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Effect of different post-harvest treatments on free amino acid content in *Fucus vesiculosus*

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Abstract

The effect of different post-harvest treatments (fresh, freezing and air-drying) and storage duration on free amino acid (FAA) content of brown seaweed *Fucus vesiculosus* were studied. The FAA profile of thalli algae was studied every 3 months over 1 year. The FAA were extracted in ethanol/water (70: 30) solution, purified by ion exchange chromatography, derivatized and determined using hight-performance liquid chromatography. Free amino acid profile was significantly ($p<0.001$) different among the fresh, freezing and air-drying seaweeds. Long-term storage of seaweeds for 12 months in both dried and frozen form contributed to increased levels of free amino acids. The total free amino acid content increased from 285.80 mg/100 g dry matter of fresh seaweeds to 597.38 mg/100 g dry matter of dried seaweeds and to 473.84 mg/100 g dry matter of the frozen form. The total content of essential amino acids increased from 49.65 mg/100 g dry matter in fresh seaweeds to 130.45 mg/100 g dry matter of dried seaweeds and to 106.70 mg/100 g dry matter of frozen seaweeds. The content of glutamic acid responsible for the taste increased from 132.36 mg/100 g dry matter of fresh seaweeds to 169.50 mg/100 g dry matter of dried seaweeds, and to 242.00 mg/100 g dry matter of frozen seaweeds. Thus, the long-term storage of dried and frozen seaweeds contributes to the improvement of their qualities.

Keywords: fucus algae, free amino acids, frozen, dried, storage.

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1. Introduction

Today sea brown seaweeds are a valuable bioresource that people strongly need in different spheres. For example, the Fucales order, including one of the most common species *Fucus vesiculosus*, which inhabits the littoral zone of the seas of the Arctic Ocean and the North Atlantic, are currently used in food industry, cosmetic production, agriculture and medicine [1--3]. These seaweeds are known as a valuable source of specific metabolites, which are investigated as potential anticancer compounds, pro- and anticoagulants, immunomodulating agents and others [3, 4].

Amino acids play a fundamental role in protein synthesis in plants and fulfill a protective role in various types of stress [5]. Seaweeds amino acids, both free and included in proteins, present a particular interest to people [2]. Aspartic acid and

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glycine affect the formation of new tissues and regulate the nervous system, lysine and isoleucine are important for the immunological system, and phenylalanine is important for thyroid function [6]. The most common amino acids in Fucus spp. is aspartic and glutamic acids, which account for 22-44% of the total amino acids [7]. Besides the increasing awareness of the useful properties of seaweeds as components of healthy and nutritious diets and the growing demand for their gastronomic value among cooks and food companies, the interest in seaweed protein and amino acids is also increasing [8, 9]. An important problem is the need to maintain a stable amino acid profile of seaweed raw materials. Since seaweed, especially in northern latitudes, can only be harvested a few months a year, seaweeds are subjected to post-harvest treatment for industrial purposes. Air- and san- drying or freezing are the most widely used treatment methods [10]. Seaweeds are then stored until at least the next harvest. However, there is surprisingly little information in scientific literature about post-harvest treatment, and most importantly, about the stability of the chemical composition of raw materials over a long storage period. First of all, this refers to information on the amino acid composition of seaweeds [7, 11--14].

In this context, in order to improve the efficiency of their practical use there is a need for comprehensive information on the stability of the amino acid profile of fucoid tallomes throughout their storage.

2. Methods and Equipment

2.1. Methods

The subject of the study was brown seaweed *Fucus vesiculosus* Linnaeus, 1753 (Phaeophyceae: Fucales). The seaweed was harvested in October, 2017 in the intertidal zone during an outflow in the Kola Bay of the Barents Sea (the area of the Abram-Cape 68058.496′ N, 33001.693′ E). Fresh seaweed in the amount of 5 kg was delivered to the laboratory 1.5 hours after harvesting, cleaned of epiphytes and sand. The seaweed tallomes were ground and free amino acids were recovered with 70% ethanol with further treatment by ion exchange chromatography.

