

THE INFLUENCE OF PRE-TREATMENT, PRESERVATION METHOD, AND STORAGE TIME ON CAROTENOID AND CHLOROPHYLL PROFILE IN NETTLE JUICES AND ITS RELATION TO COLOUR CHANGES

– Research paper –

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Abstract: One of the most valuable health-promoting plants used in the production of functional, green drinks is nettle. Acidic environments, oxygen, high temperature, and enzymes can negatively affect the colour of green juices, which is an important aspect for consumers. Therefore, the study analyzed the effect of pre-treatment, preservation method (freeze-drying, freezing, sterilization), and storage on the profile of carotenoids and chlorophylls (HPLC) in nettles juices and its correlation with colour changes (CIELAB). All the examined parameters impacted the profile of both groups of compounds and their colour. The most significant unfavorable changes were observed after sterilization. Blanching positively affected the level of coloured compounds in canned juices, with a greater enhancement being observed after a longer pre-treatment time (90 s). After storage, in frozen and freeze-dried juices, and similarly in unpreserved juices, lutein, violaxanthin and chlorophyll a predominated. However, in sterilized juices, neoxanthin and violaxanthin were completely degraded, and β -carotene and chlorophyll b were found at a significant level. During the storage of canned and frozen products, chlorophyll a was degraded to a greater extent, while in lyophilizates chlorophyll b. The transformation of neoxanthin, violaxanthin and chlorophyll a in nettle juices was determined via analysis of a^* colour parameter.

Keywords: *Urtica dioica*, blanching, freezing, freeze-drying, sterilization, colour

INTRODUCTION

The stinging nettle (*Urtica dioica*) is one of the most popular green herbal plants in the world. It is used as food, but it is also used in cosmetics and pharmacy. Additionally, chlorophyll, a natural dye, is obtained from nettle leaves on an industrial scale. Nettles have diuretic, anti-diabetic, anti-asthenia, anti-anemic, antispasmodic, antirheumatic, anticarcinogenic, antioxidant, etc. properties. Furthermore, they are a source of numerous bioactive compounds, e.g., chlorophylls, carotenoids, phenolic compounds (e.g., rutin), lectins, B vitamins, vitamins K, C, minerals (Fe, K, Ca, Mg), including trace elements (Upton, 2013; Said et al., 2015; (Paulauskiene et al., 2021). β - and

α -carotene, occurring in nettle are precursors of vitamin A. This fat-soluble vitamin is necessary to ensure proper growth of body and for the seeing process. The consumption of carotenoids is related to risk reduction of atherosclerosis and other cardiovascular diseases, protection against macular degeneration, and prevention of certain types of carcinomas, such as skin and prostate (Melendez-Martinez et al., 2022).

In Europe, nettle is most often used for nutritional purposes in the form of fresh leaves or juice, as well as for pharmaceutical purposes in the form of extracts or dried leaves. Furthermore, freeze-dried products from this plant as capsules or tablets are quite popular in North America (Upton, 2013). According to dietary recommendations, the daily consumption of dried nettle leaves for medicinal

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purposes should be 1.2–18.0 g and 15–45 mL of juice (Said et al., 2015).

Reports have shown that food processing methods affect the quality of a product, including bioactive ingredients and colour content. In the case of nettle, the colour is mainly due to chlorophylls and carotenoids, with the former being one of the most unstable plant dyes. In fresh nettle, chlorophyll a with methyl group at C-3 carbon predominates, while chlorophyll b (formyl group at C-3 carbon) is found in less quantity. Chlorophylls a and b occur in an approximate ratio of 3:1 in nettles. Also, differences can be observed in the colour of both compounds. The colour of chlorophyll a is blue-green, while the chlorophyll b colour is yellow-green (Steet and Tong, 1996). Chlorophylls also differ in stability, and their degradation can occur due to chemical or biochemical (enzymatic) reactions. Degradation of these compounds occurs with the participation of oxygen, weak acids, light, and high temperature. Chemical degradation is associated with different reactions, for example pheophytinization, hydroxylation or oxidation (Schwartz and Von Elbe, 1983; Mangos and Berger, 1997). The main reason for the green colour changes during processing of plants is the conversion of chlorophylls to pheophytins due to acidification of the environment. In these conditions, magnesium placed in the center of the tetrapyrrole ring of chlorophyll is replaced by two hydrogen ions. In consequence the colour changes from green to brown are observed. Chlorophyll a has been proven to be more resistant to temperature changes than chlorophyll b (Erge et al., 2008). Carotenoids are a group of tetraterpene pigments and have different colours, from yellow through orange to purple. The most popular carotenoid in green plants, especially in nettle is β -carotene. In addition,

e.g. violaxanthin, xanthophylls, and lutein can be found (Upton, 2013). Carotenoids are more stable than chlorophylls but are sensitive to sunlight and oxygen (Yahia et al., 2010).

The reason for the degradation of the colour compounds contained in the plant tissue, e.g., chlorophylls and phenolic compounds, is due to their transformations with the participation of enzymes (chlorophyllase, oxidases), oxygen and changes in pH. As a consequence of these changes, the tissue turns brown, which negatively affects the product's appearance. In order to prevent unfavorable changes in colour and to increase the retention of dyes in a juice during pressing, the raw material is subjected to thermal treatment, mainly blanching in water, steam, and microwaves. Heat treatment loosens and vents the tissue and inactivates the enzymes, which in turn can enhance the preservation of dyes and increase the pressing efficiency. However, this may cause their transfer to water in which the treatment is carried out (Xiao et al., 2017). Colour is one of the most important sensory determinants and it is very important to know the changes in the levels of chlorophyll and carotenoid compounds during processing and storage to minimize their degradation. To date, scientific research in this area mainly focuses on the analysis of changes occurring during the storage and processing of vegetables and fruits. There are no analogous data for stinging nettle and its processing into juices. Therefore, the presented study aimed to evaluate the effect of different blanching times, preservation methods (freezing, lyophilization, sterilization), and 6 months of storage on the profile of chlorophylls and carotenoids in nettle juices and their correlation with colour changes measured by instrumental analysis.

MATERIALS AND METHODS

The research material used for this study was nettle juice (*Urtica dioica* L.) immediately after pressing, after preservation, and after 3 and 6 months of storage. The juices were obtained by pressing using a Sana screw juicer type EUJ-707C (Omega Sana, Poland). Fresh wild nettle herb (leaves with stems) was used for processing, 60–100 cm high, before flowering. The plants were harvested at the end of May in the suburbs of the city of Krakow, near the Młynówka River (Poland).

