INFLUENCE OF STEAM TREATMENT AND DRYING ON CARROTS COMPOSITION AND CONCENTRATION OF PHENOLICS, ORGANIC ACIDS AND CAROTENOIDS

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Carrot (Daucus carota L.) is a globally used vegetable from the Apiacea family. It contains macro and micro elements, as well as various phytochemicals. The aim of the study was to determine concentration of carotenoids and organic acids, phenolic composition and antiradical scavenging activity, and colour changes during steam-blanching (for 1.5 and 3.0 min) and in dried carrots in convective and microwave-vacuum driers. Gravimetric, spectrophotometric, and high performance liquid chromatography (HPLC) methods were used for analysis. Carotenoids in fresh carrots were detected in high amounts, which decreased during thermal treatment and drying. The main organic acids in fresh carrots detected in highest amounts were oxalic, tartaric, quinic, malonic, and citric acids. Ascorbic acid concentration decreased minimally with steam processing, but significantly during drying. Fresh carrots contain minimal amounts of total phenolics, which increased during the thermal and drying processes used, while flavonoid, flavonol, flavan-3-ol and phenolic acid concentration decreased. The compound found in highest amounts by HPLC methods were 3.4-dihydroxybenzoic and 3.5-dihydroxybenzoic acids, catechin, 4-hydroxybenzoic acid, epicatechin and sinapic acid.

Key words: *carrots, steaming, drying, phenolic's, organic acids.*

INTRODUCTION

Carrots (*Daucus carota* L.) are one of the most preferred vegetables, because of their various use in culinary processes and its enriched healthy composition of phytonutrients, dietary fibre and minerals (Goncalves *et al*., 2010). They are noted for their rich concentration of antioxidants, especially β-carotene. They contain not only nutritional antioxidants such as vitamins A, C and E, but also great quantities of non-nutritional antioxidants, such as β-carotene, carotenoids, flavones, phenolic compounds etc. (Yen *et al*., 2008).

Blanching can be applied in food preparation using many methods including water, steam, vacuum-steam, in can and hot air. Temperature can range from 75–95 °C and time from 1–10 minutes. Blanching is commonly used as pretreatment for many techniques for example prior to freezing and drying (Rawson *et al*., 2011).

Convective drying, also known as hot-air drying, is the most common and widely adapted process in the food industry, requiring long drying times and high temperatures. Using this process, the colour deteriorates faster with a remarkable decrease in lightness and increase in yellowness

values in many vegetables (Karam *et al*., 2016). Microwave drying can described as ultra-rapid energy transfer providing a quick drying method (from hours to minutes). Combining microwave energy with vacuum technology, it is possible to keep temperatures within low ranges, which is essential for heat-sensitive materials, maintaining colour, less nutrient loss, and retention of flavour (Lombrana *et al*., 2010).

Conventional (thermal), modern or non-thermal (e.g. high pressure processing pulsed electric field, ultrasound/ sonication, ozone, ultraviolet), domestic (e.g. washing, peeling, and cutting) and industrial (e.g. canning, drying) processing are widely reported to degrade the level of bioactive compounds in processed food products (Tiwari and Cummins, 2013). Bioactive compounds are extra-nutritional constituents that can be found in small quantities. They are easily degraded by oxygen, light, temperature and pH. They have protective effect in diets that has been proved in many studies (Hernandez-Carrion *et al*., 2014). The aim of the current study was to determine concentrations of biologically active compounds (organic acids, phenolic compounds), their antiradical activity and colour changes of steam-blanched, convective, and microwave-vacuum dried carrots.

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MATERIALS AND METHODS

Chemicals. All used solvents and chemicals for extraction and analysis of carotenoids, organic acids and phenolic compounds were HPLC and analytical grade: gallic acid, 3.4-dihydroxybenzoic acid, 3.5-dihydroxybenzoic acid, (+)-catechin hydrate, chlorogenic acid, 4-hydroxybenzoic acid, vanillic acid, homovanillic acid, caffeic acid, (-)-epicatechin, syringic acid, vanillin, p-coumaric acid, sinapic acid, t-ferulic acid, 2-hydroxycinnamic acid, rutin hydrate, t(3)-hydroxycinnamic acid, quercetin, luteolin, kaempferol, acetone, methanol, ethanol, acetic acid, sodium hydroxide, aluminium chloride hexahydrate, sodium acetate anhydrous, sodium molybdate, sodium sulphate anhydrous, Folin-Ciocalteu reagent, magnesium carbonate basic, β-carotene, petroleum ether 40–60 °C, hydrochloric acid, sodium nitrate, m-phosphoric acid, oxalic acid, malic acid, malonic acid, fumaric acid, succinic acid, ascorbic acid, tartaric acid, quinic acid, benzoic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), potassium persulphate, distilled water.

Sample preparation and drying processing. Carrots were grown and harvested in Latvia; analysis of fresh, steam-blanched and dried carrots was conducted in the Faculty of Food Technology, Latvia University of Agriculture. Schematic procedure of carrot processing is shown in Figure 1.

Steam-blanching was done using a home steaming device Tefal VC4003 Vitamin+ (China) at temperature 95 ± 3 °C temperature for 1.5 and 3.0 minutes. Carrot discs were placed in a thin layer in a steaming chamber over boiling water. After steaming samples were taken out from container and cooled to 20 ± 2 °C (Turkmen, 2008).

