

Natural Antioxidant Changes in Fresh and Dried Spices and Vegetables

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Abstract—Antioxidants are becoming the most analyzed substances in last decades. Antioxidants act as activator for free radicals. Spices and vegetables are one of major antioxidant sources. Most common antioxidants in vegetables and spices are vitamin C, E, phenolic compounds, carotenoids. Therefore, it is important to get some view about antioxidant changes in spices and vegetables during processing. In this article was analyzed nine fresh and dried spices and vegetables- celery (*Apium graveolens*), parsley (*Petroselinum crispum*), dill (*Anethum graveolens*), leek (*Allium ampeloprasum* L.), garlic (*Allium sativum* L.), onion (*Allium cepa*), celery root (*Apium graveolens* var. *rapaceum*), pumpkin (*Curcubita maxima*), carrot (*Daucus carota*)- grown in Latvia 2013. Total carotenoids and phenolic compounds and their antiradical scavenging activity were determined for all samples. Dry matter content was calculated from moisture content. After drying process carotenoid content significantly decreases in all analyzed samples, except one - carotenoid content increases in parsley. Phenolic composition was different and depends on sample – fresh or dried. Total phenolic, flavonoid and phenolic acid content increases in dried spices. Flavan-3-ol content is not detected in fresh spice samples. For dried vegetables- phenolic acid content decreases significantly, but increases flavan-3-ols content. The higher antiradical scavenging activity was observed in samples with higher flavonoid and phenolic acid content.

Keywords—Antiradical scavenging activity, carotenoids, phenolic compounds, spices, vegetables.

I. INTRODUCTION

ANTIOXIDANTS are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Free radical damage may lead to cancer. Examples of antioxidants include beta-carotene, lycopene, vitamins C, E, A, and other substances, as phenolic compounds [1].

Fruits and vegetables contain range of phytochemicals, in addition to well-known antioxidants, such as vitamins C and E, or polyphenols, which significantly contribute to their total antioxidant activity. Epidemiological and clinical investigations have associated diets rich in fruits and vegetables with reduced risk of heart, cardiovascular, neurological and chronic diseases, and various forms of cancer. Herbs are still consumed as sources of fairly good amounts of several nutrients and it is widely accepted that herbs are significant nutritional sources of minerals. Other

nutrients such as carotenoids and phenols are found in larger quantities in these plants [2], [3].

Phenolic compounds are commonly found in vegetables. In plants these compounds have different structures, mainly esters or glycosylated forms, and may have different functional properties. Phenolics are secondary metabolites synthesized by plants, both during normal development and in response to stress conditions (infection, wounding, UV radiation and others). Phenolic compounds in plants may act as phytoalexins, antifeedants, attractants for pollinators, contributors to plant pigmentation, antioxidants and protective agents against UV light [4], [5].

Major subgroups of phenolic compounds in higher plants are phenolic acids, flavonoids, tannins, lignins, lignans, anthocyanins, stilbenes. More than 5000 different flavonoids have been described in plants. The six major subclasses of flavonoids are flavones, flavonols, flavanones, catechins or flavanols, anthocyanidins and isoflavones. Most of the flavonoids in plants are attached to sugars (glycosides), they can occasionally be found as aglycones. Environmental factors have a major effect on phenolic content. These factors may be pedoclimatic (soil type, sun exposure, rainfall) or agronomic (culture in greenhouse or fields, biological culture, hydroponic culture, fruit yield per tree, etc.). Exposure to light has a considerable effect on flavonoids. Phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase. Phenolic acids are directly involved in the response of plants to different types of stress: They contribute to healing by lignification of damage areas, they possess antimicrobial properties and their concentrations may increase after infection [6]-[8].