Pre-treatment

First, the raw material was sorted, damaged and discoloured plants were discarded. The plants were

washed under running cold tap water. After removing excess water, the nettles were cut into 10–15 cm long pieces with a sharp knife. Then, the raw material was divided into 3 parts. The first was squeezed on a screw juicer, and the other two were subjected to blanching in water for 15 s or 90 s, respectively. Blanching was done in a stainless dish, at a temperature of $98 \pm 2^\circ\text{C}$, maintaining a ratio of nettle to water of 1:5. After blanching, the nettles were put on trays (layer thickness of approx. 2–3 cm) and left for approx. 30 min at $18\text{--}20^\circ\text{C}$ to reduce the temperature to $<25^\circ\text{C}$. Then the nettles were squeezed using a screw juicer. As a result, 3 variants of juice were obtained: unblanched

(product code NB), blanched for 15 s (product code B15s), and blanched for 90 s (product code B90s).

Preservation

After squeezing, all juices were subjected to preservation by sterilization, freezing, and freeze-drying.

Sterilization. Immediately after squeezing, the juices were heated to $60\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, then poured into twist-off glass jars with a capacity of 200 mL, sealed and subjected to sterilization, where the temperature was raised to $118\text{ }^{\circ}\text{C}$ for 9 min, proper sterilization $118\text{--}121\text{ }^{\circ}\text{C}$ for 15 min, and cooling to room temperature for 14 min. After sterilization, the products were kept for 6 months in a dark room at a temperature of $18\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

Freezing. Immediately after squeezing, the juices were poured into plastic bags intended to produce ice cubes, closed, and frozen. Juices were frozen by the air-blowing method at $-40\text{ }^{\circ}\text{C}$ for approx. 90 min to reach $-25\text{ }^{\circ}\text{C}$ in the thermal center. After freezing, the juices were stored for 6 months ($-25\text{ }^{\circ}\text{C}$).

Freeze drying. Freeze drying was carried out using a freeze dryer (Gamma 1–16 LSC Christ, Germany). The juices were frozen ($-40\text{ }^{\circ}\text{C}$, 90 min) on dryer trays ($7\text{ kg}/1\text{ m}^2$) to temperature $-25\text{ }^{\circ}\text{C}$. Then frozen juices were freeze-dried for 48 h until a moisture content in samples was less than 3%. The condenser temperature was $-52\text{ }^{\circ}\text{C}$, while shelf temperature was $20\text{ }^{\circ}\text{C}$. Freeze-dried juices were packed under vacuum in OPA/PE foil bags (70 microns, FORPACK Krzysztof Buszek, Poland) using a vacuum packing machine type Boxer 42 (Henkelman, The Netherlands). The products were stored in the dark room at $18\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 6 months.

Methods

The content of dry matter, pH, chlorophylls and carotenoids in the juices was determined. In addition, the colour was determined using the instrumental method in the CIELAB system. Before the analyses, the frozen juices were thawed in a refrigerator at $4\text{--}5\text{ }^{\circ}\text{C}$ in the absence of light. Freeze-dried juices were rehydrated at room temperature for 60 min without light. Distilled water at a temperature of $18\text{--}20\text{ }^{\circ}\text{C}$ was added to the lyophilized juice, so that the content of dry matter in the juice after rehydration corresponds to the amount of dry matter in the juice before freeze drying.

Dry matter and pH

The dry matter content was determined by drying samples at $98\text{ }^{\circ}\text{C}$ to a constant weight (AOAC 1995). The pH level was determined by AOAC (1995) method.

HPLC analyses of chlorophylls and carotenoids

Extractions of chlorophylls and carotenoids were carried by HPLC method using a HPLC system (Merck-Hitachi, Darmstadt, Germany) equipped with a diode-array detector (Fiutak and Michalczyk 2020). The grade methanol in the presence of magnesium oxide was used as the extractant. The Li-Chrosphere 100 RP-18 column ($5\text{ }\mu\text{m}$; $250 \times 4\text{ mm}$; Merck, Darmstadt, Germany) was used, the elution temperature was $30\text{ }^{\circ}\text{C}$. The mobile phase consisted of methanol/acetonitrile (9:1 v/v), acetone and ammonium acetate (1 mol/l). The gradient elution program was as follows: a constant proportion of methanol/acetonitrile (80%) and changeable proportion of acetone and ammonium acetate: 0 min: 10% acetone and 10% ammonium acetate; 10 min: 16% acetone and 4% ammonium acetate; 25–50 min: 20% (acetone). Detection was conducted in the $350\text{--}750\text{ nm}$. Concentrations of neoxanthin, violaxanthin, lutein, chlorophyll a, chlorophyll b, β -carotene were calculated from the standard curves, prepared for all compounds.

Colour analysis

The colour of juices was determined by CIELAB method (reflection), using a MINOLTA CM-3500d (Konica Minolta, INC., Tokyo, Japan). The D65 illuminant, 30 mm diameter diaphragm and a 65 mm Petri dish were used. The total colour difference (ΔE) were calculated using formula (1) presented by Bernaś and Jaworska (2021):

$$\Delta E = \sqrt{(L^* - L_f^*)^2 + (a^* - a_f^*)^2 + (b^* - b_f^*)^2} \quad (1)$$

where L^* , a^* , b^* - were the colour parameters of not preserved juices, L_f^* , a_f^* , b_f^* were the colour parameters of canned, frozen and freeze-dried juices. Not preserved juices (blanched 15 s or 90 s) were compared with not blanched and not preserved juice, while canned, frozen and freeze-dried juices were compared with the corresponding not blanched, blanched for 15 s or for 90 s not preserved juices. The browning index was calculated using formulas (2) and (3) (Palou et al. 1999):

$$\text{BI} = [100(X - 0.31)]/0.17 \quad (2)$$

$$X = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*) \quad (3)$$

where L^* , a^* , b^* were the colour parameters of not preserved/preserved juices.

Statistical analysis

All analyses were carried out in triplicate. One-way analysis of variance (ANOVA) and Dunkay's test ($p < 0.05$) were used. For calculations the Statistica package (StatSoft, Inc., Tulsa, OK, USA) was used. The Pearson's correlation analysis between colour parameters (a , b), and carotenoid and chlorophyll contents was carried out.

RESULTS AND DISCUSSION

Dry matter and pH

According to Paulauskiene *et al.* (2021), fresh nettle leaves, depending on the harvest date, contain 20–24 g of dry matter, and according to Jan and Singh (2017), only 12 g/100 g fm. Nettle juice contains only 3.78 g of dry matter (Fedorovska and Polovko, 2017), which, compared to the results obtained by the authors of the study, was 0.37 g lower (Figure 1). The analyzed juice immediately after squeezing was characterized with a neutral pH, which was comparable to that reported by Fedorovska and Polovko (2017). Manzoor *et al.* (2020) determined that spinach juice had a lower pH of 6.2. The

blanching method affects the possible goal to be achieved. Traditional blanching causes inactivation of enzymes and microorganisms, and in consequence improve the shelf life of juices. However, this technique can destroy nutrients, colour, and sensory characteristics (Jabbar *et al.*, 2014). The use of blanching, regardless of the time, significantly reduced the amount of dry matter in the juice by 19–25% (Figure 1), while increasing the pH level by 0.55–0.59 (Figure 2), with greater changes observed when the time of thermal treatment was extended.

