

## Extraction of Carrot (*Daucus carota* L.) Carotenes under Different Conditions

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### Abstract

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This paper describes carotenes extraction from carrot under different conditions involving different temperatures, treatment of samples, and solvents (ethanol, 2-propanol). Carrot roots (*Daucus carota* L. cv. Nevis F1) were tested for the extraction yields of carotenes at temperatures 20°C, 40°C, and 60°C, the samples having been examined after harvest, after cold storage (stored at 5°C), and after freezing (−18°C). In connection with the technology of the carotenoid concentrate production from carrots, it was found that the solubility and yield of carotene from carrot depend on the temperature and the time of extraction as well as the treatment of the sample. It was revealed that the best extraction efficiency was achieved with the samples treated by freezing and using the extraction 60°C for 2–4 hours. Higher temperatures caused an increase in the carotenoid concentrations. The carrot variety Nevis F1 and the extraction at 60°C were used for the model production of carotenoid concentrate.

**Keywords:** carrot; extraction; carotenes; dyestuff; yield of  $\beta$ -carotene; temperature of extraction

Carotenoids are widely known as provitamin A, while there is an increasing interest in their role as antioxidants (BOHM *et al.* 2002). Anti-cancer activity and other health benefits provided by  $\beta$ -carotene include the protection against cardiovascular disease or cataract prevention (DIETMAR & BAMEDI 2001). Most studies have found beneficial associations between a higher intake of nutrients such as  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, and the outcomes associated with asthma and allergy

(DEVEREUX 2006). In recent studies, protective effects of carotenoids on bladder cancer (HUNG *et al.* 2006) etc.

Carrots are one of the best sources of  $\beta$ -carotene. The carotene content of carrots ranges from 60–120 mg/100 g, but some varieties can contain up to 300 mg/100 g (VELÍŠEK 1999). Carotens contain mainly  $\beta$ -carotene, i.e. about 80% (JESZKA 1997). The pigments are bound by proteins. Carrots can provide a significant amount of vitamin A; food

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matrices greatly affect the bioavailability of the plant carotenoids or their efficiency of conversion to vitamin A, or both (TANG *et al.* 2005). Some studies have indicated that  $\beta$ -carotene may be poorly absorbed from certain vegetables (BROWN *et al.* 1989).

The most popular methods for carotenes extraction are those using organic solvents. Most of them are toxic and expensive (SCHOEFS 2004).

The purpose of this study was to find the appropriate conditions for the extraction of  $\beta$ -carotene from carrot. The main goals were to study the influence of temperature, samples treatment, and solvents on the extraction yield of  $\beta$ -carotene, and to utilise the obtained knowledge for the establishment of the technology for obtaining biologically active concentrates of carotenoids made from the rich natural source-carrot.

## MATERIAL AND METHODS

**Carrots.** A Holland variety of carrot (*Daucus carota* L.) – Nevis F1 was cultivated in Slovakia (Komárno) and used for the laboratory determinations. The roots of carrots were analysed after harvest, after storage in cold room (4–5°C), and after freezing (–18°C) for one month. The content of carrot moisture was determined gravimetrically and the colour content in carrot was determined according to the method given in Slovak Technical Standard 56 0053.

**Extraction conditions.** Roots were cut to slices (width 2 mm, length 1 cm). The extraction yield of carotenes was observed at different temperatures (20°C, 40°C, and 60°C) using ethanol (96%, Reachem, Slovak Republic) and 2-propanol. Initially, 25 g of cut carrot samples were added to 100 g of 96% ethanol. Carrot slices were extracted in water bath (20°C, 40°C, 60°C), shaken after every 10 min, and after every hour of extraction 5 ml sample was taken and mixed with petroleum ether (20 ml). Water was added for the separation of phases, and after the separation the petroleum-ether-carotenoid phase was made up to the volume of 50 ml.

**Determination of carotenes.** The content of  $\beta$ -carotene in the petroleum-ether (Reachem, Slovak Republic) extract was determined spectrophotometrically, the absorbancy was measured at the wavelength of 450 nm using the spectrophotometer (Jenway, Great Britain). The concentration of carotenes expressed as  $\beta$ -carotene (g/100 ml) was calculated using the response factors as follows:

$$\beta\text{-carotene} = \frac{A \times d \times V}{E_{1\text{cm}}^{1\%} \times w}$$

where:

A – absorbancy

d – dilution

$E_{1\text{cm}}^{1\%}$  – coefficient of absorbancy (2592 for petroleum-ether)

w – weight of sample (g)

V – volume (ml)

**Dry matter determination.** (a) gravimetrically – moisture analysis of the samples (10 g) was performed in hot air oven at 105°C; (b) refractometry – with laboratory refractometer, to get the information about dry matter content in the extraction solvents as obtained during the technology of the carotenoid concentrate production. The results were expressed as g/100 g.