Carotenoids are structurally and functionally a very diverse group of natural pigments of the polyene type. They occur ubiquitously in all organisms capable of conducting photosynthesis, a process in which sun light is effectively converted into chemical energy. Carotenoids are important constituents of photosynthetic organelles of all higher plants, mosses, ferns and algae. They are important precursors of retinol (vitamin A); however, their main function in all non-photosynthetic organisms seems to be (photo) protection. Carotenoids are known to be very efficient physical and chemical quenchers of singlet oxygen, as well as potent scavengers of other reactive oxygen species. Carotenoids are abundantly present in fresh fruits and vegetables. Yellow-orange-red fruits and green leafy vegetables are known to be especially rich in nutritional carotenoids. In a number of studies, thermal treatment was shown to increase carotenoids accessibility, due to the disruption of cell walls and bond

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loosening. Vitamin A deficiency is a common cause of blindness and infant mortality usually is treated with commercial Vitamin A supplements. Carotenoids appear to be difficult to absorb from the green leafy vegetables that was used in trials for food supplementation programs, but absorption appears to be much better from yellow and orange fruits and vegetables [9], [10].

Drying is the one of the storage methods, which has the capability of extending the consumption period, yet maintaining products nutrition content. Product can be stored for a long period, since the activities of the microorganisms, enzymes or ferments in the material are suppressed via drying. The major disadvantage of air-drying is the longer drying period and higher drying temperature. Dry-product quality declines these disadvantages- long lasting drying period and high energy consumption. High temperatures can destroy enzymes, vitamins and determine the rancidity of fat-containing foods, but can also lead to the production of free radical scavenging substances, such as Maillard reaction products (Melanoidins and Amadori rearrangement products). More frequently both reactions induced by the transformation can take place simultaneously and can influence each other [11], [12].

The aim of this research was to analyze natural antioxidants (phenols and carotenoids) in fresh and dried celery, parsley, dill, leek, garlic, onion, celery root, pumpkin, carrot grown in Latvia region 2013. Obtained results of fresh and dried spices and vegetables were compared to determine the effect of drying to changes of natural antioxidants content. All analysis was made in Latvia University of Agriculture, Food Technology Faculty laboratories.

II. MATERIALS AND METHODS

A. Samples, Extraction Procedure

There were analyzed nine samples grown in Latvia 2013- fresh and dried at $45\pm 1^\circ\text{C}$ temperature with fan in convective dryer (celery, parsley, dill, leek, garlic, onion, celery root, pumpkin and carrot).

For phenolic compound extracts, samples were blended in equal small pieces and then 3.00g putted in flask and 30mL pure acetone was added and mechanically stirred at $18\pm 1^\circ\text{C}$ for 1h. Samples were filtrated and the residue was soaked in 30ml ethanol- water (1:1) mixture and extracted for 30min at $18\pm 1^\circ\text{C}$. Solvent was filtrated and extracts were stored at $4\pm 1^\circ\text{C}$ until further analysis. The extraction process was carried out in triplicate for each sample.

B. Moisture Content

Moisture content was determined according to AOAC 1990. Samples were dried at $105\pm 1^\circ\text{C}$ for fresh samples 2 hours and 30 minutes for dried samples. Moisture content was expressed as percent's [3]. From moisture was calculated dry matter.

C. Total Carotenoid Content

Total carotenoid content was determined according to Russian standard GOCT P 54058-2010 with modifications. Analysis was done with minimal light and at room

temperatures below $18\pm 1^\circ\text{C}$ and low humidity. Samples were blended and 1.00-2.50g weighted in centrifuge tubes, then added 0.05g magnesium carbonate (MgCO_3) and 50mL pure acetone. Tubes were centrifuged for 15min 3500rpm with Centrifuge CM-6MT Sky Line. Liquid was transferred to separating funnel, residue was centrifuged with 12.5mL pure acetone 3 times. Liquid was transferred to funnel. To purified extract (separate organic from inorganic layer and wash them) in funnel was added 25mL petroleum ether and 25mL distilled water, solution was mixed and then separated- inorganic layer was removed, organic layer was transferred to centrifuge tubes, to solution was added 1.0g sodium sulphate (Na_2SO_4). Tubes were centrifuged for 15min 3500rpm with Centrifuge CM-6MT Sky Line. Liquid was quantified transferred to 50ml flask and filled with petroleum ether till mark. This solution was used for spectrophotometrical total carotenoid analysis.

Absorption was read at 450nm JENWAY 630 Spectrophotometer, quantification was based on the β -carote standard curve with different concentrations (5.0 to 0.5 $\mu\text{g mL}^{-1}$) ($y=0.2038x + 0.0271$; $R^2=0.9938$).