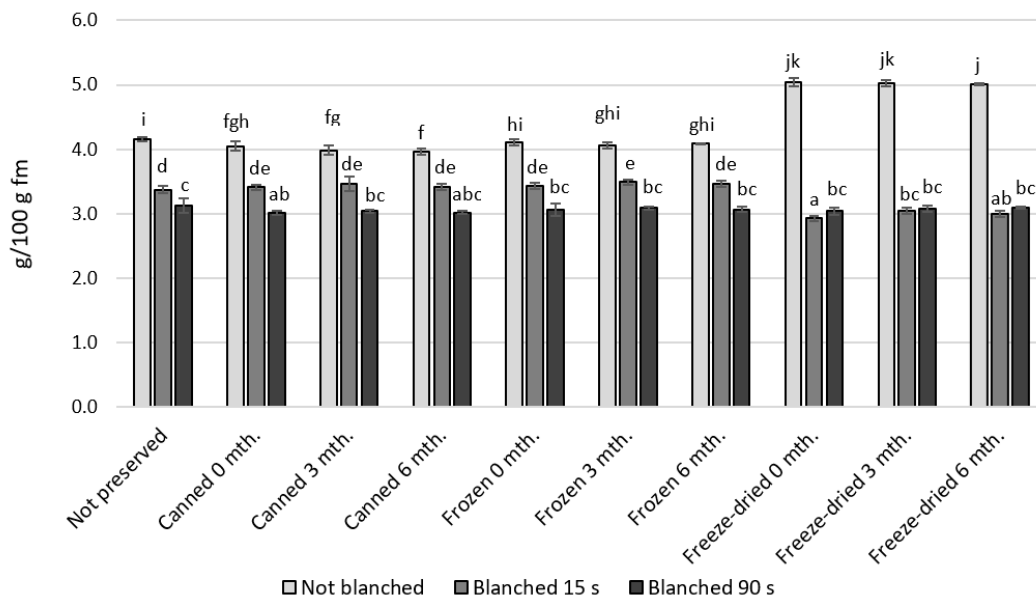


Figure 1. Dry matter content in nettle juices (g/100 g fm).

Legend: 0m, 3m, 6m – months of storage; means with different letters represent significant differences at $P < 0.05$

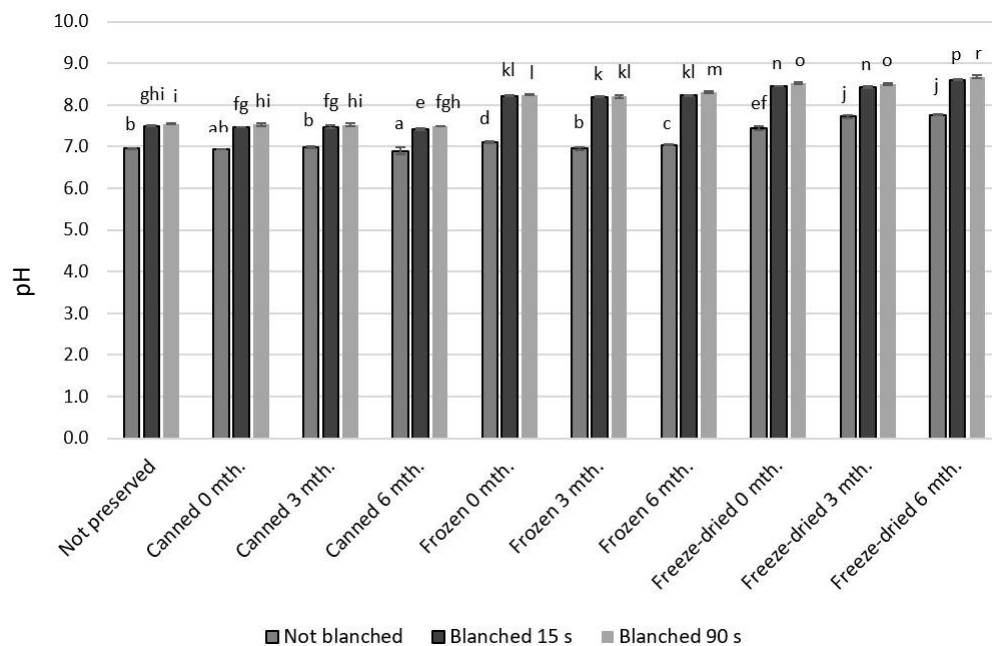


Figure 2. pH level in nettle juices. Legend: see Fig. 1

A decrease in the amount of dry matter by 0.69 g in the juice obtained from blanched raw material was also reported by Ratajski and Trajer (2017) by examining carrot juice. This phenomenon was probably due to the transfer of some soluble ingredients into the blanching water.

As a result of the preservation of juices, slight changes in the amount of dry matter were found, ranging from -3 to +21% in relation to unpreserved juices. This was not the case for the freeze-dried ones. The observed differences were probably due to the reconstitution of freeze-dried juice. The results were based on the dry matter content and subject to error, hence, to minimize this error, the level of chlorophylls and carotenoids was expressed in 100 g of dry matter. In the case of pH, the preservation caused a significant increase in the pH of frozen and freeze-dried products by 2-13%. During the 6 months' storage period, the amount of dry matter in the juices did not change. Only slight fluctuations in pH were observed, which did not exceed 4% (Figures 1, 2). Fedorovska and Polovko (2017) reported a slight pH increase from 6.62 (fresh juice) to 6.92 (juice after 27 months of storage) during the storage of nettle juices.

Carotenoids

Carotenoids, apart from chlorophylls and flavonoids, are one of the main compounds responsible for the colour of stinging nettles. β -Carotene constitutes approx. 60% of carotenoids. In addition, violaxanthin (about 15%), luteoxanthin (about 10%), lutein (13%), and α -carotene (about 1%) are present (Upton, 2013; Devkota et al., 2022). Lutein and violaxanthin were detected in the unpreserved fresh nettle juice, which constituted 42% and 33% of total carotenoids, respectively. A smaller share had α -carotene, β -carotene and neoxanthin were present in 3%, 15%, and 7%, respectively (Tables 1 and 2).

