contained more amino acids than whole plant shoots and had a better profile. According to Choi and Lee (1999), young leaves of *Agastache rugosa* contained more amino acids than older ones. The content of amino acids was also affected by the cultivar, the sowing and harvest dates (Krężel et al., 1998) and also by nitrogen, phosphorus, potassium and sulphur fertilization (Eppendorfer & Bille, 1996; Eppendorfer & Eggum, 1992).

Compared with the raw material, cooked fresh kale leaves contained significantly less amino acids in 100 g fresh matter (Table 1), as did the products prepared from frozen kale after storage, with the exception of methionine, whose content did not change in frozen kale obtained using the traditional method. In the case of frozen kale obtained using the modified method, there were significant differences at $\alpha = 0.01$, except in the content of leucine, methionine, tyrosine; and at $\alpha = 0.05$, no significant differences were recorded in the amounts of lysine, threonine, histidine, glutamic acid or serine.

In comparison with cooked fresh kale, the cooked traditional frozen product contained similar amounts of amino acids, except for an increase in the content of methionine and a decrease in proline. On the other hand, the product obtained using the modified method contained more amino acids, except for histidine, arginine, glutamic acid, serine and proline, whose content did not differ from that in cooked fresh leaves. In general, the modified frozen product prepared for consumption contained more amino acids than the traditional one. Similar levels in the two types of product were found only for methionine and histidine and, at $\alpha = 0.01$, for arginine and proline.

Compared with the raw material, the loss of total amino acids in the investigated products was broadly similar to the loss of dry matter (Table 1). The changes in dry matter content were above all affected by the leaching of constituents during blanching and cooking but they were also partly due to water absorption by starch, the level of which in kale leaves varied within the range of 1.0–1.5 g in 100 g fresh matter, depending on the cultivar and the age of the leaves (Korus, Słupski, & Kmiecik, 2005). According to Murcia, Lopez-Ayerra, Martinez-Tome, and Garcia-Carmona (2001), prolonging thermal processing in water brought about increased losses of amino acids in fresh matter of florets and stems of broccoli, the losses being different in the two parts of broccoli.

Table 1
Amino acid composition of raw and processed leaves of kale, in milligrams of 100 g of product

Amino acid	Leaves		Goods prepared for consumption from frozen leaves		LSD*	
	Raw	Cooked	Blanched before freezing	Cooked before freezing	$\alpha = 0.01$	$\alpha = 0.05$
Isoleucine	156 ± 10	98 ± 5	104 ± 6	133 ± 7	15.4	11.0
Leucine	299 ± 10	236 ± 9	239 ± 4	282 ± 14	21.7	15.5
Lysine	221 ± 10	192 ± 6	196 ± 9	230 ± 11	19.6	13.9
Cystine	58 ± 3	41 ± 3	45 ± 2	54 ± 5	8.0	5.7
Methionine	72 ± 4	53 ± 4	67 ± 3	66 ± 2	7.6	5.4
Total sulphur amino acids	130	94	112	120	13.6	9.7
Tyrosine	122 ± 7	95 ± 4	91 ± 5	110 ± 7	12.6	9.0
Phenylalanine	186 ± 9	139 ± 6	143 ± 7	168 ± 7	16.2	11.6
Total aromatic amino acids	308	234	234	278	28.6	20.4
Threonine	164 ± 9	133 ± 8	135 ± 7	156 ± 8	17.2	12.3
Valine	207 ± 12	138 ± 7	143 ± 8	176 ± 10	20.3	14.4
Histidine	106 ± 6	95 ± 4	96 ± 5	100 ± 1	9.6	6.9
Total essential amino acids	1591	1220	1259	1475	119.0	84.9
Arginine	229 ± 10	192 ± 10	181 ± 9	200 ± 10	21.3	15.2
Aspartic acid	349 ± 17	282 ± 11	278 ± 10	314 ± 11	27.9	19.9
Glutamic acid	450 ± 34	397 ± 25	359 ± 23	418 ± 19	56.1	40.0
Serine	163 ± 11	136 ± 7	134 ± 7	154 ± 8	18.3	13.1
Proline	434 ± 17	300 ± 11	246 ± 11	273 ± 10	27.1	19.3
Glycine	190 ± 13	151 ± 8	149 ± 7	175 ± 10	20.7	14.7
Alanine	215 ± 12	159 ± 6	155 ± 7	189 ± 9	18.8	13.4
Total non-essential amino acids	2030	1617	1502	1723	188.5	134.4
Total amino acids	3621	2837	2761	3198	305.7	218.0
Dry matter g/100 g of product	18.08	13.95	13.54	14.90		

*LSD: least significant difference.