**Technology of carotenoid concentrate production** (Figure 1). According to our laboratory results, following main steps of carrot processing were established and tested to gain carotenoid concentrate in semi-manufacturing conditions.

Washing of carrot – carrot roots were washed with water. Clean roots of Nevis F1 of similar size in the amount of 30 kg were analysed for dry matter content and carotenes content (dry matter – 9.79%, carotenes – 15.17 mg/100 g).

Cutting – roots were cut to slices (2 mm).

Water content reduction – 2-propanol (26 l) was used at low temperatures 12 hours. After pressing, presswork was received that was used for the next extraction, and in the obtained solvent (43 l – 37.2 kg), the carotene content (0.45 mg/100 g) and, refractometrically, dry matter (3.45%) contents were determined.

Extraction – single-stage extraction with 2-propanol. The presswork (8.6 kg) and 35 l of 2-propanol were heated together (60°C) and mixed every 10 minutes. Every 30 min, 2 ml sample was taken, made up to the volume of 50 ml with 2-propanol, and the absorbancy (450 nm) was read. The extraction was performed until the third hour of extraction. The products of this step were the solvent and the presswork.

Pressing – the presswork were pressed during 20 min, under the pressure 1.8 kPa, then dried (70°C) and the contents of carotenes (13 mg/100 g) and dry matter (22%) were determined in the presswork after extraction (4.9 kg). The extract (33 l) was evaluated for the dry matter (0.279%)