The seaweed samples were divided into 2 groups of 2 kg each. The first set of samples was frozen in a freezer at -25±2°C. The second group was dried in a greenhouse. The greenhouse was equipped with a ventilation system and the internal temperature was 15°C at night and up to 25°C in the middle of the day. The average relative humidity was 50±5%. After 5 days, the samples of dried seaweed were placed into a container and stored in a room with a controlled temperature of 20±20°C and humidity of 45±5%.

The amino acid composition is determined by high performance liquid chromatography (HPLC) using a reversed-phase C18 column and PITC (phenyl isothiocyanate) pre-derivatization samples. UV detection at 254 nm. The amino acid analysis was performed by a modified procedure on a Shimadzu LC-20AD Prominence liquid chromatograph (Japan) with a Shimadzu SPD-M20A Prominence photodiode array detector and a 250x4.6 mm Supelco C18.5 μm (US) chromatographic column as described in [11, 13].

All measurements were performed in 3-fold repetition. The results are presented as mean values ± SD (standart deviation). The statistical validity of differences in the level of free amino acids was confirmed by a single-factor dispersion analysis (one-way ANOVA) with a level of significance $P<0.05$ in the Statistica software.

2.2. Chemicals and reagents

HPLC-grade acetonitrile, phenyl isothiocyanate (PITC), orto- phosphoric acid, sodium acetate, DL-Methionine sulfone were purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water used for the preparation of all reagents, eluents, and buffers was obtained from a Milli-Q-simplicity 185 system (Millipore, Bedford, MA, USA). All solutions and reagents were filtered through 0.45 μm MS® Nylon membrane filters. The amino acid standards alanine (Ala), aspartic acid (Asp), arginine (Arg), glutamic acid (Glu), glycine (Gly), hydroxyproline (Hyp); histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser),, tyrosine (Tyr), threonine (Thr), tryptophan (Trp) and valine (Val) were from Sigma-Aldrich (Steinheim, Germany).

2.2.1. Diagrammatic representation

Figure 1 shows the total free amino acid content of frozen and dried seaweed stored for 3, 6, 9 and 12 months compared to fresh seaweed. The amino acid profile of dried and frozen seaweed samples stored for 3, 6, 9 and 12 months compared to the free amino acid content of fresh seaweed is shown in Table 1.

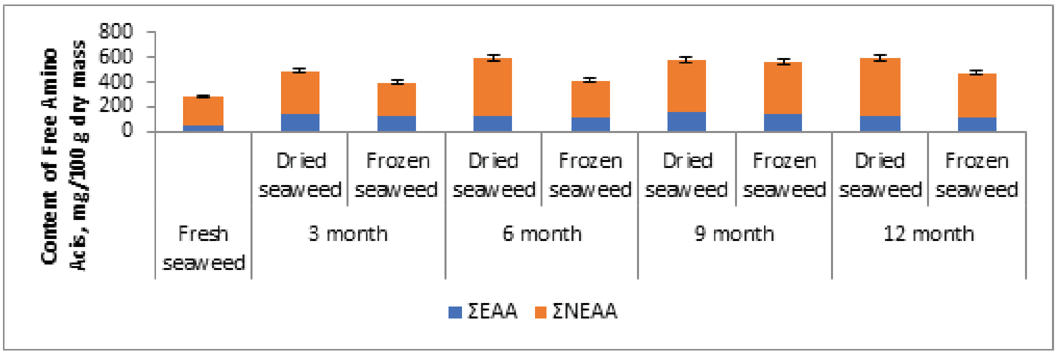

Figure 1: Free amino acid (in mg amino acid 100 g-1 dry mass algae), free essential amino acid (∑ EAA, in mg amino acid 100 g-1 dry mass algae) and free non-essential amino acid (∑ NEAA, in mg amino acid 100 g-1 dry mass algae) of *Fucus vesiculosus* fresh, dried and frozen.

3. Results

The data presented in Figure 1 and Table 2 showed that the total value of free amino acids, both essential and non-essential, during post-harvesting preservation and storage increased compared to fresh seaweeds. The most significant differences make more than 2 times (F (4, 10) = 576.70, p = 0.0000) for fresh and dried samples. An important indicator of the amino acid profile is the ratio of essential to non-essential amino acids. The results of the study showed that during the storage of both dried and frozen seaweed tallomes this indicator increased.