Diasolua Ngudi, Kuo, and Lambein (2003) found that the losses of amino acids in dry matter of cooked cassava (*Manihot esculenta* Crantz) leaves increased with a greater quantity of cooking water and a longer cooking time. Depending on the sample, they usually amounted to 58% of the content found in raw leaves. It is obvious that these high losses were also greatly affected by the ratio between the weight of cassava leaves and their area. In the present investigation, the optimum ratio of water to weight of kale (arrived at in preliminary experiments) was applied in cooking and blanching, and the length of thermal processing was that required to achieve sufficient inactivation of enzymes in the case of blanching, or consumption consistency in the case of cooking.

In comparison with the protein in the raw material, that in cooked fresh leaves contained significantly less isoleucine, valine and proline and significantly more lysine, histidine, glutamic acid and, at $\alpha = 0.05$, arginine (Table 2). Additionally, in the traditional frozen product, the content of tyrosine and alanine was significantly lower, while no significant differences were recorded in the content of lysine, histidine or glutamic acid. The total content of amino acids, in the traditional frozen product, was also significantly lower than in the raw material. In the modified frozen product, the amounts of amino acids were similar to those in the raw material, except for lower proline and higher lysine content; additionally the content of isoleucine and valine was lower at $\alpha = 0.05$.

Compared with that in cooked fresh leaves, the protein in the frozen products usually contained similar or significantly lower levels of amino acids. The traditional frozen product was characterized by a lower content of tyrosine and arginine, aspartic acid, glutamic acid, proline and alanine and, at $\alpha = 0.05$, of leucine and serine; the content of total amino acids was also lower. The modified frozen product contained less histidine, glutamic acid and proline and, at $\alpha = 0.05$, less aspartic acid and total amino acids; this sample contained more arginine. Comparing the two frozen products, that obtained using the modified method contained more isoleucine and, at $\alpha = 0.05$, leucine, lysine, tyrosine, valine and alanine. However, the differences in total amino acid content were not significant.

During the preparation of food, the side chains of some protein-bound amino acids can react chemically with each other or with other molecules present in the food, resulting in a reduction in nutritive value (Sherr, Lee, & Jelesiewicz, 1989). Some of these reactions are reversible; however, subsequent reactions are not reversible and amino acids are destroyed (Baxter, 1995).

Many authors stress that the parameters of technical and culinary processing affect the amino acid composition of protein in different ways, depending on the raw material. This is probably due to differences in structural composition.

According to Lisiewska, Słupski, Kmiecik, and Gębczyński (2004), blanching and refrigerated storage brought

Table 2
Amino acid composition of raw and processed leaves of kale, in grams of 16 gN

Amino acid	Leaves		Goods prepared for consumption from frozen leaves		LSD*	
	Raw	Cooked	Blanched before freezing	Cooked before freezing	$\alpha = 0.01$	$\alpha = 0.05$
Isoleucine	3.30 ± 0.21	2.69 ± 0.13	2.60 ± 0.14	3.03 ± 0.15	0.348	0.248
Leucine	6.31 ± 0.22	6.50 ± 0.26	5.96 ± 0.10	6.42 ± 0.32	ns	0.364
Lysine	4.68 ± 0.21	5.28 ± 0.18	4.88 ± 0.22	5.22 ± 0.23	0.455	0.324
Cystine	1.23 ± 0.07	1.12 ± 0.09	1.13 ± 0.06	1.22 ± 0.12	ns**	ns
Methionine	1.52 ± 0.08	1.45 ± 0.12	1.67 ± 0.09	1.50 ± 0.05	ns	0.133
Total sulphur amino acids	2.75	2.57	2.80	2.72	ns	ns
Tyrosine	2.58 ± 0.16	2.62 ± 0.11	2.25 ± 0.12	2.49 ± 0.15	0.288	0.206
Phenylalanine	3.94 ± 0.19	3.83 ± 0.18	3.56 ± 0.18	3.82 ± 0.17	ns	ns
Total aromatic amino acids	6.52	6.45	5.81	6.31	0.670	0.478
Threonine	3.47 ± 0.19	3.67 ± 0.22	3.35 ± 0.18	3.55 ± 0.18	ns	ns
Valine	4.37 ± 0.24	3.79 ± 0.20	3.55 ± 0.19	3.99 ± 0.24	0.473	0.337
Histidine	2.25 ± 0.14	2.61 ± 0.11	2.40 ± 0.11	2.27 ± 0.01	0.226	0.161
Total essential amino acids	33.65	33.56	31.35	33.51	ns	ns
Arginine	4.85 ± 0.22	5.27 ± 0.27	4.50 ± 0.21	4.55 ± 0.24	0.515	0.367
Aspartic acid	7.37 ± 0.37	7.75 ± 0.31	6.91 ± 0.26	7.14 ± 0.25	0.651	0.464
Glutamic acid	9.52 ± 0.73	10.89 ± 0.68	8.94 ± 0.58	9.50 ± 0.43	1.329	0.948
Serine	3.44 ± 0.22	3.75 ± 0.20	3.34 ± 0.18	3.49 ± 0.18	ns	0.314
Proline	9.17 ± 0.36	8.24 ± 0.31	6.11 ± 0.26	6.20 ± 0.23	0.637	0.454
Glycine	4.02 ± 0.27	4.15 ± 0.21	3.72 ± 0.19	3.98 ± 0.22	ns	ns
Alanine	4.54 ± 0.24	4.36 ± 0.17	3.86 ± 0.18	4.29 ± 0.20	0.436	0.311
Total non-essential amino acids	42.91	44.41	37.38	39.15	4.439	3.166
Total amino acids	76.56	77.97	68.73	72.66	7.161	5.107
N g/100 g of fresh matter	0.76	0.58	0.64	0.70		