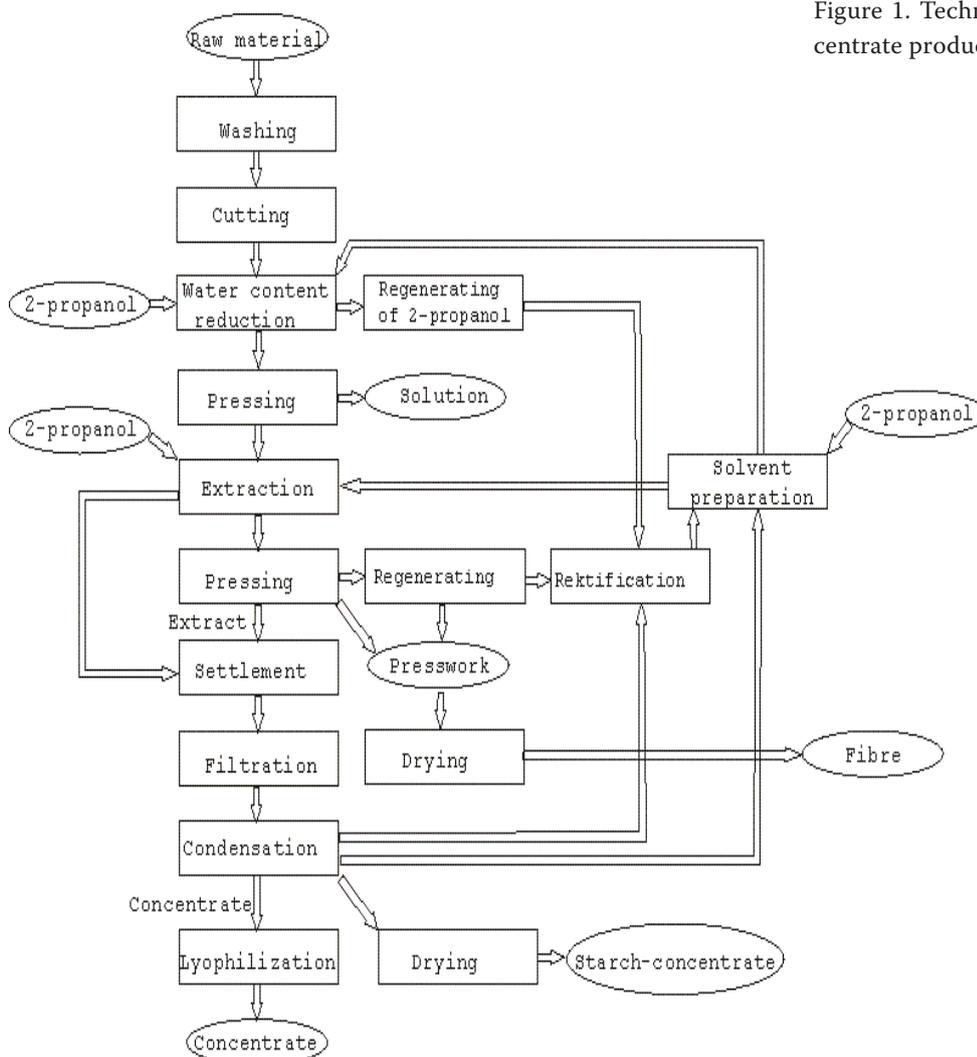


Figure 1. Technology of carotenoid concentrate production

and carotene contents (7.04 mg/100 g) as well. Distilled water was added to the extract (2 l).

Condensation of the extract – the extraction product was condensed in the vacuum evaporator. The pressure of 2–3 kPa and the temperature of 40°C were used until the solution of 4 l was obtained. Under a higher pressure of 960 hPa and the temperature of 50°C, 2-propanol and water were removed. The product of this step – the concentrate (1 l) was analysed for the dry matter (21.887%) and carotenes – 82.51 mg/100 g contents.

Lyophilisation – Lyovac GT 2 was used for lyophilisation. The product achieved was a powdered concentrate of carotenes (177.07 g – 508 mg/100 g carotene contents).

Another treatment of the carotenoids concentrate (157.66 g), was drying it on corn starch at lower temperatures (max. 40°C); the sample was diluted – 10 g of starch: 10 ml of concentrate-SCC.

**Statistical evaluation.** The results of the laboratory examinations were tested using Multifactor Analysis of Variance.

## RESULTS AND DISCUSSION

### Extraction yield of $\beta$ -carotene from fresh samples by the influence of time, treatment, and temperature

The processing of foods involves changes in the structural integrity of the matrix which produces both negative (loss of carotenoids due to oxidation) and positive (increased bioavailability) effects (LIVNY *et al.* 2003). Light, heat etc. promote isomerisation of carotenoids. Oxidative degradation, the principal cause of extensive losses of carotenoids, depends on the availability of oxygen and is stimulated by factors such as light.

At 20°C, the yield of carotenes from the fresh after-harvest sample was slightly affected by the time and temperature (not shown). After 5 h of extraction, the fresh sample showed 1.59 mg/100 g of carotene yield, the velocity of extraction being very slow.

With the extraction at 40°C, the yield of carotenes (2.47 mg/100 g) was higher compared to that at 20°C while the highest extraction yield was found at 60°C (Figures 2 and 3). At 60°C, the extraction yield of carotenes was found to be high already after 10 min of extraction, which can be explained by a good release of carotenes from the disturbed texture of the carrot at 60°C. At 60°C, the extraction maximum was found in the second hour of extraction (4.22 mg/100 g). After this time, the extraction yield of  $\beta$ -carotene decreased (Table 1). Compared to the third and fourth hours, the extraction was almost the same as the result of the degradation and loss of carotenes. Naturally,  $\beta$ -carotene exists in the all-*trans* form. After processing, some part of all-*trans* form is converted into its different *cis*-isomers (AMAN *et al.* 2005). CALVO *et al.* (2007) state, that in the extractions performed with ethanol at 60°C, the yield of lycopene and its isomers was lower than at 50°C, that could indicate an extensive isomerisation at the high temperature with ethanol, but the oxidative degradation being the predominant reaction.

Optimal time for the best extraction of carotenes seems to be 2 to 4 h, depending on the treatment of the sample; at lower temperatures (40°C) and with fresh samples, a longer time (min 5 h) is needed for the extraction CALVO *et al.* (2007) found the highest  $\beta$ -carotene concentration from

lyophilised and milled skin of tomatoes at 25°C while the times assayed did not have a great influence on the  $\beta$ -carotene yield. RAFAJLOVSKA *et al.* (2007) showed that the increase of temperature positively influences the mass transfer processes. The increased colour yields also result from the changes in the cellular structure of the biological matrix. On the other hand, the increase of the extraction temperature may cause raw material browning. Due to this circumstance, it is required to establish the optimal extraction temperature.

