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Publication date:
2004

Document version:
Final published version

Citation for pulished version (APA):
Larsen, E., & Christensen, L. P. (2004). *A simple saponification procedure for the quantitative determination of carotenoids in green vegetables*. Poster session presented at 3rd International Congress on “Pigments in Food, more than colours…” , Quimper, France.

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A Simple Saponification Method for the Quantitative Determination of Carotenoids in Green Vegetables

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Introduction

The carotenoids are one of the most important group of natural pigments because of their wide distribution in the plant and animal kingdoms, structural diversity and numerous functions. Owing to the possible health promoting properties of carotenoids on human health (1), the carotenoid composition of foods has been analyzed extensively (2,3). Saponification with sodium hydroxide or potassium hydroxide has long been an integral part of both vitamin A and carotenoid or pro-vitamin A analyses (2–5). For carotenoids, saponification is carried out with the deal aim of eliminating chlorophylls that interfere in the spectrophotometric assay and for removing unwanted lipids. A decrease in the carotenoid content is usually observed during saponification varying from a few percent to 100% depending on the concentration and structure of the carotenoid and on the saponification procedure.

Aim

The aim of the present work is to develop a simplified saponification procedure with a minimum of degradation/isomerization of carotenoids.

Materials and methods

Plant materials and chemicals. Samples of fresh (green pepper, green chili, broccoli, different kinds of lettuce, cucumber, squash, and celeriac) and frozen (peas, beans, parsley, chives, kale, spinach, and Brussels sprouts) vegetables were obtained from a local grocery store and frozen (–20°C) until use. Ambersep 900 OH was purchased from Aldrich and washed with distilled water prior to use.

Extraction, saponification, and determination of pigments. Frozen samples of the vegetables (5.0 g) were homogenized 60 s in acetone (20 mL) in a centrifugation vial using a ultra-turax blender. After 1 h the mixture were centrifuged (12,000 rpm) and part of the supernatant (3 mL) was filtered and transferred directly to a HPLC vial. Another part of the supernatant (10 mL) was transferred to a vial containing Ambersep 900 OH (1.5 g) and a small stirring bar. After the mixture was stirred for 30 min, 3 ml supernatant was transferred directly to a HPLC vial. Separations were performed on a LiChrospher 100 RP-18 column (5 μm; 244 × 4 mm i.d.) (6). The column temperature was 30 °C and the mobile phases consisted of solvent A (80% MeOH–20% H_2O) and solvent B (100% EtOAc). Separations were performed by the following solvent gradient: 0 min 20% B, 2.5 min 22.5% B, 20–22.5 min 50% B, 24–26 min 80 % B, 31–34 min 100% B, 42–47 min 20% B. The flow rate was 1 mL/min.

Figure 1. Before and after action of Ambersep 900 OH on spinach extract.

Results and discussion

The saponification of extracts of green vegetables usually involves adding methanolic potassium or sodium hydroxide and either refluxing the solution or leaving it under nitrogen for many hours. Afterwards, the mixture needs to be extracted with water to remove the alkali salts. It is obvious that these operations are time and labor intensive. In spite of that, a saponification step might be justified to remove unwanted chlorophylls and fatty acids in order to improve resolution and reduce HPLC analysis time.

We have found that just adding a strongly basic resin (Ambersep 900 OH) to acetone extracts is very efficient in removing chlorophylls (Fig. 1). During stirring, the solution changes color from green to yellow and the polymeric beads changes color to slight green. To avoid long reaction times, stirring is necessary and usually it will take about 30 min to remove the chlorophylls from the solution. A likely action of Ambersep 900 OH on the chlorophylls is shown on Fig. 2. Preliminary results indicate that the method is very mild and works well with a large variety of green vegetables, e.g. green pepper, green chili, broccoli, cucumber, squash, celeriac, different kinds of lettuce, peas, chives, kale, beans, parsley, spinach and Brussels sprouts. Minor changes in the carotenoid profile are sometimes observed.

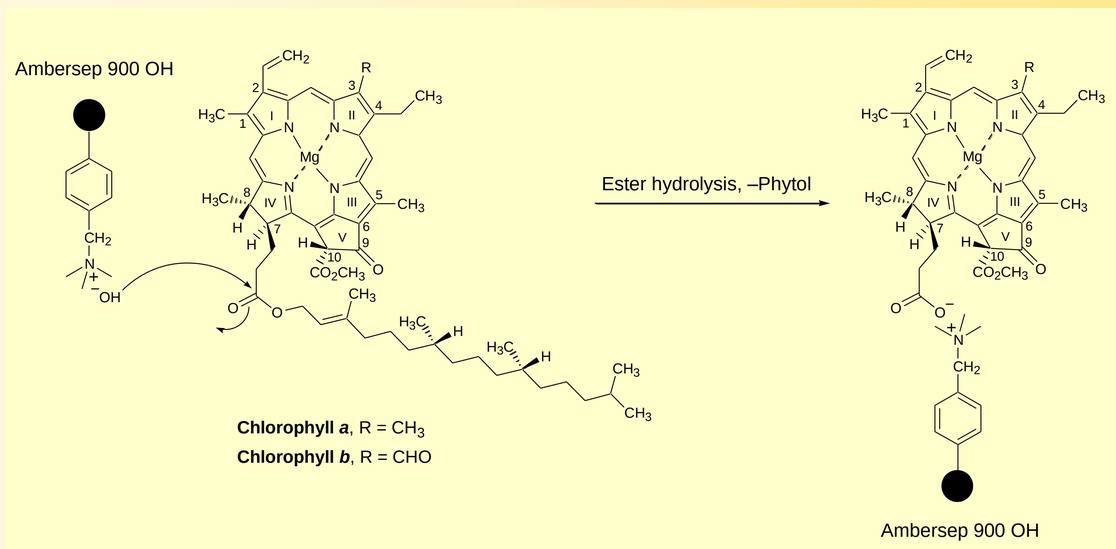

Figure 2. Reaction between Ambersep 900 OH and chlorophylls.

The extracts (before and after action of Ambersep 900 OH) were analyzed by HPLC-DAD and the chromatograms showed complete removal of the chlorophylls while the concentration of the carotenoids were unchanged (Fig. 3).

Figure 3. HPLC chromatograms before and after action of Ambersep on parsley extract. The xanthophylls (5–16 min) are unchanged while the chlorophylls (18–25 min) are completely removed.

Conclusion

The method described here for the selective removal of chlorophylls from extracts of green vegetables is very simple and reliable compared to the standard methods used for basic hydrolysis. This should allow more laboratories to introduce a chlorophyll removal step to shorten the HPLC analysis time for carotenoid analysis of green vegetables.

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