Evaluation of Extraction Methods for the Analysis of Carotenoids for Different Vegetable Matrix

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Abstract. In this study, different solvents were used to achieve the maximum extractibility of total carotenoids. The extracted total carotenoids were estimated using UV- visible spectrophotometer. Carotenoids from vegetable matrix can be used as a food colorant, food additive, cosmetics, antioxidants and nutraceuticals.

Keywords: *Cucurbita maxima* L., *Hippophae Rhamnoides*, *Zea mays* L., total carotenoids

Introduction. In pumpkins, carotenoid is a natural plant pigment which is responsible to give the orange colour. Sea buckthorn fruits grows widely in Europe, central Asia and temperate regions of South Asia, India and China, ranging in altitude of a minimum of 60 m asl to a maximum of 5200 m asl. The juice provides a nutritious beverage, high in suspended solids and very high in carotenes (Beveridge T. *et al.*, 1999). Maize (*Zea mays* L.) is one of the important cereal crops and used as staple food for human being, feed for animals and for diverse processed products across the continents. Amongst the three major cereals, only maize kernel contains coloured carotenoid compounds that can be converted into vitamin A in the human and animal body (Mishra and Singh, 2010).

Aims and objectives. The aim of the paper was to study the extraction of two methods and differences in the carotenoid content of six sample, by using spectrophotometer analysis.

Materials and methods. The experimental material comprised 6 samples: pumpkin pulp, pumpkin juice, sea buckthorn fruits, sea buckthorn juice and two corn hybrids. The pumpkin and sea buckthorn fruit was bought from the supermarket. Samples were cut to reduce size and directly extracted and analyzed. Juice extraction from pumpkin and sea buckthorn is carried out using pressing technique. The two samples come from a corn research station. The corn kernels was ground with a laboratory mill. The samples were stored at 4 °C. Analytical methods for the study of carotenoids include basically the preparation of the sample, organic solvent extraction and quantification of these. All chemicals were obtained from Sigma-Aldrich or Merck (Darmstadt, Germany).

Procedure 1. Room temperature rehydration and extraction with mortar and pestle. Rehydration allows efficient penetration of the extraction solvent into the samples tissues. Weight 3 g the ground sample in a glass, add sufficient water to cover the ground sample and let stand for 30 min. Add about 20 ml of cold acetone (acetone refrigerated for about 2 hours) and let stand for 15 min. Filter with suction through a Buchner funnel. Put the solid in a mortar grind well with the pestle and add about 50 ml of cold acetone and grind again with the pestle to extract the carotenoids. Three or four extractions/filtrations are usually enough, but if the residue is still colored, repeat again. Was put in a separatory funnel all filter contents, add 50 ml of diethyl ether

and wash three times (3x200 ml) with saturated NaCl solution. The upper phase was collected and concentrate the carotenoid solution in the round-bottom flask in a rotary evaporator (temperature must not exceed 35°C). Recovery was done with petroleum ether. It may be necessary to concentrate or dilute the carotenoid solution (the absorbance should be between 0.2 and 0.8). The total carotenoid content present in the extract was measured at λ 450 nm using UV-visible spectrophotometer (UV – 1700 series Shimadzu).

Procedure 2. Weight the sample (the sample weight as in procedure 1), add about 30 ml methanol/ ethyl acetate/petroleum ether (1/1/1, v/v/v) and 0.1 % BHT (w/v). The sample was put on a magnetic stirrer for 1 hour. Filter with suction through a Buchner funnel. Put the solid in a beaker, add about 30 ml of methanol/ ethyl acetate/petroleum ether and was again put on magnetic stirrer for 1 hour. Return the residue to the mortar, add 30 ml methanol/ ethyl acetate/petroleum ether and macerate again. The filtering and separation was made in the same way as described in procedure 1, but the recovery was made with methanol.

Results and Discussion. The results showed a total carotenoids content at pumpkin pulp of 47,2 $\mu\text{g/g}$ dry matter for procedure 1 and 53,38 $\mu\text{g/g}$ dry matter for procedure 2. In some studies done by Lucia Maria Jaeger de Carvalho *et al.* (2012) and Norshazila *et al.* (2012) reported that values obtained from pumpkin pulp was ranged from 7,05 to 138,56 $\mu\text{g/g}$ dry matter. The carotenoid content values are according with the data from literature and for the other samples.

Fig. 1. Total carotenoids content from six samples P1-P6

The results ($\mu\text{g/g}$ Dw) for total carotenoids content noted as: P1-pumpkin pulp, P2-pumpkin juice, P3-sea buckthorn fruits, P4-sea buckthorn juice, P5-corn hybrids, P6-corn hybrids.

Conclusion. The study showed that the extraction with methanol/ethyl acetate/petroleum ether was more efficient for the pumpkin pulp and pumpkin juice samples meanwhile for the corn sample the cold acetone method appears to be the efficient one. For the sea buckthorn and sea buckthorn juice samples good results were obtained by both extraction methods.

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