Results were calculated according to equation:

$$X = 4.00 \cdot A \cdot \frac{V}{m},$$

where X: carotenoid content $\text{mg } 100\text{ g}^{-1}\text{ DW}$, 4.00: coefficient to transition to optical absorption, A: read optical absorption of samples, V: petroleum ether volume in the cuvette, (3.5 mL), m: weight of the analyzed samples.

Results were expressed as $\text{mg } \beta$ -carote equivalents per 100 gram dry weight ($\text{mg } \beta\text{-CE } 100\text{ g}^{-1}\text{ DW}$) [13].

D. Phenolic Compounds

Total Phenolic Content was determined according to the Folin- Ciocalteu method [14] with modifications. To 500 μL of extracted sample were added 2.5mL of 0.2N Folin- Ciocalteu reagent and 2.0mL 0.75g mL^{-1} sodium carbonate solution. The resulting solution was mixed and allowed to stand for 30 minutes at $18\pm 1^\circ\text{C}$ in dark place. Absorption was read at 760nm using JENWAY 630 Spectrophotometer. Quantification was based on the standard curve generated with 0.120mg mL^{-1} of gallic acid ($y=10.226x + 0.0792$; $R^2= 0.999$). Results were expressed as milligram gallic acid equivalent per 100 gram dry weight ($\text{mg GAE } 100\text{ g}^{-1}\text{ DW}$) [14].

Total Flavonoid Content was determined by [15] with modifications. To 500 μL of extracted sample were added 2.0mL distilled water and 150 μL NaNO_2 ($5\text{g} \cdot 100\text{ mL}^{-1}$) and left to incubate for 5min. After that was added 150 μL AlCl_3 ($10\text{g} \cdot 100\text{ mL}^{-1}$) and incubate for 6min. Then 1mL 1M NaOH and 1.2mL distilled water was added. Solution was mixed and incubated at $18\pm 1^\circ\text{C}$ in dark for 20 min. Absorbance was measured at 510nm using JENWAY 630 Spectrophotometer. A standard curve was constructed based on a range of catechin hydrate concentrations (from 0.4 to 0.01mg mL^{-1}) ($y=3.0347x - 0.0405$; $R^2=0.9982$). Results were expressed as milligram catechin equivalent per 100 gram in dry weight ($\text{mg CE } 100\text{ g}^{-1}\text{ DW}$) [15].

Total Phenolic Acid Content was determined by [16] with

modifications. To 500 μ L of extracted sample were added 2.5mL distilled water, 500 μ L 0.5M HCl, 500 μ L Arnov reagent (10g Na₂MoO₄·2H₂O and 10g NaNO₂ were dissolved in 100mL with distilled water), 500 μ L 1.0M NaOH and 500 μ L distilled water. Solution was mixed and absorbance was measured at 490nm using JENWAY 630 Spectrophotometer. As a blank solution was used extraction solvent instead of extract. A standard curve was constructed based on a range of caffeic acid concentrations (0.3 to 0.001 mg mL⁻¹) ($y=3.3933x - 0.0225$; $R^2=0.9951$). Results were expressed as caffeic acid equivalent per 100 gram in dry weight (mg CAE 100g⁻¹ DW) [16].

Total Flavonols Content was determined by [17] with slight modifications. To 2.0ml sample extract were added 2.0mL AlCl₃ solution in ethanol (20g L⁻¹) and 6 mL CH₃COONa solution in ethanol (50g L⁻¹). Solution were mixed and incubated for 2.5 h at 18 \pm 1 $^{\circ}$ C temperature in dark. As a blank solution was used extraction solvent instead of extract. Absorbance was measured at 440 nm using JENWAY 630 Spectrophotometer. A standard curve was constructed based on a range of rutin hydrate concentrations (0.2 to 0.001 mg mL⁻¹) ($y=5.35x + 0.0456$; $R^2=0.9994$). Results were expressed as rutin equivalent per 100 gram in dry weight (mg RE 100 g⁻¹ DW) [17].