Approximately 3.00 ± 0.01 kg carrot discs were dried in a convective drier. Drying in a chamber type convective drier with air circulation was done in a Memmert UF55 model (Germany) with controlled speed circulation (1.2 ± 0.1 m·s^{-1}) at 45 ± 1 °C temperature for 12 to 14 hours. Cut samples were placed on a perforated mesh (Ø 0.185 m, bolts square — 0.030 m^2) with holes (Ø 0.002 m); mesh occupancy — 6.70 kg·m^{-2}. Samples were dried till constant non-changing moisture content (approximately from $4.00 \pm 0.10\%$ till $9.00 \pm 0.10\%$) (Schweiggert *et al.*, 2007)

Drying with a microwave-vacuum drier was done using an industrial Musson-1 (OOO Ingredient, Russia). The necessary amount of carrot (from 2.0–4.0 kg) was prepared for drying (fresh, steamed — 1.5 min, steamed — 3.0 min) and placed in the drying cylinder (height 540 mm, diameter 350 mm), which was placed in microwave-vacuum drier (Microwave-vacuum drier Musson-1, 2007). The drying parameters were programmed pressure — 7.5 / 9.3 kPa, rotation speed of cylinder — 6 min^{-1}, number of cycles — one. Samples were dried till moisture content 7.0–9.0% for two hours.

Two different pre-treatments were used before microwave-vacuum drying: steam-blanching for 1.5 and 3.0 min (procedure described previously). Immediately after cooling, samples were dried in a microwave-vacuum drier as described previously. Samples were immediately analysed after the above treatments.

Moisture content. Moisture content was determined according to the AOAC standard method. Fresh samples were dried at 105 ± 1 °C for 2 hours (Mazzeo 2011), and dried samples at 70 ± 1 °C (Sulaiman, 2011) for 3.0 hours. Moisture content was expressed as a percentage.

Total carotenoid concentration. Total carotenoid concentration was determined according to the Russian standard ГОСТ Р 54058-2010 with modifications (Anonymous, 2011). Samples were ground and milled, 1.00–2.50 g weighed into centrifuge tubes, and 0.05 g magnesium carbonate and 50.0 mL of acetone were added. Tubes were centrifuged for 15 min at 3500 rpm. Then the liquid was transferred to a separating funnel; the residue was centrifuged with 12.5 mL pure acetone 3 times (15, 10 and 5 min) and the liquid was added to a separation funnel. To purify the extract in funnel, 25.0 mL petroleum ether and 25.0 mL water were added, the solution was mixed and separated; as a result the inorganic layer was removed and the organic layer was transferred to centrifuge tubes; 1.00 g sodium sulphate was added to the solution. Tubes were centrifuged for 15 min at 3500 rpm; the obtained solution was used for spectrophotometric analysis. Absorption was read at 450 nm and quantification was based on the β-carotene standard curve with different concentrations (0.50 to 5.00 μg mL^{-1}). Obtained results were expressed as mg β-carotene equivalents per 100 gram dry weight (mg β-CE 100 g^{-1} DW).

Extraction procedure of phenolic compounds. For phenolic compound extracts, all samples were blended or milled in equal small pieces and then 5.00 ± 0.01 g for fresh and 2.50 ± 0.01 g for dried mass was placed in a flask, 50.0 mL pure acetone was added and mechanically stirred at 18 ± 1 °C for 1 h. Samples were filtered and the residue was soaked in 50.0 mL ethanol-water (1:1) mixture and extracted for 30 min at $18 \pm$ °C. The solvent was filtered and
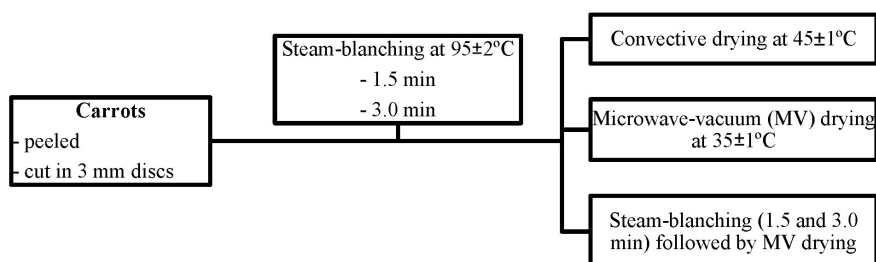

Fig. 1. Scheme of processing steps of carrots.

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the extract was stored at 4 ± 1 °C until further analysis (max. storage time was two days). The extraction process was carried out in triplicate for each sample (Luthria, 2008; Garcia-Salas 2010).

Total phenolic compound concentration. Phenolic was determined according to the Folin-Ciocalteu method with modifications — 2.5 ml of 0.2 N Folin-Ciocalteu solution in water and 2.0 ml·7.5 g·100 ml^{-1} sodium carbonate solution were added to 0.50 ml of previously extracted samples. The resulting solution was mixed and allowed to stand for 30 min at 18 ± 1 °C. Quantification was based on a standard curve generated with 0.120 mg·ml^{-1} of gallic acid. Results were expressed as milligram gallic acid equivalent per 100 gram dry weight (mg GAE·100 g^{-1} DW) (Pande *et al*. 2010).

Total flavonoid concentration. Flavinoid concentration was determined by the Olivera *et al*. (2008) method with modifications. To 0.50 ml sample extract, 2.0 ml water and 0.15 ml sodium nitrite (0.05 g·ml^{-1}) were added; the obtained solution was left to incubate for 5 min. Then 0.15 ml aluminium chloride (0.10 g·ml^{-1}) was added and the solution incubated for 6 min; then 1.0 ml·1M NaOH and 1.2 ml of water were added. The solution was mixed and incubated at 18 ± 1 °C in the dark for 20 min. Absorbance was measured at 510 nm. A standard curve was constructed based on a range of catechin hydrate concentrations (from 0.4 to 0.01 mg·ml^{-1}). Results were expressed as milligram catechin equivalent per 100 gram in dry weight (mg CE 100 g^{-1} DW).