The content of Asp and Thr in dried seaweed increased significantly after 6 months of storage by 5.3 times and 3.1 respectively. After 9 months of storage, the content of Asp and Thr decreased, and after 12 months it was higher than that of fresh seaweed by 6.3 times for Asp, and by 3.1 times for Thr. The content of Glu after 6 months of storage was 1.2 times higher than that of fresh seaweed, this tendency was preserved after 9 and 12 months of storage.

Ala, Pro, His, Val and Arg showed an increase in content in dried seaweed after 3 months of storage by 1.6-3.0 times, but then a slight decrease in content after 9 and 12 months of storage for Ala, Pro, Val and His. Arg level during storage decreased gradually and after 12 months of storage it decreased to values comparable to those for fresh seaweed.

The level of Phe, Tyr, Met, Lys, Leu and Ile increased 1.5-4.9 times after 3 months of dry seaweed storage, and was high after 6, 9 and 12 months of storage. However, after 12 months of storage, the Lys level 5.2 times increased the level of fresh seaweed.

For Cys (Cysteine + Cystine) and Hyp, an increase of dried seaweed content after 3 and 6 months of storage was revealed, and then the Hyp content did not change until the end of the storage period, and the Cys level decreased to the initial level of fresh seaweed.

The content of Ser and Trp after 3 months of storage decreased by 1.5-1.8 times compared to fresh seaweed. Later the Ser content remained substantially unchanged and the Trp content decreased. As a result, by the end of the storage period, the content of these amino acids was 1.6-2.2 times lower than that of fresh seaweed.

The Gly level did not change over 6 months of storage, followed by an increase of 1.3 times compared to fresh seaweed, which did not change after 12 months of storage.

The total content of free amino acids of seaweeds stored under freezer conditions was 1.4-2.0 times higher than fresh seaweed, and the level of the sum of essential acids further increased by 2.1-2.8 times (F (4, 10) = 161.22, p = 0.0000).

The trends of the majority of free amino acids in frozen seaweed samples were similar: in 3 months the level of amino acid increased 1.3-3.0 times compared to fresh seaweed, in 6 months of storage the content remained the same or decreased to the level in fresh seaweed, in 6 months the level of amino acids increased again, and in 12 months the content of free amino acids was comparable to that of fresh seaweed at the beginning of the experiment.

The exception was Glu, Trp, Hyp: the level of these amino acids in frozen samples did not change significantly after 3 and 6 months of storage. After 9 months of storage, the Glu and Hyp levels were the highest for the entire storage period, and for Trp the maximum value was revealed after 12 months of storage.

The level of Pro content and the dynamics of its change during storage was different from all other studied amino acids: during the whole period of storage its content decreased and in 12 months of storage it decreased by 1.5 times compared to fresh samples.

TABLE 1: Amino acid composition (in mg amino acid 100 g^{-1} dry mass algae), free essential amino acid (∑ EAA, in mg amino acid 100 g-1 dry mass algae) and free non-essential amino acid (∑ NEAA, in mg amino acid 100 g-1 dry mass algae) of *Fucus vesiculosus* fresh, dried and frozen.

	Fresh seaweed	3 month		6 month		9 month		12 month	
		Dried seaweed	frozen seaweed	Dried seaweed	frozen seaweed	Dried seaweed	frozen seaweed	Dried seaweed	frozen seaweed
1	2	4	5	6	7	8	9	10	11
Essential amino acids (EAA)									
Leu	1.51 ± 0.09	3.08 ± 0.13	2.77 ± 0.33	3,35 ± 0.19	2.54 ± 0.16	3.21 ± 0.13	3.27 ± 0.13	2,80 ± 0.18	3.92 ± 0.15
Phe	28.25 ± 3.83	88.06 ± 5.90	83.43 ± 1.13	70.96 ± 2.78	86.50 ± 6.17	108.08 ± 17.32	93.46 ± 1.54	72.33 ± 4.05	75.20 ± 3.74