Total Flavan-3-ol (Proanthocyanidin) Content was determined by [18]. To 1.0mL sample extract were added 2.5mL freshly prepared vanillin solution (1g 100 mL⁻¹ dissolved in ethanol) and 2.5mL HCl solution (8% prepared in ethanol). Solution were mixed and incubated in water bath at 30 \pm 1 $^{\circ}$ C temperature for 20min. Absorbance was measured at 500nm using JENWAY 630 Spectrophotometer. A standard curve was constructed based on a range of catechin hydrate concentrations (12.0 to 0.1mg mL⁻¹) ($y=0.0425x + 0.0845$; $R^2=0.9899$). Results were expressed as milligram catechin equivalent per 100 gram in dry weight (mg CE 100g⁻¹ DW) [18].

E. Antiradical Scavenging Activity

The antiradical scavenging activity of extracts was determined on the radical scavenging ability in reacting with stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical according to [19] with modifications. 4mg of DPPH reagent was dissolved in 100ml pure ethanol and 3.5mL of this solution was added to 0.5mL sample extract. This mixture was shaken and kept in dark at 18 \pm 1 $^{\circ}$ C for 30min, absorbance was measured at 517nm using JENWAY 630 Spectrophotometer. The radical percentage was calculated using the following equation:

$$\%ASA = \left(\frac{A_{blank} - A_{sample}}{A_{blank}} \right) \cdot 100$$

where A_{blank} was the absorbance of the control reaction (full reaction, without analyzed sample extract) and A_{sample} was the absorbance of the analyzed samples. The antiradical activity was expressed as TROLOX (6-hydroxy-2,5,7,8-tertamethylchroman-2-carboxylic acid) equivalent antiradical

activity (μ mol TE 100 g⁻¹ DW) [19].

The antiradical scavenging activity of extracts was determined on the radical scavenging ability in reacting with ABTS^{•+} radical according to [20] with modifications. ABTS^{•+} radical solution was made from 7mM ABTS and 2.45mM potassium persulfate solutions in ratio 1:1 dissolved in water. This solution was left in dark for 12-16h, at the end given solution is blue green color (active for 3 days). For analysis dissolved ABTS^{•+} radical solution in ethanol absorption $A=0.70\pm 0.20$. To 100 μ L analyzed extract was added 3.5mL ABTS^{•+} solution, mixed and after 6min absorbance was measured at 734nm using JENWAY 630 Spectrophotometer. Results were expressed as TROLOX (6-hydroxy-2,5,7,8-tertamethylchroman-2-carboxylic acid) equivalent antiradical activity (μ mol TE 100g⁻¹ DW) (calibration curve 1000 to 10 μ mol) [20].

F. Statistical Analysis

All analysis was triplicate and results are presented as a mean value \pm standard deviation (SD) and analyzed using Microsoft Office 2007 software. Statistically significant differences between results were calculated at the level of confidence $\alpha=0.05$. In order to find out if the differences in mean values estimated were statistically significant, the one-way analysis of variance was applied

III. RESULTS AND DISCUSSION

Moisture content was analyzed both for fresh and dried samples. Dry mater (Table I) was calculated from samples moisture content and was used for further data analysis to express results to dry matter.

TABLE I
DRY MATER CONTENT IN FRESH AND DRIED SPICES AND VEGETABLES

	Dry mater in fresh samples, %	Dry mater in dried samples, %
Celery	17.57 \pm 0.64	90.66 \pm 0.94
Parsley	25.30 \pm 0.55	91.22 \pm 0.68
Dill	18.40 \pm 0.22	93.04 \pm 0.52
Leek	11.07 \pm 0.24	87.33 \pm 2.04
Onion	6.20 \pm 0.42	78.92 \pm 4.95
Garlic	45.59 \pm 0.38	87.44 \pm 1.23
Celery root	12.96 \pm 0.26	90.46 \pm 1.11
Carrot	11.60 \pm 0.07	84.87 \pm 3.13
Pumpkin	8.21 \pm 0.73	89.14 \pm 1.49

The highest phenolic compound content was in spices, least in vegetables. The highest total phenolic and flavonoid content was observed in dried dills, lowest phenols in fresh carrot and lowest flavonoid content in fresh garlic (Table II).