Total flavonol concentration. Flavonol concentration was determined by the Ložiene *et al*. (2007) method with slight modifications. To 2.0 ml sample extract, 2.0 mL AlCl$_3$ solution in ethanol (20 g·l^{-1}) and 6.0 ml of CH$_3$COONa solution in ethanol (50 g·l^{-1}) were added and mixed, the obtained solution was incubated for 2.5 h at 18 ± 1 °C in the dark. During incubation, the solution was mixed and was filtered just before measurement. Absorbance was measured at 440 nm. A standard curve was constructed based on a range of rutin hydrate concentrations (0.2 to 0.001 mg·ml^{-1}). Results were expressed as rutin equivalent per 100 gram in dry weight (mg RE·100 g^{-1} DW).

Total flavan-3-ol (proanthocyanidin) concentration. Flavan concentration was determined by the Zam (2012) method with modifications. To 1.0 ml sample extract, 2.5 mL freshly prepared vanillin solution in ethanol (1 g·100 ml^{-1}) and 2.5 ml·8% HCl solution in ethanol were added. The solution was mixed and incubated in a water bath at 30 ± 1 °C for 20 min and then quickly cooled to 18 ± 1 °C. Absorbance was measured at 500 nm. A standard curve was constructed based on a range of catechin hydrate concentrations (12.0 to 0.1 mg·ml^{-1}). Results were expressed as milligram catechin equivalent per 100 gram in dry weight (mg CE·100 g^{-1} DW).

Total phenolic acid concentration. Phenolic acid concentration was determined by the Gawlic-Dziki (2012) method

with modifications. To 1.0 ml of extract, 2.5 ml water, 0.5 ml 0.5M HCl, 0.5 ml Arnov reagent (10.0 g Na$_2$MoO$_4$*2H$_2$O and 10.0 g NaNO$_2$ dissolved in 100 ml water), 0.5 ml 1M NaOH and 0.5 ml water were added. The solution was mixed and then absorbance was measured at 490 nm. As a blank solution, extraction solvent instead of extract was used. A standard curve was constructed based on a range of caffeic acid concentrations (0.3 to 0.001 mg·ml^{-1}). Results were expressed as caffeic acid equivalent per 100 gram in dry weight (mg CAE·100 g^{-1} DW).

Determination of concentration of individual phenolic compounds. The previously mentioned extraction procedure was used. The obtained solution was distilled using a Rotatory evaporator (Heidolph Rotary Evaporator, Germany) till constant mass. Distillation temperature was 55 ± 5 °C (Ti *et al*., 2014). The evaporated mass was dissolved in 5.0 ml pure methanol. The methanol extract was centrifuged and filtered with a 0.45 μm Millipore membrane just before analysis; pure solution was used for high performance liquid chromatography (HPLC) analysis.

Individual phenolic compounds were determined using modified the HPLC-DAD method (Shimadzu LC 20 Prominence, Japan). The method was based on chromatographic separation of phenolic compounds and their retention time. The analysis was performed at 30.0 ± 0.1 °C. The mobile phase consisted from A – distilled water; B – methanol; C – acetic acid. Injection volume was 10 μl. The analytic column PerkinElmer C18 (4.6 mm × 250 mm × 5 μm) was used in the DAD SPD-M20A column. Total time of analysis was 78 min (Chen *et al*., 2001; Wang and Zuo, 2011; Baydar and Baydar, 2013, Lazarova *et al*., 2014). Parameters changed during analysis. The gradient elution was as follows: 0–2 min, 0–15% B, 2.5–2.4% C; 2–12 min, 15–18% B, 2.4–2.2% C; 12–20 min, 18–20% B, 2.2–1.8% C; 20–30 min, 20–25% B, 1.8–1.6% C; 30–40 min, 25–30% B, 1.6–1.4% C; 40–50 min, 30–45% B, 1.4–1.0% C; 50–55 min, 45–55% B; 1.0–0.9% C; 55–65 min, 55–85% B; 0.9–0.6% C; 65–70 min, 85–100% B, 0.6–0.0% C; 70–73 min, 100–0% B, 0.0–2.5% C; 78 min stop. Flow was also changed during analysis: 0–12 min, 1.0–0.8 ml·min^{-1}; 12–20 min, 0.8–0.6 ml·min^{-1}; 20–25 min, 0.6–0.5 ml·min^{-1}; 25–35 min, 0.5–0.4 ml·min^{-1}; 35–45 min, 0.4–0.8 ml·min^{-1}; 45–50 min, 0.8–0.7 ml·min^{-1}; 50–55 min, 0.7–0.8 ml·min^{-1}; 55–65 min, 0.8–0.85 ml·min^{-1}; 65–70 min, 0.85–1.0 ml·min^{-1}.

Twenty-one standard compound mixtures were prepared at concentrations 0.05 to 1.00 mg·ml^{-1} and diluted in pure methanol. Solution was stored at 4 ± 1 °C temperature. The identification of phenolic compounds in the samples was done by comparing retention times of individual phenolic compounds in the reference vs. tested solution (qualitative analysis).

The concentration of those compounds was assessed based on comparison of peak areas obtained from the reference analysis (quantitative determination). Analysis was done in

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duplicate. Results were expressed as milligrams per 100 gram of dry weight sample (mg·100 g^{-1} DW).

Antiradical scavenging activity. The antiradical scavenging activity of extracts was determined on the radical scavenging ability in reaction with stable 2.2-diphenyl-1-picryl-hydrazyl (DPPH) free radical according to the Afify (2012) method with modifications. Absorbance was measured at 517 nm using a JENWAY 630 Spectrophotometer. The antiradical activity was expressed as TROLOX (6-hydroxy-2.5.7.8-tertamethylchroman-2-carboxylic acid) equivalent antiradical activity (mmoL TE·100 g^{-1} DW).