Long time of storage, temperature, light and oxygen presence, water activity, can cause to the loss of carotenoids during processing and storage, and these losses are elevated at higher temperatures. One of the least stable carotenoids is lycopene. Lutein is quite stable, and more stable than β -carotene and α -carotene under the same time and storage conditions (Melendez-Martinez et al., 2022). Due to blanching, a 60-78% reduction in the level of most of the analyzed compounds was observed compared to the juices of unblanched plants (code NB). However, in our case, α -carotene was the exception, which increased 4.0-4.5 times (Table 1, 2). Independent of specific characteristic, slightly greater changes were noted when using a shorter (15 s) than longer (90 s) blanching time. The

ratio of individual carotenoids to each other also changed, but this did not depend on the blanching time. Lutein (37%) and α -carotene (27%) were the most abundant, while violaxanthin, which predominated in the unblanched juice (code NB), accounted for only 22-23% in blanched juices. The detected changes in the level of carotenoids were probably due to exposure to high temperatures over a short time, causing isomerization and transformation, for example, into volatile and non-volatile compounds. In addition, the presence of oxygen and some metals (catalysts) in the tissue or environment could contribute to the auto-oxidation of certain compounds, e.g., β -carotene. Iron is an example of an element involved in these reactions, which is present in large quantities in nettles (Boon et al., 2010; Repajic et al., 2021).

Due to the seasonality of occurrence and short shelf life, juices obtained from herbaceous plants require preservation. One of the most commonly used methods is pasteurization, but for juices with a pH > 4.5, sterilization is required. An alternative approach is freezing or drying. However, air drying uses high temperature and oxygenation, causing large losses of labile compounds, e.g., dyes (Melendez-Martinez et al., 2022). As a result of the preservation of nettle juices, significant changes in the level of carotenoids and their mutual proportions were observed (Table 1, 2). The total amount of carotenoids decreased by 28-95%, with the smallest changes being related to unpreserved juices of sublimation droughts. In most products, the degradation of individual carotenoids occurred, except for juices obtained from the raw material blanched for 15 s (code B15s), in which an increase in the level of β -carotene by 32-69%, violaxanthin by 31-49%, and neoxanthin by 31-66%, and lutein by 30-40% was determined. The presence of α -carotene was not found in any of the products. The share of individual carotenoids depended on the preservation method. Lutein predominated in sterilized and freeze-drying (72-85% and 45-48% of total carotenoids, respectively), and violaxanthin (37-53%) in frozen juices. β -Carotene, depending on the preservation method, accounted for 12-21% of the total carotenoids.

According to Melendez-Martinez *et al.* (2022), thermal treatment negatively affects the level of carotenoids in vegetables. However, this depends on the type of treatment and carotenoid as well as the vegetable species. Certain carotenoids, e.g., lutein, are susceptible to molecule fragmentation, which in turn deteriorates the product's colour and

reduces its biological activity (Becerra et al., 2020). Steaming can be used instead of boiling in water to reduce carotenoid losses. In turn, in the case of tomatoes, the use of heat treatment, despite the

reduction in the amount of lycopene, can increase its bioavailability because, due to the isomerization reaction, it can change from the *trans* form to the more absorbable *cis* form (Unlu et al., 2007).

Table 1. Carotenoids in juices from *Urtica dioica*, mg/100 g dm

	Preservation method	Pre-treatment method	Period of storage [months]		
			0	3	6
β-carotene	Not preserved	NB	55.6±2.8p	-	-
		BL15s	12.2±1.0cde	-	-
		BL90s	16.1±0.6fg	-	-
	Canned	NB	2.8±0.2a	9.4±8.8bc	nd
		BL15s	16.2±2.2fg	20.7±1.2i	12.1±0.8cde
		BL90s	17.4±0.9gh	25.8±5.8j	19.4±1.5hi
	Frozen	NB	36.2±1.5l	45.2±0.9m	52.1±4.1no
		BL15s	9.4±0.5bc	50.4±3.4n	52.5±3.0no
		BL90s	12.1±1.2cde	29.2±1.1k	65.7±2.0r
	Freeze-dried	NB	38.0±1.7l	43.7±1.7m	53.4±0.9op
		BL15s	20.7±1.7i	11.7±1.4cde	8.5±0.5b
		BL90s	13.4±1.0def	13.9±1.2ef	10.6±0.4bcd
Violaxanthin	Not preserved	NB	125.9±2.4o	-	-
		BL15s	31.0±0.5cd	-	-
		BL90s	38.9±2.5ef	-	-
	Canned	NB	nd	nd	nd
		BL15s	nd	nd	nd
		BL90s	nd	nd	nd
	Frozen	NB	63.7±2.7i	72.7±3.4j	91.6±5.8m
		BL15s	40.6±1.0f	83.7±2.0l	89.9±8.7m
		BL90s	30.3±1.5c	45.6±3.1g	102.5±3.7n
	Freeze-dried	NB	78.4±2.6k	52.8±1.9h	55.3±2.5h
		BL15s	46.2±1.6g	19.8±0.7b	14.6±1.1a
		BL90s	34.9±1.5de	28.5±2.1c	20.7±1.5b
Neoxanthin	Not preserved	NB	26.3±1.5h	-	-
		BL15s	7.4±0.8ab	-	-
		BL90s	8.8±0.5bcd	-	-
	Canned	NB	nd	nd	nd
		BL15s	12.2±0.8e	nd	nd
		BL90s	6.5±0.5a	nd	nd
	Frozen	NB	18.3±0.6g	15.8±0.9f	30.8±1.9i
		BL15s	10.4±1.2d	18.3±1.3g	36.4±3.1j
		BL90s	8.3±0.2abc	10.2±0.9cd	39.4±0.7k
	Freeze-dried	NB	18.2±1.4g	15.6±0.6f	29.6±1.3i
		BL15s	9.6±0.9cd	6.5±0.5a	14.7±2.2f
		BL90s	7.5±0.2ab	8.7±0.5bcd	19.0±1.3g

Legend: NB – not blanched; BL - blanched; “-“ not applicable; nd – not detected; significant differences see Fig. 1