TABLE II
TOTAL PHENOL AND FLAVONOID CONTENT IN FRESH (A) AND DRIED (B)
SAMPLES

		Total phenols ^a	Total flavonoids ^b
Celery	A	330.84 ± 6.60	249.67 ± 8.67
	B	1961.00 ± 41.48	449.21 ± 10.60
Parsley	A	1209.83 ± 3.80	192.53 ± 9.43
	B	3449.25 ± 92.51	158.07 ± 6.35
Dill	A	1848.53 ± 4.40	1213.76 ± 15.38
	B	4593.74 ± 65.84	3664.65 ± 23.65
Leek	A	214.23 ± 7.87	320.30 ± 4.80
	B	622.62 ± 33.35	102.03 ± 1.79
Onion	A	126.63 ± 3.21	538.29 ± 17.79
	B	1663.84 ± 14.68	97.11 ± 3.35
Garlic	A	225.25 ± 4.42	78.14 ± 3.44
	B	475.95 ± 18.25	88.52 ± 2.20
Celery root	A	363.50 ± 7.04	325.33 ± 6.16
	B	441.76 ± 13.86	79.73 ± 4.05
Carrot	A	108.64 ± 3.41	318.73 ± 5.26
	B	544.53 ± 24.03	66.64 ± 2.47
Pumpkin	A	166.10 ± 7.36	475.87 ± 12.24
	B	672.19 ± 16.27	166.64 ± 0.87

Data are mean ± SD

^a Expressed as mg GAE·100 g⁻¹ dry weight (DW)

^b Expressed as mg CE·100 g⁻¹ dry weight (DW)

Total phenol content in parsley is 1247.8±0.9mg GAE 100g⁻¹ product; in celery 1637.1±0.1mg GAE 100g⁻¹ product; in dill 1399.8±2.4 mg GAE 100g⁻¹ product; in onion 774.0±1.1mg GAE 100g⁻¹ product. The total flavonoid content in parsley is 119.0±0.6 mg CE 100g⁻¹ product; in celery is 887.0±4.1mg CE 100g⁻¹ product; in dill is 650.1±4.8mg CE 100g⁻¹ product [21].

Total flavonol, flavan-3-ol and phenolic acid content varies in wide range and are shown in Table III.

TABLE III
TOTAL FLAVONOL, FLAVAN-3-OL AND PHENOLIC ACID CONTENT IN FRESH
(A) AND DRIED (B) SAMPLES

		Flavonols ^c	Flavan-3-ols ^d	Phenolic acids ^e
Celery	A	20.63 ± 0.62	n.d.	185.50 ± 2.55
	B	436.73 ± 1.51	2025.20 ± 36.48	584.96 ± 5.39
Parsley	A	60.01 ± 0.45	n.d.	139.76 ± 3.90
	B	258.18 ± 1.66	5769.96 ± 23.84	184.65 ± 1.03
Dill	A	376.76 ± 0.69	n.d.	1061.10 ± 2.89
	B	873.48 ± 2.92	5194.86 ± 41.81	2525.01 ± 10.97
Leek	A	n.d.	n.d.	238.44 ± 4.01
	B	n.d.	n.d.	78.61 ± 0.30
Onion	A	n.d.	n.d.	221.35 ± 6.34
	B	173.84 ± 1.85	n.d.	123.86 ± 0.60
Garlic	A	9.22 ± 1.06	2397.35 ± 53.55	66.51 ± 1.34
	B	169.16 ± 2.80	2188.94 ± 33.36	39.20 ± 0.55
Celery root	A	33.32 ± 1.72	7166.61 ± 144.48	389.33 ± 28.11
	B	18.28 ± 0.20	714.71 ± 19.13	84.43 ± 0.55
Carrot	A	n.d.	n.d.	264.22 ± 2.36
	B	43.48 ± 0.53	n.d.	61.56 ± 1.67
Pumpkin	A	n.d.	n.d.	373.32 ± 3.33
	B	41.77 ± 0.41	1226.66 ± 51.27	191.34 ± 2.41

Data are mean ± SD

^c Expressed as mg RE·100 g⁻¹ dry weight (DW)

^d Expressed as mg CE·100 g⁻¹ dry weight (DW)

^e Expressed as mg CAE·100 g⁻¹ dry weight (DW)

Flavonols, flavan-3-ol, and phenolic acid contents increase in spices after drying. Only problem is that flavan-3-ols are not detectable in fresh celery, parsley, dill and pumpkin samples. Flavon-3-ols are in higher concentrations in fresh garlic and celery root, but decreases after drying.