The antiradical scavenging activity of extracts was determined on the radical scavenging ability in reaction with ABTS^{*+} radical according to the Erkan (2011) method with modifications. Absorbance was measured at 734 nm using a JENWAY 630 Spectrophotometer. Results were expressed as TROLOX equivalent antiradical activity (mmoL TE·100 g^{-1} DW) (calibration curve 10 to 1000 µmoL).

Individual organic acids. Approximately 10.00 ± 0.01 g fresh or 5.00 ± 0.01 g dried samples were weighed in flasks and 50.0 ml freshly prepared m-phosphoric acid in distilled water (pH = 3.00 ± 0.20) was added. The samples were mixed at 18 ± 1 ^{o}C temperature for 2 h and then filtered two times through Whatman filter paper and before injection in a chromatograph centrifuge and then filtered through 0.45 µm filter. A pH equal or lower than 5.00 is necessary in the mobile phase to maintain the stability of vitamin C during the analysis (Martin-Belloso *et al.*, 2012; Isabelle *et al.*, 2010).

Determination of oxalic, tartaric, quinic, malic, malonic, ascorbic, citric, fumaric, and succinic acid concentration was carried out according to Sherer *et al.* (2012) with modifications, using a Shimadzu LC-20 Prominence (Japan) liquid chromatograph, equipped with detector DAD SPD-M20A, auto sampler adjusted to 10 µl volume injection, and Alltech C18 column (5 µm particle size, 250 × 4.6 mm, kept at 30 ± 0.1 ^{o}C temperature). The mobile phase consisted of acetonitrile and 0.05 M KH$_2$PO$_4$ (ratio 1:99) (pH = 2.80 ± 0.02 adjusted with o-phosphoric acid) using an isocratic elution with a flow rate 1.00 ml·min^{-1}. Detection was done at 210 nm. Total analysis time was up to 10 minutes.

Determination of salicylic and benzoic acid concentration was carried out according to Bae *et al.* (2014) with modifications. It was used Shimadzu LC-20 Prominence (Japan) liquid chromatograph, equipped with detector DAD SPD-M20A, auto sampler adjusted to 10 µl volume injection, Alltech C18 column (5 µm particle size, 250 × 4.6 mm, kept at 25 ± 0.1 ^{o}C). The mobile phase consisted from acetonitrile and 0.025 M KH$_2$PO$_4$ (ratio 45 : 55) (pH = 2.55 ± 0.02 adjusted with o-phosphoric acid) using a gradient elution with a flow rate 1.50 ml·min^{-1}. Detection was at 210 nm. Total analysis time was up till 5.5 minutes.

Colour analysis. Colour intensity of samples was measured with a Color Tec-PCM (New York, USA). Before measure-

ment samples were blended in a uniform mass: fresh samples with a Braun Multiquick 5 (Poland), and dried samples with a Knifetec^{TM} 1095 mill (Foss, Sweden). Samples were placed in plastic petri plates in monogamous mass and then placed on the top plate. Using the equipment, a sample diameter of 80 mm was analysed for determination of visual colour with a computer program.

Characterising colour CIE L*a*b* space, amplitude L* represents colour intensity (L$_0$ – dark, L$_{100}$ – light), a* – the amount of red (–a – +a), b* – yellow and blue colour amount (–b – +b). Changes of colour components, compared with fresh samples (control) was characterised by the sum of the square difference — total difference ΔE*, obtained from using L*a*b* values.

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2},$$

where: ΔE* – total colour difference, which characterises sample colour changes compared with a fresh sample (control); L* – sample colour intensity characterising the value using pre-treatment; L$_0$* – sample colour intensity characterising the value in fresh samples (control); a* – sample colour components green-red characterising values using pre-treatment; a$_0$* – sample colour components green-red characterising the value in fresh samples; b* – sample colour components blue-yellow characterising values using pre-treatment; b$_0$* – sample colour components blue-yellow characterising the value in fresh samples (Zhu *et al.*, 2014; Karasu *et al.*, 2015).

Data statistical analysis. Moisture, carotenoid, total phenolic compounds concentration were determined in triplicate, individual phenolic and organic acid compound concentration — in duplicate; colour analysis — in ten replicates. Results were calculated and presented as a mean value ± standard deviation (SD) using Microsoft Office 2010 software. Statistically significant differences between values were calculated at the level of confidence α = 0.05 using one-way analysis of variance.

RESULTS

Moisture content of fresh carrots was 89.78 ± 0.90% and it was minimally affected by steam-blanching. Carotenoids were the main compounds in carrots and concentration ranged from 185.17 ± 0.55 to 282.86 ± 8.59 mg β-CE 100 g^{-1} DW. Total phenolic compound concentration ranged from 100.37 ± 0.84 to 593.05 ± 29.13 mg GAE·100 g^{-1} DW. Total phenolic acid concentration in carrots ranged from 97.50 ± 1.54 to 309.75 ± 10.24 mg CAE·100 g^{-1} DW, and total flavonoid concentration from 135.70 ± 4.53 to 458.08 ± 2.96 mg CAE·100 g^{-1} DW (Table 1).

A slight increase in carotenoid concentration was observed in 1.5 min steam-blanched carrots, while longer steam processing resulted in a decrease. The highest increase (67.4% higher than initial carotenoid concentration) was observed in microwave vacuum (MV) dried carrots.