Table 2. α -carotene and lutein in juices from *Urtica dioica*, mg/100 g dm

	Preservation method	Pre-treatment method	Period of storage [months]		
			0	3	6
α -carotene	Not preserved	NB	10.0 $\pm$ 0.5a	-	-
		BL15s	37.6 $\pm$ 2.4b	-	-
		BL90s	46.8 $\pm$ 0.4c	-	-
	Canned	NB	nd	nd	nd
		BL15s	nd	nd	nd
		BL90s	nd	nd	nd
	Frozen	NB	nd	nd	nd
		BL15s	nd	nd	nd
		BL90s	nd	nd	nd
	Freeze-dried	NB	nd	nd	nd
		BL15s	nd	nd	nd
		BL90s	nd	nd	nd
Lutein	Not preserved	NB	159.2 $\pm$ 4.3p	-	-
		BL15s	51.1 $\pm$ 2.5cd	-	-
		BL90s	63.4 $\pm$ 3.7ef	-	-
	Canned	NB	15.7 $\pm$ 1.4ab	57.5 $\pm$ 3.5de	16.5 $\pm$ 1.6ab
		BL15s	71.6 $\pm$ 3.9gh	80.6 $\pm$ 0.5i	22.1 $\pm$ 2.0b
		BL90s	75.6 $\pm$ 0.3hi	79.0 $\pm$ 4.2i	20.3 $\pm$ 1.0b
	Frozen	NB	55.0 $\pm$ 1.4d	103.0 $\pm$ 4.6j	124.7 $\pm$ 2.7mn
		BL15s	16.3 $\pm$ 2.5ab	111.5 $\pm$ 7.0k	126.4 $\pm$ 5.n
		BL90s	9.9 $\pm$ 0.5a	72.1 $\pm$ 4.5gh	142.7 $\pm$ 5.5o
	Freeze-dried	NB	111.7 $\pm$ 6.6k	116.3 $\pm$ 6.7jk	119.0 $\pm$ 6.0lm
		BL15s	66.3 $\pm$ 3.5fg	54.7 $\pm$ 5.0d	46.0 $\pm$ 3.1ec
		BL90s	50.9 $\pm$ 2.3cd	58.2 $\pm$ 4.7de	66.8 $\pm$ 4.9fg
	Sum of carotenoids	NB	376.7 $\pm$ 7.8t	-	-
		BL15s	139.6 $\pm$ 3.6j	-	-
		BL90s	172.2 $\pm$ 6.9l	-	-
	Canned	NB	18.5 $\pm$ 1.5a	66.9 $\pm$ 4.1cd	16.5 $\pm$ 1.6a
		BL15s	100.0 $\pm$ 5.6gh	101.3 $\pm$ 0.3gh	34.2 $\pm$ 2.5b
		BL90s	99.5 $\pm$ 1.2gh	104.8 $\pm$ 5.7ghi	39.7 $\pm$ 1.4b
	Frozen	NB	173.2 $\pm$ 3.8l	236.7 $\pm$ 9.6mn	299.2 $\pm$ 12.1r
		BL15s	76.7 $\pm$ 4.4e	263.8 $\pm$ 11.4p	305.3 $\pm$ 18.5r
		BL90s	60.7 $\pm$ 1.1c	157.1 $\pm$ 7.2k	350.3 $\pm$ 10.1s
	Freeze-dried	NB	246.2 $\pm$ 12.3no	228.4 $\pm$ 3.8m	257.3 $\pm$ 10.5op
		BL15s	142.7 $\pm$ 5.4j	92.7 $\pm$ 6.6fg	83.7 $\pm$ 4.3ef
		BL90s	106.7 $\pm$ 4.2hi	109.2 $\pm$ 4.3hi	117.0 $\pm$ 4.0i

Legend: see Fig. 1 and Tab. 1

During the first three months of storage of sterilized and frozen juices, an increase in the total amount of carotenoids by 1-261% was observed, while for freeze-dried juices, changes in the range from -35% to +2% were found. Lutein was observed in all juices (42-96% of total carotenoids), followed by β -carotene (14-25% of sterilized) or violaxanthin (21-32% of frozen and freeze-dried). During the next 3 months of storage, the level of total carotenoids in sterilized juices decreased significantly by 62-75%

compared to the previous analysis stage. However, for frozen food, an increase of 16-123% was observed, and for freeze-drying droughts, changes in the range from -10 to +13% occurred. After the end of the storage period, lutein was present in the juices (41-100% of total carotenoids), but its share in frozen and freeze-dried products slightly decreased compared to the earlier stage. In frozen juices, the level of β -carotene increased the most by 4-125%, violaxanthin by 8-125%, and neoxanthin

by 95-287%, while in freeze-dried neoxanthin by 90-127%.

The detected changes in the level of individual carotenoids may stem from their mutual transformations, especially β -carotene, which undergoes the hydroxylation reaction to zeaxanthin, followed by conversion to violaxanthin in the presence of zeaxanthin epoxidase, and finally to neoxanthin via neoxanthin synthase (xanthophyll cycle) (Simkin *et al.*, 2008). This phenomenon was observed in our analyzes, where, among the analyzed products, the highest amount of violaxanthin and neoxanthin were found in frozen juices, and then freeze-dried, in which both enzymes were probably active. In contrast, these carotenoids were not found in sterilized juices, in which the enzymes were inactivated. The described transformations of violaxanthin and neoxanthin are a mechanism of plant adaptation due to the lack of natural light, hence, our observations were in agreement since all studied juices were stored in the absence of light. Additionally, the amount of violaxanthin was higher than that of neoxanthin. Fedorovska and Polovko (2017) showed that after 27 months of storage, the nettle juices at a temperature of 2-4 °C in dark bottles, showed only 5% loss compared to fresh juice.

After 6 months of storage, the highest level of total carotenoids was found in frozen juices (299-350 mg/100 g dm) and the lowest in sterilized (17-40 mg) (Table 1, 2). The pre-treatment applied before squeezing significantly affected the carotenoids content, with the greatest differences observed between the juices freeze-drying. Blanching, positively influenced the number of carotenoids in sterilized juices, but negatively impacted their content in freeze-dried. However, in the case of frozen juices, the best effect occurred in blanching for 90 s, where 15 s did not affect the content of the compounds in the juice. Regardless of the preservation method, blanching for 90s ensured greater carotenoids stability in the juice than for 15s.

Chlorophylls

Chlorophylls play a vital role in acceptability of green food, especially vegetables juices. They are not stable and can decompose into brown compounds under acidic pH, light, processing, storage and enzyme activity. The degradation of chlorophylls is a consequence of chlorophyllase action in high temperature and acidic conditions. This enzyme changes chlorophylls into chlorophyllide, which can be transformed into pheophorbide or pheophytin (Takamiya and Tsuchiya, 2000; Gauthier-Jaques *et al.*, 2001). As a result of chemical reactions involving, among

others, pheophytin is formed upon replacement of Mg in the chlorophyll to H ions. In turn, decarbomethoxylation can occur and lead to the conversion of pheophytin to pyropheophytin (Diop Ndiaye *et al.*, 2011). Various blanching solutions have been applied to stabilize chlorophyll in green plants during processing, such as alkaline water solutions or zinc and copper chloride solutions (Al-Dabbas *et al.*, 2017).