#### Extraction yield of $\beta$ -carotene from treated samples by the influence of time, temperature and treatment

Increased carotenes yields due to storing and freezing of the samples were shown as compared to the after-harvest samples (Table 1). The best solubility of carotenes was found at 60°C, the heat treatment according to DUTTA *et al.* (2005) such as blanching, cooking, and streaming help to release the carotenoids bound by protein and render them easily extractable. Hence, the carotenes content increased from 4.12 mg/100 g fresh sample to 5.68 mg/100 g frozen sample already after the first hour of extraction at 60°C (Table 1), meaning an increase of 37%. After the fourth hour of extraction at 60°C, the increase caused by freezing was from 3.98 mg/100 g in the fresh sample to 6.45 mg/100 g in the frozen sample, which means 62% higher yield of carotene.

Storage of the samples increased the extraction yield of carotenes, but a statistically significant effect of cold storage was confirmed only at 60°C. Comparing the fresh after-harvest carrots and the

Table 1.  $\beta$ -carotene yield (mg/100 g) at temperatures of 40°C and 60°C from different treated samples of carrot cv. Nevis F1

Time of extraction (min)	Treatment of samples at 40°C			Time of extraction (min)	Treatment of samples at 60°C		
	after harvest	cold storage	freezing		after harvest	cold storage	freezing
60	2.11	2.35	2.52	10	2.59	2.9	3.63
120	2.14	2.56	2.61	60	4.12	5.22	5.68
180	2.19	2.59	2.64	120	4.22	5.29	6.22
240	2.3	2.61	2.7	180	4.05	5.14	6.33
300	2.47	2.69	2.69	240	3.98	5.11	6.45
CV*	3.82	4.75	2.87 <sup>+</sup>	CV	2.50	1.57 <sup>+</sup>	5.51

\*CV = coefficient of variations (%) – measuring after extractions 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> hours

Table 2. Multifactor analysis of variance (extraction at 40°C and 60°C) – main effects

Temperature (°C)	Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Significance level
40	samples treatment	0.43068	2	0.21534	58.49	0.0000 <sup>++</sup>
	time of extraction	0.1419067	4	0.0354767	9.636	0.0038 <sup>++</sup>
60	samples treatment	8.742333	2	4.3711667	45.129	0.0000 <sup>++</sup>
	time of extraction	10.785573	4	2.6963933	27.839	0.0001 <sup>++</sup>

stored and frozen samples, the samples treated by freezing showed the best and the highest release of carotene, the influence of freezing having been statistically significant at 40°C and 60°C as well. According to VAN HET HOF *et al.* (2000), the processing such as the mechanical homogenisation or heat treatment has the potential to enhance the bioavailability of carotenoids from vegetables (from 18% to a sixfold increase). The better resorption of carotenes from the heat-treated carrot is explained by the disruption of the cells, the nutrients being better utilised in the body. Enzymes can also affect the cells disruption.

In our case, it was statistically confirmed that with increasing time, the extraction yield of  $\beta$ -carotene is higher (Table 2). The statistical evaluation of the influence of the extraction time showed a significant difference between, the first hour and the fourth hour of extraction at 40°C. In the case of 60°C, a significant difference was found between, the extraction yields after 10 min and the first hour of extraction, then between 10 min and the second hour, and the third hour and the fifth hour of extraction as well.

### Role of solvent

Carotenoids are soluble in apolar solvents, including edible fats and oils. Because carotenoids are liposoluble, they are usually extracted from the plant sources with organic solvents such as chloroform, hexane, acetone, petroleum ether, etc. (BELITZ *et al.* 2004). The samples can contain large amounts of water; water-miscible organic solvents such as ethanol are also used. One of the problems is the elimination of the residual solvents to obtain a safe extract; this can be avoided by using food grade solvents such as ethanol. CALVO *et al.* (2007) found that the yield of each carotene from peel powder of tomatoes was noticeably higher with extraction performed with ethanol than with that using ethyl acetate.

Ethanol was chosen as the extractive solution in our laboratory conditions. Another solution for the model production of carotenoid concentrate tested was 2-propanol; the extraction of  $\beta$ -carotene into this solution was higher as compared to ethanol (Figure 2). Therefore, to obtain the carotenoid concentrate in semi-manufacturing conditions, 2-propanol was used as solvent. Cv. Nevis F1 with 60°C extraction was used for the experimental production of carotenoid concentrate (Figure 1).