CAROTENOID AND PHENOLIC COMPOUND CONCENTRATION AND THEIR ANTIRADICAL ACTIVITY IN PROCESSED CARROTS

	Fresh[*]	1.5 min SB[*]	3.0 min SB[*]	45 °C	MV	1.5 min SB + MV	3.0 min SB + MV
Carotenoids	190.73 ± 2.32	197.38 ± 2.86	185.17 ± 0.55	200.49 ± 2.86	282.86 ± 8.59	174.48 ± 2.64	171.32 ± 4.10
Phenolics	114.44 ± 0.88	100.37 ± 0.84	106.04 ± 10.99	593.05 ± 29.13	381.69 ± 12.03	402.94 ± 10.66	385.72 ± 4.40
Phenolic acids	305.58 ± 2.66	300.84 ± 6.72	309.75 ± 10.24	156.44 ± 2.53	97.50 ± 1.54	131.69 ± 1.22	181.33 ± 5.58
Flavonoids	458.08 ± 2.96	447.53 ± 4.90	387.91 ± 2.62	167.07 ± 1.12	135.70 ± 4.53	168.44 ± 2.45	185.37 ± 1.79
Flavonols	129.17 ± 4.56	216.79 ± 6.33	129.62 ± 2.69	30.43 ± 1.81	83.86 ± 3.38	66.43 ± 1.48	25.63 ± 3.87
Flavan-3-ols	n.d.	n.d	n.d.	4653.06 ± 28.90	3121.91 ± 45.93	765.77 ± 29.05	n.d.
ABTS	10.71 ± 1.17	13.10 ± 2.94	52.14 ± 3.09	662.14 ± 7.96	518.33 ± 4.42	674.05 ± 10,58	635.48 ± 14.68
DPPH	115.50 ± 2.22	128.42 ± 4.16	143.42 ± 3.40	218.63 ± 11.88	206.13 ± 0.63	431.44 ± 4.06	463.63 ± 4.38

Results were expressed as average amount with standard deviation; n.d., not detected; SB, steam blanching; 45 °C, convective drying; MV, microwave–vacuum drying; [*]Priecina and Karklina, 2014

The total phenolic concentration minimally changed with steam processing, while with convective drying content resulted in an increase by 80.7%; with MV processing phenolic concentration increased by 70.0% and 71.6%.

The phenolic acid concentration decreased with drying process by 48.8% with convective and by 68.1% with MV drying; steam-blanching for 1.5 min helped to maintain acid concentration with convective and decreased concentration by 56.9% with MV drying and with 3.0 min processing concentration decreased by 40.7%. Many hypotheses have been raised to explain phenolic acid concentration changes during cooking: partial release from bounded form leading to increase in the soluble form, a decrease of the soluble fraction due to thermal degradation and an increase of the bound form caused by interactions of phenolic acids with macromolecules of the food matrix (Zaupa *et al*., 2015).

Flavonoids were the second major subgroup of phenolic compounds. Their concentration ranged from 135.70 ± 4.53 to 458.08 ± 2.96 mg CE·100 g^{-1} DW. With thermal and drying, total flavonoid concentration decreased significantly. After short-term steam blanching, the concentration changed minimally, while in a longer time it decreased by 15.3%; with convective drying by 63.5%, and with MV processing it decreased by 70.4%, 63.2% and 59.5%, respectively. Heat processing followed by MV drying helps to maintain flavonoid concentration more than using only the drying process.

Flavan-3-ols (proanthocyanidins) were not detected in fresh and steamed carrots, while in convective and MV dried samples they were detected in significant amounts. Using 1.5 min steam-blanching followed by MV drying, the total concentration decreased by 75.5% compared with MV dried carrots, but with 3 min pre-processing flavan-3-ols were not detected.

The antiradical activity of processed carrots is determined by two different radicals and both had a tendency to increase minimally during blanching, but significantly increased with drying.

Carrots contain a wide range of phenolic compounds, including both simple and complex phenolics. Phenolic compounds in the highest concentration were 3.4-dihydroxybenzoic acid, 3.5-dihydroxybenzoic acid, catechin, quercetin and luteolin (Table 2).

From the analysed 21 phenolic compounds, 11 were detected in fresh samples, 12 in steam-blanched carrots, and 18 in convective and microwave-vacuum drier processed carrots, in different concentrations depending on used method.

Organic acids in carrots were detected in various concentrations and changed differently depending on processing type. The highest concentrations of analysed organic acids were observed in dried carrots (Table 3).

Principal component analysis (PCA) shows simple visualisation of complex data according to the similarity of the grouped data. In this study, PCA was applied to access the relationships between carrot processing methods depending on the organic acid and phenolic composition. The first and second principal components (PC1 and PC2, respectively) explained 44.24% and 22.03% of the variation (Fig. 2).

Figure 2 (a) shows clustering of the carrots in three main groups. The fresh and steam-blanched samples were separated from convective dried (45 °C) and MV drier processed samples. Higher concentration of ascorbic acid, benzoic acid, 3.4-dihydroxybenzoic acid, fumaric acid, malic acid, gallic acid, rutin, quercetin and luteolin was observed in fresh and steam-blanched carrots. In convective dried carrots tartaric acid, oxalic acid, salicylic acid, citric acid, succinic acid, 4-hydroxybenzoic acid, and 2-hydroxycinnamic acid were detected in highest concentration. In MV processed carrots quinic acid, malonic acid, kaempferol, (+)-catechin, (-)-epicatechin, vanillin, 3.5-dihydroxybenzoic acid, homovanillic acid, syringic acid, t-ferulic acid, chlorogenic acid, p-coumaric acid, sinapic acid, caffeic acid, and 3-hydroxycinnmic acid had higher concentration. The PCA loading plots results depict similarity of methods based on chemical composition (Fig. 2b).