The chlorophyll content determined in unpreserved juice from fresh nettle herb was similar to that reported in the literature for nettles (Upton, 2013) and mint leaves (Straumite *et al.*, 2015). In nettle juice, chlorophyll is predominated (76% of total chlorophylls), similar to mint leaves (>80%) analyzed by Straumite *et al.* (2015) and spinach leaves (70%) studied by Kidmose *et al.* (2005). As a result of blanching, chlorophylls in the juices were significantly reduced by 69-74%, while the proportion of chlorophyll a to b remained unchanged (Table 3). This occurred due to the transference of part of the chlorophyll to the blanching water. Kidmose *et al.* (2005) showed that as a result of steam blanching of spinach leaves for 3-15 min, a significant reduction in the amount of chlorophyll a and b occurred, with the largest changes observed for the longest processing time, amounting to 25-34%. At the same time, the authors noted an increase in the level of pheophytin a and b, while pheophytinization was observed only after blanching for 15 min (code B15s).

Preserving nettle juices usually caused significant chlorophyll degradation by 2-97%, with the largest changes observed after sterilization (60-97%) and the smallest after freezing (2-37%). Except for frozen juices, chlorophyll b underwent high levels of degradation compared to chlorophyll a (Table 3). Chlorophyll a was still dominant in most juices (69-86% of total chlorophylls). The only exception was unblanched nettles juice (code NB), subjected to sterilization, where chlorophyll b was dominant (76%). During 6 months of storage, chlorophylls in the juices were further degraded, with the most stable juices being those subjected to freezing (a decrease of 7-9%) and the least stable being the canned ones (a decrease of 87-100%).

According to Kidmose *et al.* (2005), air drying of spinach (75 °C, 3 h) followed by 6 months of storage resulted in higher reduction of chlorophylls than steam blanching followed by 6 months of frozen storage. Fedorovska and Polovko (2017) showed that chlorophyll degraded by 10% when nettle juices were stored for 27 months compared to fresh juice. Ozola *et al.* (2019) showed that freezing leaves of edible wild plants caused an increase in the amount of chlorophyll, unlike drying.

Table 3. Chlorophylls in juices from *Urtica dioica*, mg/100 g dm

	Preservation method	Pre-treatment method	Period of storage [months]		
			0	3	6
Chlorophyll a	Not preserved	NB	1224.1±55.3o		
		BL15s	318.8±11.4g		
		BL90s	383.8±7.9h		
	Canned	NB	9.9±0.4a	5.3±0.6a	nd
		BL15s	102.4±6.1b	23.1±0.6a	nd
		BL90s	138.4±6.7c	22.6±0.5a	nd
	Frozen	NB	380.9±23.0h	735.0±41.3j	770.1±29.8k
		BL15s	37.7±6.9a	312.6±3.7fg	446.1±30.0i
		BL90s	32.2±1.1a	320.3±16.2g	390.6±12.1h
	Freeze-dried	NB	766.8±37.6jk	785.8±33.0kl	808.8±33.8l
		BL15s	193.2±3.6d	184.9±4.1d	147.3±4.4c
		BL90s	159.9±7.2cd	280.5±22.6ef	265.8±14.0e
Chlorophyll b	Not preserved	NB	397.2±16.6o		
		BL15s	98.9±5.1j		
		BL90s	118.1±2.4k		
	Canned	NB	7.2±0.4ab	32.3±2.2ef	nd
		BL15s	46.6±3.4g	33.7±0.3ef	19.2±1.8bcd
		BL90s	60.0±1.86h	38.0±3.6fg	24.3±2.2de
	Frozen	NB	136.1±6.6l	240.4±15.3m	231.0±13.5m
		BL15s	10.9±0.6abc	65.5±3.2hi	93.4±6.1j
		BL90s	6.4±0.8a	73.3±5.0	75.5±4.1i
	Freeze-dried	NB	263.2±11.5n	262.5±9.0n	334.1±17.5o
		BL15s	39.0±0.7fg	33.1±1.7ef	22.2±0.9cde
		BL90s	37.2±3.2fg	60.1±3.6h	58.9±6.4h
Sum of chlorophylls	Not preserved	NB	1621.3±72.0o		
		BL15s	417.6±16.4h		
		BL90s	501.9±7.8ij		
	Canned	NB	17.1±0.8ab	37.6±1.8ab	nd
		BL15s	149.0±8.0c	56.8±0.4b	19.2±1.8ab
		BL90s	198.4±5.1de	60.6±3.7b	24.3±2.2ab
	Frozen	NB	517.0±27.6j	975.4±56.5k	1001.1±40.2kl
		BL15s	48.5±7.4ab	378.1±4.3gh	539.4±35.1j
		BL90s	38.6±1.8ab	393.5±21.1h	466.1±16.2i
	Freeze-dried	NB	1029.9±49.0lm	1048.3±40.0m	1143.0±49.9n
		BL15s	232.0±4.1e	218.0±3.2e	169.5±3.6cd
		BL90s	197.1±10.2de	340.5±25.9fg	324.7±17.2f

Legend: NB – not blanched; BL - blanched; “-” not applicable; nd – not detected; significant differences - see Fig. 1

According to reports, low temperature enhances the preservation of chlorophylls, and short freezing time promotes the decomposition of plant cells, possibly extracting more pigments from plants. It should be noted, that a spectrophotometric method was employed to determine chlorophylls, which, unlike the HPLC method, is less specific and accurate, which may cause measurement errors. When sterilized and frozen nettle juices were stored,

chlorophyll a decomposed to a greater extent, while chlorophyll b was degraded after freeze-drying. According to the literature, chlorophyll a degrades more easily (2.5 times) than chlorophyll b, especially at low pH (Koca et al., 2007). Pre-treatment before preservation affected chlorophylls' stability during the storage of the nettle juices, and depended on the preservation method. In the case of sterilized juices, blanching reduced the loss of

chlorophyll b, while maintaining the stability of chlorophyll a. However, for frozen and freeze-drying juices, a positive effect was observed for blanching for 90 s (code B90s), which stabilized chlorophyll b and, to a small extent, chlorophyll a.

Colour

Colour is one of the most essential parameters for evaluating changes in the level of individual dyes. Depending on the transformations that occur in the

product, its colour may emit different shades. In the case of nettle leaves, the most important index is parameter a (green-red colour). The green colour characteristic of fresh nettles is actually a mixture of green (chlorophylls) and yellow (carotenoids). In the unpreserved juice from the unblanched nettle herb (code NB), the colour plane shifted towards green ($a < 0$) and yellow ($b > 0$), while the brightness (L) was quite low (Table 4).