	Fresh seaweed	3 month		6 month		9 month		12 month	
		Dried seaweed	frozen seaweed	Dried seaweed	frozen seaweed	Dried seaweed	frozen seaweed	Dried seaweed	frozen seaweed
1	2	4	5	6	7	8	9	10	11
Lys	1.33 ± 0.03	5.39 ± 0.10	3.43 ± 0.09	4.95 ± 0.25	1.79 ± 0.29	5.42 ± 0.73	4.38 ± 0.42	6.98 ± 0.70	2.16 ± 0.18
Val	3.35 ± 0.20	6.42 ± 0.37	5.36 ± 0.30	6.53 ± 0.19	4.62 ± 0.14	5.95 ± 0.27	6.02 ± 0.23	6.35 ± 0.26	4.39 ± 0.16
Ile	1.48 ± 0.13	2.97 ± 0.15	2.37 ± 0.20	3.09 ± 0.13	2.32 ± 0.22	2.92 ± 0.05	2.57 ± 0.07	2.90 ± 0.23	2.18 ± 0.24
Thr	7.27 ± 0.44	15.07 ± 0.40	11.32 ± 0.35	22.70 ± 0.21	12.18± 0.28	19.27± 0.51	13.77 ± 0.44	22.78 ± 0.44	9.92 ± 0.54
Met	0.32 ± 0.07	1.60 ± 0.04	0.97 ± 0.09	1.19± 0.12	0.80± 0.07	1.46 ± 0.10	1.69 ± 0.14	1.55 ± 0.17	1.33 ± 0.16
His	3.88 ± 0.15	7.22 ± 0.29	7.66 ± 0.39	4.56 ± 0.16	5.84 ± 0.28	3.63 ± 0.28	6.34 ± 0.46	6.70± 0.35	3.63 ± 0.38
Trp	2.58 ± 0.10	1.41± 0.15	2.88 ± 0.12	1.84 ± 0.19	3.40± 0.23	1.94 ± 0.17	1.19 ± 0.12	1.98 ± 0.10	6.11 ± 0.37
∑EAA	49.65 ± 3.78	138.50 ± 6.74	124.17 ± 5.62	125.36 ± 4.96	119.64 ± 4.09	160.79 ± 8.96	139.58 ± 7.64	130.45 ± 5.37	106.70 ± 3.14
Non-essential amino acids (NEAA)									
Asp	24.08 ± 2.25	34,99 ± 1.70	40,54 ± 2.82	126,60 ± 6.43	41,15 ± 3.12	117,06 ± 3.81	60,84 ± 1.44	151,79 ± 14.95	33,68 ± 1.26
Glu	132.36 ± 5.71	139,49 ± 7.56	140,50 ± 2.21	163,11 ± 3.04	175,67 ± 16.10	160,50 ± 8.55	252,69 ± 10.67	169,50 ± 3.10	242,00 ± 11.24
Hyp	0.16 ± 0.03	0,37 ± 0.03	0,18 ± 0.03	0,52 ± 0.08	0,24 ± 0.02	0,52 ± 0.10	0,58 ± 0.09	0,48 ± 0.06	0,43 ± 0.06
Ser	8.09 ± 0.11	5,43 ± 0.12	8,36 ± 0.41	5,67 ± 0.48	13,00 ± 0.59	5,12 ± 0.22	9,31 ± 0.74	6,21 ± 0.41	10,51 ± 0.19
Gly	3.05 ± 0.58	4,03 ± 0.08	5,28 ± 0.34	3,69 ± 0.25	6,61 ± 0.52	4,58 ± 0.24	5,92 ± 0.31	5,05 ± 0.21	6,41 ± 0.41
Ala	41.70 ± 4.56	123.55± 4.86	52.44 ± 2.69	120.24± 6.43	37.00 ± 0.38	94.46 ± 3.69	64.71± 1.99	99.25 ± 5.40	44.31 ± 2.07
Pro	13.95 ± 0.31	25.58± 1.79	13.07 ± 0.47	24.03 ± 1.70	10.72 ± 0.97	20.10 ± 1.55	8.98 ± 0.14	22.18± 1.52	9.42 ± 0.64
Tyr	2.26 ± 0.25	8.69± 0.45	6.86 ± 0.19	8.03 ± 0.24	3.05 ± 0.18	10.85 ± 0.34	8.08 ± 0.21	8.06 ± 0.38	3.94 ± 0.14
Cys[1]	6.43 ± 0.93	11.35 ± 1.06	8.53± 0.80	13.55 ± 1.28	6.06 ± 0.68	13.84 ± 2.85	17.76 ± 2.79	6.95 ± 0.50	10.51 ± 1.07
Arg	3.29 ± 0.59	7.39 ± 0.54	3.63 ± 0.40	6.05 ± 0.82	3.45 ± 0.51	4.12 ± 0.49	2.38 ± 0.17	2.42 ± 0.13	2.14 ± 0.19
∑AA	285.80 ± 22.51	492.08 ± 34.10	399.57 ± 7.64	590.64 ± 14.49	413.97 ± 7.03	580.29 ± 25.37	561.79 ± 5.08	597.38 ± 12.74	473.84 ± 10.70
EAA/AA	0.17	0.28	0.31	0.21	0.28	0.27	0.25	0.22	0.23