Colour is a major physical characteristics of food from a consumers' perspective. Colour in carrots during thermal and drying was analysed using the L*a*b* spectrum and colour differences ΔE (Table 4)

PHENOLIC COMPOSITION OF PROCESSED CARROTS (mg·100 g^{-1} DW)

	Fresh	1.5 min SB	3.0 min SB	45 °C	MV	1.5 min SB + MV	3.0 min SB + MV
Gallic acid	0.294	1.578	1.934	0.334	0.413	0.051	0.029
3,4-dihydroxybenzoic acid	8.531	3.465	1.653	2.414	n.d.	1.955	1.726
3,5-dihydroxybenzoic acid	n.d.	0.653	0.511	1.666	1.068	1.865	2.286
(+)-catechin	n.d.	2.086	1.772	0.819	1.899	1.044	4.058
Chlorogenic acid	n.d.	n.d.	n.d.	0.473	0.860	0.901	1.741
4-hydroxybenzoic acid	n.d.	n.d.	2.828	7.112	0.433	1.597	2.355
Homovanillic acid	n.d.	n.d.	n.d.	0.277	0.319	0.377	0.203
Caffeic acid	n.d.	n.d.	n.d.	n.d.	0.386	0.270	0.380
(-)-epicatechin	n.d.	n.d.	n.d.	1.729	2.523	2.123	2.511
Syringic acid	0.085	0.154	0.162	0.477	0.398	0.393	0.364
Vanillin	n.d.	0.145	0.128	0.542	0.547	0.594	0.664
p-coumaric acid	n.d.	0.082	0.187	0.357	1.302	0.246	0.254
Sinapic acid	0.038	0.109	0.119	2.570	11.686	8.373	14.172
t-ferulic acid	0.028	n.d.	n.d.	0.092	0.097	0.219	0.252
2-hydroxycinnamic acid	0.104	0.045	0.204	0.401	0.025	0.248	0.177
Rutin	0.853	n.d.	n.d.	0.181	0.410	0.207	0.249
3- hydroxycinnamic acid	0.038	n.d.	0.009	0.185	3.999	0.313	0.196
Quercetin	16.739	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Luteolin	2.844	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Kaempferol	0.028	0.009	n.d.	n.d.	0.041	0.010	0.082
Total phenolic's by HPLC	29.583	8.327	9.507	19.629	26.407	20.786	31.699

Results were expressed as average amount with 10% standard deviation; n.d., not detected; SB, steam blanching; 45 °C, convective drying; MV, microwave vacuum drying

ORGANIC ACIDS OF STEAM-BLANCHED AND DRIED CARROTS (mg·100 g^{-1} DW)

	Fresh[*]	1.5 min SB[*]	3.0 min SB[*]	45 °C	MV	1.5 min SB + MV	3.0 min SB + MV
Oxalic acid	316.46	228.07	139.49	953.78	331.94	146.82	89.75
Tartaric acid	840.63	776.10	559.82	1553.01	n.d.	552.23	798.41
Quinic acid	n.d.	n.d.	1030.42	n.d.	2898.31	3846.49	935.89
Malic acid	168.19	301.42	1818.20	n.d.	n.d.	n.d.	n.d.
Malonic acid	n.d.	n.d.	n.d.	817.10	1281.86	2757.70	1168.83
Ascorbic acid	127.84	123.71	94.11	109.80	97.75	82.62	70.13
Citric acid	1074.49	321.94	215.79	4163.34	1378.58	1108.75	60.42
Fumaric acid	104.31	9.98	22.07	0.03	n.d.	n.d.	15.93
Succinic acid	14.57	79.63	n.d.	780.32	158.97	24.39	420.77
Salicylic acid	n.d.	0.60	n.d.	3.36	0.18	0.79	1.01
Benzoic acid	1.83	0.42	0.78	0.59	0.29	0.27	0.13
Total org. acids	2648.33	1841.87	3880.69	8381.33	6147.87	8520.07	3561.28

Results were expressed as average amount with 10% standard deviation; [*]Priecina and Karklina (2015); SB, steam blanching; 45 °C, convective drying; MV, microwave vacuum drying

With steam-blanching samples minimally changed in lightness, while 3.0 min steam processing produced visual changes (ΔE > 10). Carrots became significantly lighter with convective and MV drying, but carrots subjected to steam-blanching followed by MV drying maintained a darker colour than dried carrots without pre-processing. More visual changes were observed in dried samples without pre-processing.

DISCUSSION

Food processing (including thermal process) induces matrix disruption in lipophilic carotenoids, thereby increasing their bioavailability (Van Buggenhout et al., 2010). In plant tissues carotenoids can exist in cis and trans forms and during processing a portion of the trans form carotenoid is either lost or converted to the cis form and its derivatives, result-