### Technology of carotenoids concentrate production and its application

Due to our steps of the established technology (Figure 1), two forms were obtained of colouring concentrate and dietary fibre as the most interesting products in the food industry. Both concentrates were tested for their application to food products (FIKSELOVÁ *et al.* 2007). According to these results and due to the fact (ŠULC *et al.* 2007) that lyophilisation can cause some loss or degradation of the valuable biologically active components (e.g. antioxidants), we recommend to produce this colouring concentrate by drying the extract on edible starch or another medium at low temperatures. Carotenoid concentrate can

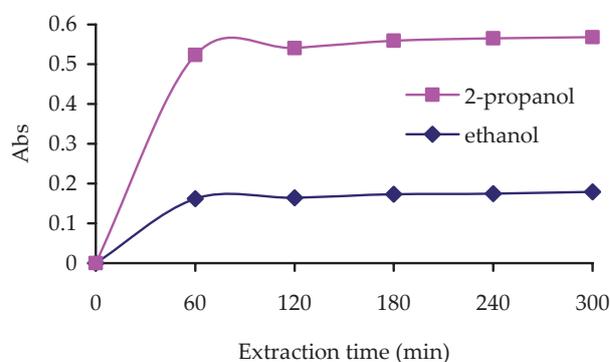


Figure 2. Comparison of different solvents for carotenes extraction from carrot

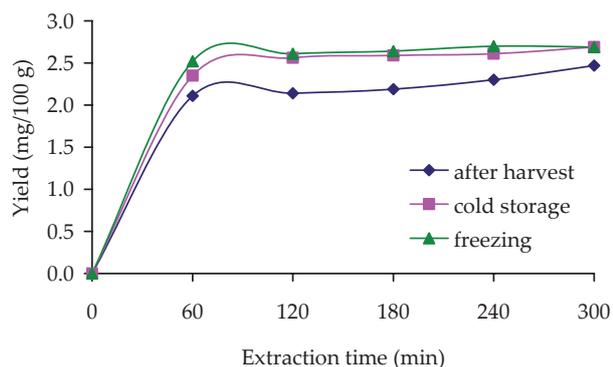


Figure 3.  $\beta$ -carotene yield obtained during extraction of differently treated samples of carrot cv. Nevis F1 with ethanol at 40°C

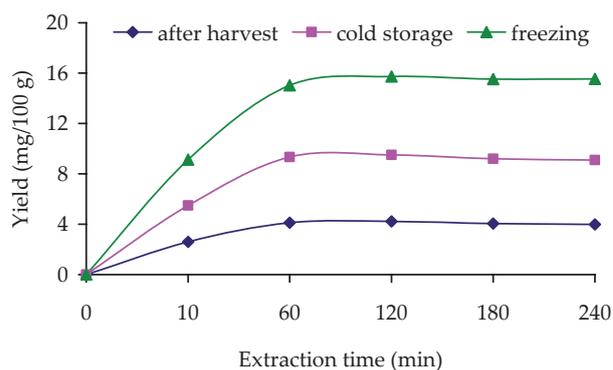


Figure 4.  $\beta$ -carotene yield obtained during extraction of differently treated samples of carrot cv. Nevis F1 with ethanol at 60°C

be used as a natural dyestuff for the application to margarine, ice creams, various cheese products, beverages, sauces, meat, confectionery, etc. to gain better quality of the products. Functional foods refer to foods that provide an additional health benefit above and beyond their pure value. This term is used for many product concepts, ranging from vitamin or mineral enriched foods to the use of herb and plant extracts (SASS 2007). FIKSELOVÁ and NÉMETH (2007) claim that functional foods are also the products derived from the naturally occurring substances as a part of the daily diet and possessing particular physiological benefits when ingested, but can also be defined as foods with altered bioavailability.

KESHARLAL *et al.* (www.wikipatents.com) prepared pharmacologically and biologically active compositions containing carotenoids in combination with micro- and macro-nutrients from carrots and used them in formulas for health care and nutrition applications. Their process includes sequentially treated carrot juice with a carboxylic acid and a saccharide to obtain a carotenoid fraction rich in micro- and macro-nutrients in proportions compatible with those originally found in the natural state.

## CONCLUSION

We can conclude that the temperature, increased up to 60°C, positively influences the extraction yield of carotenes from carrot, however, for the technology of carrot processing we recommend to use lower temperatures of approximately 40–60°C. A positive effect was also shown by freezing or cold storage of carrot, the time needed for the extrac-

tion depending on the treatment of the sample and temperature used (approximately 2–4 h). 2-propanol is a more suitable solvent in terms of the carotenes yield as compared to ethanol.

The described model technology for obtaining carotenoids can be modified (temperature of extraction, more-stages extraction). Besides carotenoids concentrate, another interesting by-product gained is dietary fibre, with excellent water binding capacity, which can be therefore used for functional foods production (as bread or pastry supplement).

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