[1] Cysteine+ Cystine

4. Discussion

Freezing is one of the most popular ways of long-term product storage. By converting most of the liquid water in foods into ice, freezing significantly slows down physical and biochemical changes associated with food degradation, as well as the growth and reproduction of biodegradation. Generally, freezing preserves taste, texture and nutritional value of food better than any other preservation technology. For this reason, many studies were aimed at improving the quality of frozen fruits, vegetables, meat and fish products [15]. Although there have been many biological studies on cryopreservation of seaweed [16], to our knowledge there have been no studies on the chemical composition of frozen seaweed. In the Russian Federation, only sea cabbage -- Laminaria seaweed -- is used for food purposes. In accordance with the current regulatory documentation [17], the recommended shelf life of ice-frozen sea cabbage from the date of its manufacture is 12 months at a storage temperature of not more than -18°C. At the same time the quality indicators of this type of food products are only organoleptic properties, and physical and chemical properties normalize only the amount of mineral impurities.

Pharmacopoeia of different countries, including Russia, normalize the content of polysaccharides and iodine in dried tallomes of brown seaweed setting the shelf life of 3 years [18, 19]. However, no document normalizes amino acid content for both dried and frozen seaweed tallomes.

The studied *F. vesiculosus* tallomes are listed in the European Food Safety Authority (EFSA). Previous studies demonstrated the presence of food-important molecules in seaweed, including proteins and amino acids [1]. However, many of these potentially useful chemical components may be lost in post-harvest treatment of seaweed, which reduces the effective value of some compounds [20]. Therefore, the drying process used during seaweed post-harvest treatment was covered in several works. For example, Chan et al. [21] considered freeze drying, oven drying and sun drying, as well as effects on total amino acids, fatty acids, minerals and vitamin C in *Sargassum hemiphyllum* (brown seaweed). Sun drying and freeze drying were found to reduce the loss of some important chemical components compared to oven drying. In another study [22] on *Saccharina latissima*, the effects of lyophilic and convection drying in air at 25, 40 and 70°C were studied. The results of the study showed that, apart from iodine, other chemical components did not change under the influence of drying. In a recent study Silva et al. [23] compared the chemical composition of *Ulva rigida*, *Gracilaria sp*. and *F.vesiculosus* seaweed tallomes dried in an oven (25, 40 and 60°C) compared to fresh seaweed, and showed that the extraction of polyphenols and their activity are significantly influenced by the drying method. In literature we failed to find information on the long-term storage of dried or frozen seaweed and the impact of this process on the composition of free amino acids. Besides, the proportion of essential amino acids increases, which improves the useful properties of seaweed if eaten. A substantial increase in Glu levels, with which the specific taste of seaweed is generally associated, will also improve the taste of seaweed thus making them more commercially attractive. Most likely redox and enzymatic processes associated with protein degradation contributed to the increase of free amino acids. The only amino acid whose content decreased when stored for 12 months in frozen fucus tallomes is Pro compared to fresh samples.

5. Conclusion

In conclusion, it may be said that long-term storage of dried and frozen thalli algae *F. vesiculosus* led to a significant increase in the content of the majority of free amino acids and may be used to improve the quality of fresh algae.

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Conflict of Interest

The authors have no conflict of interest to declare.

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