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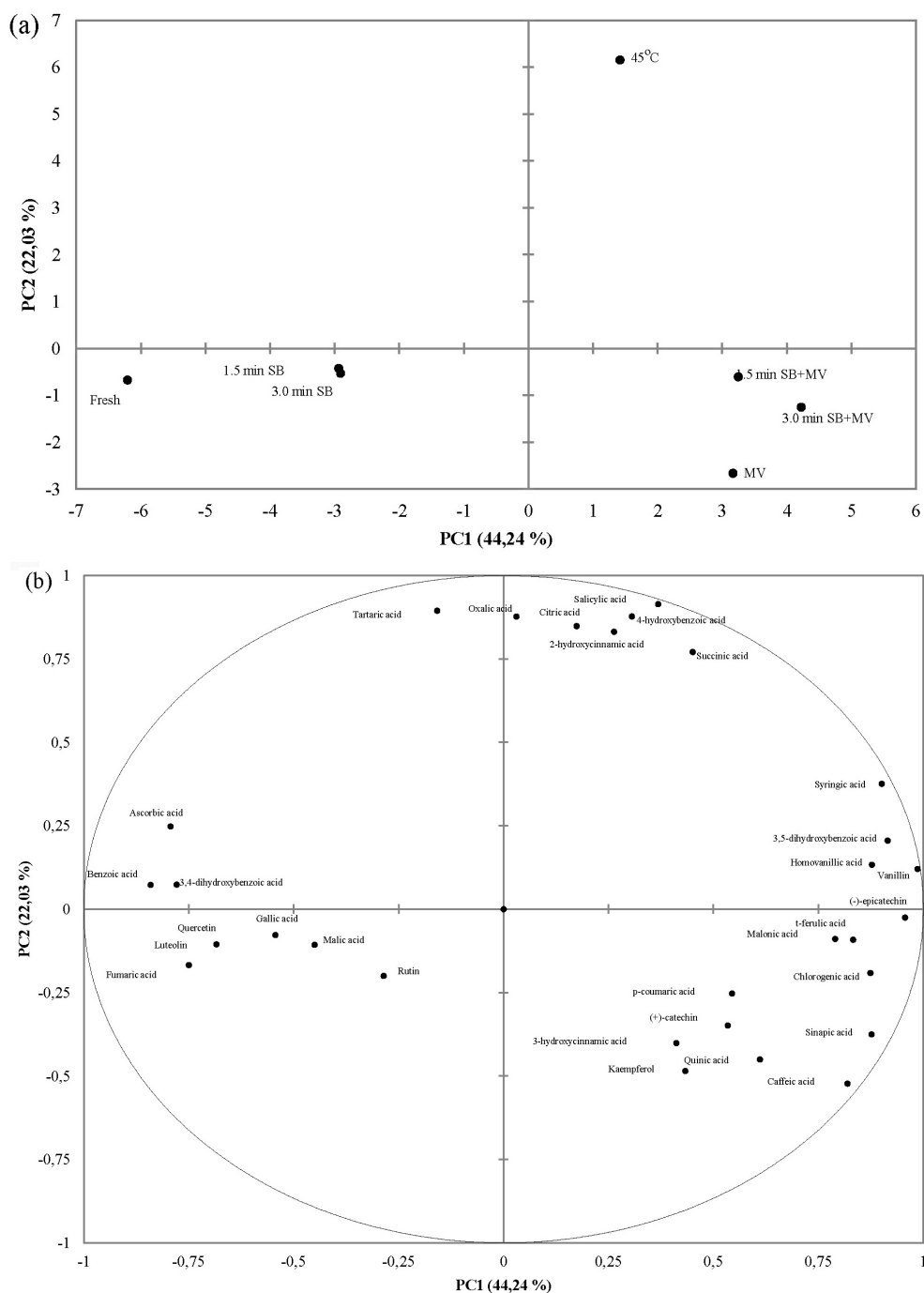Proc. Latvian Acad. Sci., Section B, Vol. 72 (2018), No. 2.

Fig. 2. (a) – scores and (b) – loading plots of principal component PC1 and PC2 of the PC analysis. Organic acid and phenolic composition data was determined after seven processing methods. SB, steam blanching; 45 °C, convective drying; MV, microwave vacuum drying.

ing in increase of the total carotenoid concentration (Sharma *et al.*, 2012). Decrease in carotenoid concentration is due to oxidation, which depends on oxygen and can be stimulated by heat and light. Also it can lead to reduction of pigment levels (carotenoids in plants are responsible for yellow and red colours of vegetables) (Murador *et al.*, 2014).

Increase in phenolic compound concentration can occur due to thermally induced extraction of antioxidant molecules, which previously were complexed or polymerised, or retention of active compounds caused by inactivation of enzymes involved in catabolism of phenolics (Kapoor and Aggarwar,

2015). According to Guillen *et al.* (2017), decrease in phenolic concentration can be explained as a result of phenolic breaking down by heat, while an increase can be due to increase in the level of free flavonols caused by thermal treatment. It was suggested that anthocyanidins are unstable in nature and degradation can be caused by oxidation reactions, leading to formation of colourless phenolic aglycones (chalcone), which further transform into coumarin glycoside derivatives (Tiwari and Cummins, 2013).

Both of the used radical solutions have a specific analysis range — the ABTS radical solution estimates activity of not only phenolic compounds, but also of carotenoids, ascorbic

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COLOUR PARAMETER CHANGES IN CARROTS USING STEAM-BLANCHING AND DRYING PROCESSES

	L	a	b	ΔE
Fresh	45.25 ± 0.07	17.09 ± 0.08	44.80 ± 0.34	-
1.5 min SB	48.49 ± 0.09	16.73 ± 0.15	48.63 ± 0.39	5.02 ± 0.08
3.0 min SB	48.03 ± 0.08	16.01 ± 0.26	57.12 ± 0.60	12.67 ± 0.31
45 ^{o}C	72.19 ± 0.19	17.40 ± 0.22	39.68 ± 0.47	27.42 ± 0.23
MV	72.33 ± 0.11	15.22 ± 0.23	39.19 ± 0.30	27.71 ± 0.16
1.5 min SB+MV	69.66 ± 0.23	14.71 ± 0.35	47.03 ± 0.37	24.62 ± 0.32
3.0 min SB+MV	66.64 ± 0.15	17.15 ± 0.28	47.56 ± 0.43	21.57 ± 0.23

SB, steam blanching; 45 ^{o}C, convective drying; MV, microwave vacuum drying

acid and other compounds as whole matrix, while the DPPH solution was specifically designed for determination of antiradical activity of phenolic compounds (Schaich *et al.*, 2015). Besides structural characteristics (differences in hydroxylation, glycosylation, and methoxylation) of the phenolic compounds, the contents of the individual phenolics also contributes to the overall antioxidant activity (Natic *et al.*, 2015).