Table 4. Colour parameters in juices from *Urtica dioica*

	Preservation method	Pre-treatment method	Period of storage [months]		
			0	3	6
L*	Not preserved	NB	12.10±0.33h	-	-
		BL15s	10.58±0.10f	-	-
		BL90s	9.73±0.13e	-	-
	Canned	NB	18.00±0.22k	17.83±0.11k	19.10±0.23l
		BL15s	12.13±0.03h	11.93±0.02h	12.78±0.11j
		BL90s	11.96±0.12h	11.44±0.01g	12.44±0.08i
	Frozen	NB	8.00±0.04bc	7.40±0.21a	7.88±0.47b
		BL15s	12.91±0.43j	7.50±0.04a	7.47±0.06a
		BL90s	11.37±0.68g	7.23±0.06a	8.11±0.35bc
	Freeze-dried	NB	9.70±0.25e	9.01±0.09d	9.09±0.10d
		BL15s	7.47±0.07a	7.29±0.07a	7.28±0.06a
		BL90s	8.29±0.02c	8.17±0.03bc	8.28±0.06c
a*	Not preserved	NB	-5.37±0.24a	-	-
		BL15s	-4.01±0.16e	-	-
		BL90s	-4.95±0.11b	-	-
	Canned	NB	0.87±0.03r	1.47±0.14s	1.77±0.22t
		BL15s	-2.02±0.11n	-0.55±0.04o	0.20±0.04p
		BL90s	-2.50±0.23m	-0.49±0.09o	0.32±0.20p
	Frozen	NB	-3.16±0.24jk	-3.06±0.09k	-3.22±0.09ijk
		BL15s	-2.74±0.07l	-3.63±0.05g	-3.71±0.09fg
		BL90s	-3.06±0.01k	-3.43±0.04h	-4.32±0.03cd
	Freeze-dried	NB	-4.80±0.07b	-3.77±0.03fg	-4.47±0.10c
		BL15s	-3.38±0.01hi	-3.06±0.03k	-3.27±0.08hij
		BL90s	-4.25±0.07d	-3.79±0.05fg	-3.89±0.10ef
b*	Not preserved	NB	12.22±0.54r	-	-
		BL15s	8.00±0.14l	-	-
		BL90s	7.46±0.22k	-	-
	Canned	NB	17.41±0.13s	17.78±0.07t	18.27±0.12u
		BL15s	9.85±0.05o	9.13±0.07n	9.86±0.13o
		BL90s	10.25±0.18p	8.65±0.01m	9.65±0.15o
	Frozen	NB	6.96±0.05j	6.47±0.26gh	6.82±0.30ij
		BL15s	3.96±0.46a	5.41±0.04cd	5.66±0.04de
		BL90s	3.67±0.40a	4.67±0.06b	6.18±0.50fg
	Freeze-dried	NB	9.77±0.48o	8.25±0.25l	8.93±0.17mn
		BL15s	5.39±0.12cd	4.80±0.06b	5.19±0.09c
		BL90s	6.60±0.03hi	5.76±0.10e	5.92±0.17ef

Legend: NB – not blanched; BL - blanched; “-“ not applicable; significant differences - see Fig. 1

Similar observations were described by Straumite *et al.* (2015) in the analysis of fresh mint leaves. Instrumental colour measurements somewhat reflected those of chemical analysis because there were more chlorophylls in the juice than carotenoids, but chlorophyll a predominated, which is blue-green rather than yellow-green. Since nettle juice was a mixture of many coloured compounds, sometimes unknown, it was difficult to precisely determine the level of each colour. As a result of blanching nettles, a significant darkening of the juice 13-20% (L) and a reduction in the level of a parameter by 8-25% and b by 35-39% were noted. This increased the browning index (BI) by 49-56%. In the case of parameter a, greater changes were detected after 15 s of blanching (code B15s), and in the case of parameter b, after 90 s (code B90s). Preservation significantly affected the colour of the studied juices, which depended on the preservation method and the type of the analyzed parameter. Sterilization and freezing resulted in the brightening of the juice, but reduced the green colour saturation. In turn, freeze-drying caused the darkening of the juices and decreased the green and yellow colour saturation. Only in the case of sterilization was an increase in the level of yellow colour observed. Sledz and Witrowa-Rajchert (2012) found that microwave-convective drying of fresh leaves of six species of herbs caused a significant decrease in a and b parameters, except for rocket leaves.

According to the report, the changes in the green and yellow colour resulted from significant fluctuations in chlorophyll content in *Apiaceae* herbs. Nevertheless, chlorophyll degradation was not the only reason for colour changes in the other herbs.

The storage of nettle juices usually affected their colour, with the greatest changes recorded in canned juices (a) and frozen juices from blanched plants (L and b), compared to non-stored ones. In the first case, an increase of 104% in the share of red colour (samples NB) or 110-113% decrease (samples B15s and B90s) occurred. In the second case, darkening of the juice by 29-42% and an increase in the share of yellow colour by 43-68% occurred. A change from green to yellow or red resulted from unmasking of the carotenoids by destroying chlorophyll. The most stable products were observed from freeze drying. Analysis of ΔE parameter revealed that after the end of storage, the colour of the unpreserved juice obtained from unblanched herb was the closest to the colour of juices from raw material blanched for 90 s (code B90s), preserved by freeze-drying and freezing (Figure 3). In contrast, sterilized juices obtained from unblanched herb exhibited the most colour change. Qamar *et al.* (2019) showed that during storage for 21 days, barley and wheatgrass grass juices darkened (L^*) and an increase in the level of the a and b parameters was observed.