*LSD: least significant difference, **ns: non significant.

about a significant decrease in the content of protein in dill leaves and in the level of isoleucine, tyrosine and phenylalanine but increased the content of aspartic acid, glutamic acid and proline. The level of the remaining amino acids did not significantly differ from that in the raw material. However, Diasolua Ngudi et al. (2003) recorded a similar content of amino acids in the protein of cooked cassava leaves, using five samples harvested in different localities; the only exceptions were alanine, with a considerably increased content, and methionine, the content of which was considerably reduced.

The 1st limiting amino acids in leaf vegetables were, depending on the source, the sulphur containing amino acids leucine and lysine (Choi & Lee, 1999; Eppendorfer & Bille, 1996; Ishida et al., 2000; Lisiewska et al., 2004; Wallace, Marfo, & Plahar, 1998). In the kale leaves investigated in the present study, lysine was the 1st limiting amino acid with respect to protein quality (Table 3). The CS index for fresh and cooked leaves was 81 and 91, respectively; for traditional and modified frozen products, 84 and 90, respectively. The 2nd limiting amino acid was leucine. The lowest CS value of 90 for leucine was found in the traditional frozen product; for the remaining samples, the CS index varied in the range of 96–98. According to Ayaz et al. (2006), lysine was the limiting amino acid in kale, the CS index being 95. Eppendorfer and Bille (1996) reported that in this vegetable the amino acids limiting the quality of protein were the sulphur containing amino

Table 3
Amino acids indexes of raw and processed leaves of kale according to FAO/WHO (1991)

Index	Amino acid	Leaves		Goods prepared for consumption from frozen leaves	
		Raw	Cooked	Blanched before freezing	Cooked before freezing
CS	Isoleucine	118	96	93	108
	Leucine	96	98	90	97
	Lysine	81	91	84	90
	Cystine + methionine	110	103	112	109
	Tyrosine + phenylalanine	103	102	92	100
	Threonine	102	108	99	104
	Valine	125	108	101	114
	Histidine	118	137	126	119
EAA		106	105	99	105

CS: chemical score index, EAA: essential amino acid index.

acids, whose CS index was 79–89, depending on the fertilizers applied.

The total quality of protein can be expressed by an EAA index. The lowest, 99, was found for the traditionally frozen product; values for the remaining samples were broadly similar at 105–106. According to Akpanyung, Udoh, and Akpan (1995), Dewanji and Matai (1996), and Rao et al. (1990), leaf vegetables are a good source of amino acids. The present investigation suggests that processed as well

as fresh kale leaves can be included among these vegetables.

4. Conclusions

Kale leaves, both fresh and processed, can be a good source of amino acids. The 1st limiting amino acid was lysine (CS 81–91) and the 2nd leucine (CS 90–98). Essential amino acids as a proportion of total amino acids was 44% and 43% for fresh and cooked leaves, respectively and 46% for each of the frozen products.

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