Increase in 4-hydroxybenzoic acid concentration can be explained by the effect of thermal processing, which caused degradation of anthocyanin to benzoic derivatives, particularly 4-hydroxybenzoic acid. Increased temperature in combination with increased pH causes degradation leading to various benzoic derivatives (Patras *et al.*, 2010). Increase of syringic acid concentration under MV conditions can be explained by accelerated thermal degradation of anthocyanin glycoside (malvidin-3-glycoside) under MV and acidic conditions (Zhao *et al.*, 2013). As phenolic acids are susceptible to oxidation and degradation, exposure to light, oxygen and heat during food processing accelerates destruction of phenolic acids (Ravichandran *et al.*, 2012). The obtained results showed that processing at low temperatures and using MV drying can limit the effect of oxygen and thus limit destruction of the studied phenolic acids.

3-hydroxycinnamic acid was found in low amounts that decreased with thermal processing, but increased with drying — with convective drying by 79.5% and the highest increase (by 99%) was observed with MV drying. Rutin was detected in fresh carrots, but not after steam-blanching; with convective drying rutin concentration decreased by 78.8%, while with MV processing by 51.9%, 75.7%, and 70.8%. In fresh carrots quercetin and luteolin was found in high concentration, but was not detected after thermal and drying processes.

The predominant phenolic acids in carrots are chlorogenic acid, caffeic acid, p-hydroxybenzoic acid, ferulic acid and other cinnamic acid isomers (Shahidi and Ambigaipalan, 2015). In our study chlorogenic acid had the highest concentration among phenolic acids.

Decrease in concentration of rutin, quercetin and kaempferol during thermal and drying processing can be explained by oxidation. Quercetin is unstable and is easily degraded in a slightly alkaline environment, while photodegradation has no significant effect (Dall'Acqua *et al.*, 2012).

The main organic acid with antioxidant properties is ascorbic acid. In carrot it was found in high amounts; short term steam processes minimally affected its concentration, while longer steaming decreased concentration to 73.6% of the initial content. After convective drying carrots maintained 85.9% of the ascorbic acid concentration, while after MV drying the concentration was 76.5%, 64.6%, and 54.9% of initial amount. Degradation of bioactive compounds (ascorbic acid and anthocyanin) can be explained by oxidation during thermal processing and drying (Rawson *et al.*, 2011).

Principal component analysis (PCA) was used to explain differences in chemical composition between processing methods (Šumec *et al.*, 2016).

CONCLUSIONS

The importance of the presented research lies in the underlining of the bioactive compounds of steam-blanched and differently dried carrots that need to be considered to understand composition changes during processing and loss of these compounds. It was shown that carrots are a rich source of carotenoids and short-term steam-blanching and the selected drying techniques minimally changes carotenoid concentration. HPLC analysis was used to identify and quantify amounts of phenolic compounds and organic acids by comparison with standards. Flavonoids, benzoic derivatives and main organic acids with antioxidant properties had the highest concentration in fresh and thermal processed carrots; organic acids and two phenolic acids had highest concentration in convective dried carrots, while most of the identified phenolic compounds (mostly phenolic acids) were observed in high concentration in MV drier processed carrots.

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APSTRĀDES AR TVAIKU UN KALTĒŠANAS IETEKME UZ BURKĀNU FENOLU SASTĀVU, ORGANISKAJĀM SKĀBĒM UN KAROTINOĪDU SATURU

Burkāni (*Daucus carota* L.) ir pasaulē plaši izmantoti dārzeņi no Apiacea dzimtas, kas satur makro un mikro elementus, kā arī dažādus fitoķīmiskos savienojumus. Pētījuma mērķis bija analizēt karotenoīdus, organiskās skābes, fenolu savienojumu sastāvu un to antiradikālo aktivitāti, kā arī krāsu izmaiņas pēc apstrādes ar tvaiku (1,5 un 3,0 min) un kaltētos burkānus, kas kaltēti konvekcijas un mikroviļņu–vakuuma kaltēs. Analīzei tika izmantotas gravimetriskās, spektrofotometriskās un augstas izšķirtspējas šķidrumu hromatogrāfijas (HPLC) metodes. Karotenoīdi svaigos burkānos tika konstatēti lielā daudzumā, tomēr pēc termiskās apstrādes un kaltēšanas procesiem kopējais saturs samazinājās. Galvenās organiskās skābes, kas tika identificētas augstākās koncentrācijās svaigos un apstrādātos burkānos, bija skābeņskābe, vīnskābe, hīniskās, maloniskās un citronskābes. Pēc apstrādes ar tvaiku askorbīnskābes saturs samazinājās minimāli, bet pēc kaltēšanas procesa tā saturs ievērojami samazinājās. Svaigie burkāni satur minimālu kopējo fenola savienojumu saturu, un pēc termiskās apstrādes un visiem kaltēšanas procesiem šis saturs palielinājās, bet flavonoīdu, flavonolu, flavan-3-olu un fenolskābju saturs samazinājās. Izmantojot HPLC metodi, 3.4-dihidroksibenzoskābe un 3.5-dihidroksibenzoskābe, katehīns, 4-hidroksibenzoskābe, epikatehīns un sinapīnskābe bija augstākās koncentrācijās par citām identificētajām vielām.

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