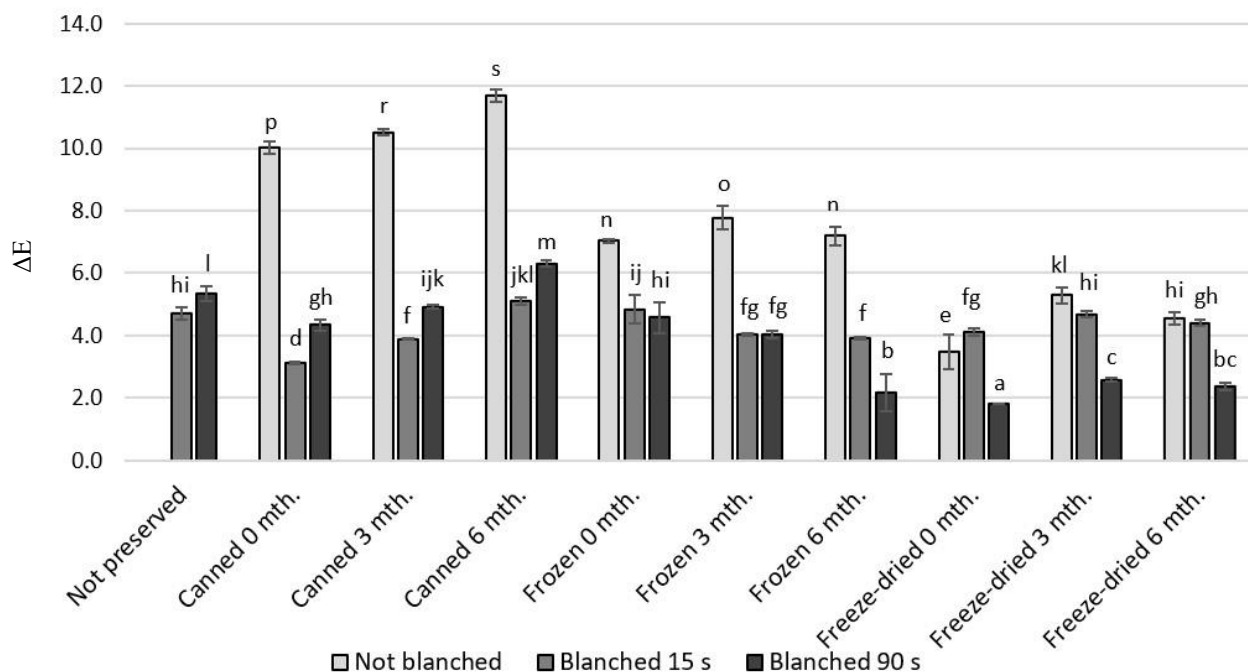


Figure 3. ΔE in nettle juices.

Legend: not preserved juices (blanched 15 s or 90 s) were compared with not blanched and not preserved juice; canned, frozen and freeze-dried juices were compared with the corresponding not blanched, blanched for 15 s or for 90 s not preserved juices; significant differences - see Fig. 1

Based on Pearson's correlation coefficients, it was shown that the relationship between the level of parameter a and carotenoids and chlorophylls content is to be negative and quite strong (neoxanthin, violaxanthin, chlorophyll a) (r from -0.67 to -0.70) or moderate (lutein, β -carotene, chlorophyll b) (r from -0.52 to -0.57). Only the α -carotene level was not dependent on parameter a. For parameter b, only the content of neoxanthin was negatively, and moderately correlated with its level because, in the case of the remaining compounds, the relationship was at weakest. Straumite *et al.* (2015) in fresh mint leaves observed a weak correlation between the content of carotenoids and colour b parameter and between a colour parameter and chlorophyll content.

Browning is a big problem in green-tinted plant products. As mentioned above, the cause of such a phenomenon may be due to the transformation of

chlorophylls or phenolics caused by the activity of enzymes, oxygen and changes in pH. The sterilization of juices showed an increase in the browning index (BI) by 15-78% and a decrease by 30-79% after freezing (Figure 4). Among the preservation methods, the lowest impact on the browning index was by freeze-drying, which caused changes in the level of the BI parameter from -19% to +10% in relation to juices after pre-treatment. 6 months of storage of canned and dried juices had little effect on the browning index, in contrast to freezing storage, which in blanched herb juices caused an approx. 3-fold increase. After storage, the highest BI was found in juices from unblanched herbs, subjected to sterilization. Preservation of juices by freezing and freeze-drying effectively reduced browning, especially in juices from unblanched herbs, and to a lesser extent, from blanched herbs.

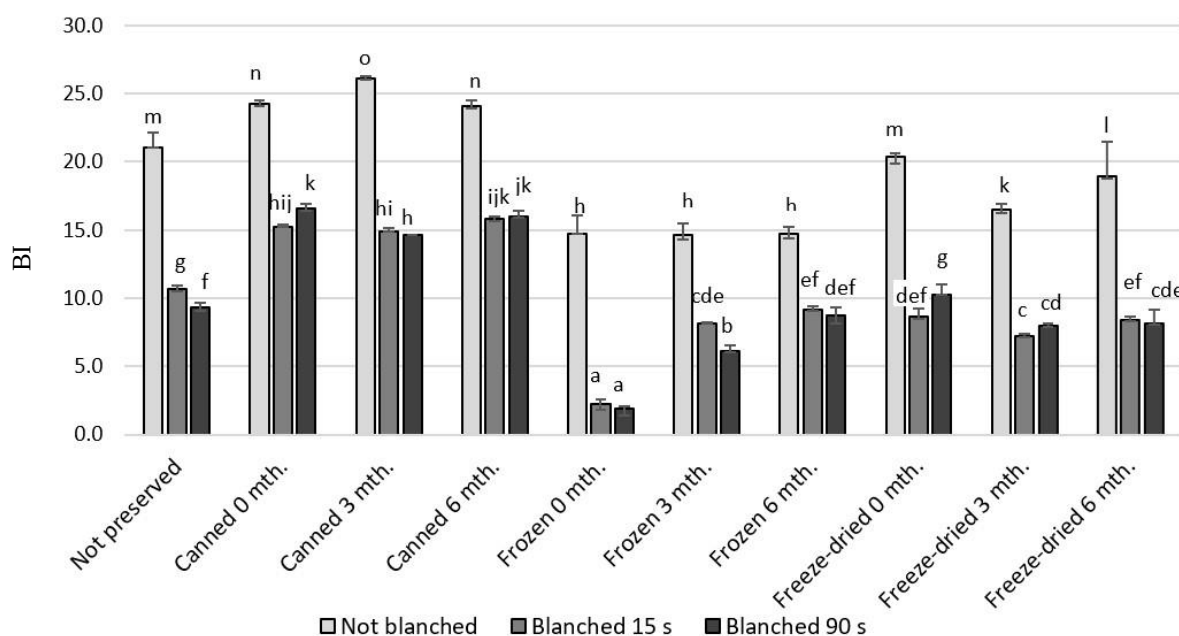


Figure 4. Browning index (BI) in nettle juices

Legend: means with different letters represent significant differences at $P < 0.05$

CONCLUSION

The applied pre-treatment, preservation, and storage influenced the amount and profile of carotenoids and chlorophylls in the juices, and, thus, their colour. After 6 months of storage in frozen and freeze-dried juices, similar to unpreserved juice, lutein, violaxanthin and chlorophyll predominated. Neoxanthin and violaxanthin were completely degraded in sterilized juices, and β -carotene and chlorophyll b were present in a significant level. Blanching benefited the level of carotenoids and chlorophylls only in sterilized juices. During the

storage of canned and frozen products, chlorophyll a was degraded to a greater extent, while in the case of lyophilizates it was chlorophyll b. Juice preservation by freezing and freeze-drying effectively reduced browning, especially in juices from unblanched herbs, and to a lesser extent, from blanched herbs. After storage, the colour of juices made from blanched for 90 s nettle herb, preserved by freeze-drying and freezing, had the most comparable colour to the raw juice. However, sterilized juices obtained from unblanched herb

displayed the greatest colour change. By measuring the level of a* colour parameter, it was possible to assume the transformations of neoxanthin,

violaxanthin and chlorophyll in sterilized, frozen and freeze-dried nettle juices.

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