Total carotenoid content varies in wide range and are shown in Table IV.

TABLE IV
TOTAL CAROTENOID CONTENT IN FRESH AND DRIED SAMPLES

	Fresh samples, mg β-carotene E 100 g ⁻¹ DW	Dried samples, mg β-carotene E 100 g ⁻¹ DW
Celery	278.04 ± 1.26	250.21 ± 3.49
Parsley	148.14 ± 1.33	308.09 ± 3.29
Dill	341.64 ± 3.25	271.49 ± 1.87
Leek	38.04 ± 0.26	22.42 ± 0.08
Onion	0.26 ± 0.08	0.19 ± 0.02
Garlic	0.71 ± 0.04	0.42 ± 0.02
Celery root	4.73 ± 0.11	0.55 ± 0.03
Carrot	88.21 ± 0.95	88.92 ± 0.72
Pumpkin	11.58 ± 0.26	8.60 ± 0.02

The highest carotenoid content was determined in fresh dill and lowest in dried onion. In most cases, carotenoid content significantly decreases, because they are depending from light, pressure and processing. Only exception was parsley- after drying carotenoid content significantly increases. From data can be found that the highest content was in green and orange pigmented samples. Carotenoids from green leafy vegetables have least bioavailability (absorbs) in human organism than from yellow or orange fruits and vegetables [10].

For extracts for all samples are determined antiradical scavenging activity using DPPH and ABTS radical solutions (Table V).

TABLE V
ANTIRADICAL SCAVENGING ACTIVITY OF FRESH (A) AND DRIED (B) SAMPLES

		ABTS, μM TE 100 g ⁻¹ DW	DPPH μM TE 100 g ⁻¹ DW
Celery	A	738.57 ± 3.27	448.83 ± 2.81
	B	870.00 ± 6.27	609.67 ± 2.90
Parsley	A	346.07 ± 15.69	335.29 ± 2.06
	B	870.36 ± 5.38	669.88 ± 1.02
Dill	A	826.21 ± 26.04	456.96 ± 0.78
	B	872.50 ± 4.99	688.21 ± 0.78
Leek	A	n.d.	33.83 ± 0.78
	B	864.64 ± 9.06	504.04 ± 1.79
Onion	A	n.d.	93.63 ± 1.84
	B	868.57 ± 1.60	660.71 ± 0.78
Garlic	A	n.d.	135.08 ± 0.78
	B	546.43 ± 4.16	190.71 ± 1.79
Celery root	A	n.d.	68.11 ± 0.78
	B	863.93 ± 8.29	143.42 ± 2.12
Carrot	A	n.d.	114.88 ± 1.02
	B	537.14 ± 9.77	374.04 ± 3.12
Pumpkin	A	n.d.	135.08 ± 1.28
	B	875.71 ± 8.11	309.67 ± 16.21

There is commonality that fresh spices are more active than fresh vegetables using both radicals, but not increased

differences using ABTS radical in dried samples. Using DPPH radical solution, and after drying antiradical scavenging activity increases and correlates with total phenols increasing in samples. During analysis, the best percent range for analyzing activity for ABTS radical was from 20-100%, but for DPPH radical in range from 7 till 100%. Less active radical solution was celery roots, the highest antiradical scavenging activity has dills. The polarity of plant radical scavenging components is important factor defining extracts activity. One of high polarity phenolic compounds are simple phenolic compounds and flavonoids [17].

IV. CONCLUSIONS

During drying analyzed vegetables and spices lose a significant amount of total carotenoids. The content of phenolic compounds in spices was higher than in vegetables after drying, but in dried vegetables was determined the highest flavan-3-ols content. Antiradical scavenging activity has close correlation with flavonoid and phenolic acid content in samples – higher content flavonoid and phenolic acid, higher activity. Less antiradical scavenging activity was observed in those samples what has highest flavan-3-ol content.

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