

# Enzymatic Method: The best choice for extraction of carotenoids of Alfalfa

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## ABSTRACT

Carotenoids are natural compounds present in animals and plants. They have important applications in food and drugs and are usually extracted from natural materials. Alfalfa is rich in carotenoids. Commercial methods have been described for extracting carotenes and xanthophylls from alfalfa. In the present study, carotenoids were extracted and measured from alfalfa by acid, alkaline and enzyme extraction methods. The results showed different contents of the carotenoids in different methods of extraction. The carotenoids level when enzyme was used for the extraction from Alfalfa was significantly more than acid and alkaline. This finding shows that the pigment was probably attached to proteins in alfalfa. In this experiment, sodium hydroxide could also replace enzymatic methods. The amount of pigment was followed by sodium hydroxide treatment after enzymatic method.

**KEY WORDS:** Alfalfa, Carotenoids, Extraction.

## 1. INTRODUCTION

Nowadays medicinal plants are widely used to treat or prevent various diseases (Asadbeigi, 2014; Karamati, 2014; Bahmani, 2016; Shaygannia, 2016; Delfan, 2014). They are also used for preparation of new drugs and coloring ingredients for industrial purposes (Bahmani, 2013; Sarrafchi, 2016; Bahmani, 2014; Saki, 2014; Bahmani, 2014). Pigments are natural products and the major attributes to the quality of a food product, affecting the appearance and acceptance of the product, affecting the appearance and acceptance of the product (Sowbhagya, 2010). Carotenoids are one of the most important pigments in plants. Carotenoids were first discovered in carrots, from which in 1831 a compound that was named 'beta-carotene' was isolated. They are essential for photosynthesis and in general for life in the presence of oxygen, and for numerous biological functions. These components are known to provide a range of biological effects (provitamin, antioxidant, coloring, etc.). The role of carotenoids as a source of pigment and in immune defense system has been established. They have antioxidant activity and provitamin function.

Several methods have described to extract these pigments from plant materials. Carotenoids are usually extracted by using different solvents. Carotenoids have been extracted using organic solvents but the heightened public awareness of health issues and the application of more restrictive regulations have stimulated other technologies (Macas-Snchez, 2010). The most effective known solvents are products of the petroleum industry which are toxic for human consumption (Safarnejad, 1996).

Alfalfa (*Medicago sativa* L.) is a valuable forage crop which is grown in areas of limited rainfall, high temperature where the land is often salt affected (Ishida, 2009). It is recognized as the oldest plant for forage. This plant has good natural source of raw materials such as carotenoids. The application of the different methods in the recovery of carotenoids from alfalfa such as solvent extraction method has been widely used. The aim of this study was to use non solvent extraction techniques and compare these techniques for extracting carotenoids from Alfalfa.

## 2. MATERIALS AND METHODS

**The test materials:** Alfalfa was collected in West of Iran and identified by Dr. Atousa Ziaei. Then it was air dried in the shade and powdered.

**Extraction of carotenoids by trypsin:** Five g of sample was placed in test tube and dissolved in de-ionized water. Separation of carotenoids was done by trypsin. 2% of trypsin was added to samples and heated at 37°C for 120 min. The hydrolysate was then centrifuged and the supernatant was used for experiments.

**Extraction of carotenoids in alkaline condition:** The sample (2 g) was placed in test tube and dissolved in the NaOH. Two concentrations 0.1 and 0.01N were chosen. The samples were kept in 4°C for 48 h. The hydrolysate was then centrifuged and the supernatant was used for experiments.

**Extraction of carotenoids in acid condition:** 2 g of samples were placed in test tubes. For induction acid condition, HCl and H<sub>2</sub>SO<sub>4</sub> were chosen. Two concentrations 0.1 and 0.01 N were used for each acid. The samples were kept in 4°C for 48 h. The hydrolysates were then centrifuged and the supernatant was used for experiments.

**Determination of total carotenoids of extraction:** Total carotenoids were determined by  $\beta$  carotene standard curve and by spectrophotometric method at 470 nm. The total carotenoids content of samples was calculated on the basis of the standard curve of  $\beta$  carotene (Sowbhagya, 2010).

**Statistical Analysis:** The concentration of carotenoid was calculated using the standard curve obtained by commercial  $\beta$  carotene:  $Y=0.1566x-0.002$ ,  $R^2=1$ . The evaluation was made by comparing groups using analysis of T-Test in SPSS software. The difference more than 95% ( $p \leq 0.05$ ) was considered significant.

### 3. RESULTS

The results of this study were summarized and expressed as mean  $\pm$ SD in Table 1. The level of carotenoids in enzymatic method ( $6.1 \pm 0.53$  g) was significantly different compared to the ones in other groups ( $p < 0.05$ ). The amount of pigment was followed by sodium hydroxide and acid treatment, respectively (Table 1). The amounts of carotenoids were significantly different between groups according to normality in acid and alkaline. The  $p$  value, in HCl group was 0.01. The change in alkaline condition was also observable ( $p = 0.02$ ). The carotenoids amount in  $H_2SO_4$  was slight difference recorded.

**Table.1. Level of carotenoids (mg/g)**

Acid condition				Alkaline condition NaOH(N)		Enzymatic condition
HCl(N)		H <sub>2</sub> SO <sub>4</sub> (N)				
0.1 (a)	0.01 (b)	0.1 (c)	0.01 (d)	0.1 (e)	0.01 (f)	Trypsin(%2)(g)
0.86± 0.01	1.18± 0.02	0.96± 0.01	1.38± 0.01	1.5± 0.03	2.4± 0.16	6.1± 0.53

### DISCUSSION

In this study, the maximum extraction yields of carotenoids were obtained in enzymatic condition. Over the years, numerous procedures have been proposed for the isolation of colors from plant materials. Generally, the methods of extraction for pigments are solvent extraction. But, the application of enzymes for separation is also reported in literature. The enzymes can lose the structural integrity of plant materials (Thaipong, 2005). Therefore, the color extraction will be enhanced. In our study, the most amounts of carotenoids were also obtained from alfalfa in enzymatic method. In the previous study it was also revealed that trypsin recovered highest amount of carotenoids from shrimp wastes (Babu, 2008).

The described process offers enzymatic condition as a selective extraction of carotenoids which can be used for food dyes. These data will also provide evidence for the connection between the protein and carotenoids. In this process, protein-pigment complexes were separated with a protease enzyme. The carotenoprotein complexes are usually water-soluble (Pilbrow, 2010).

In this work, an alternative extraction process for carotenoids alkaline condition was proposed. In the present study, sodium hydroxide extraction was more effective than acid extraction. In food industries, sodium hydroxide is used for different purpose including peeling of potatoes, solubilizing plant proteins, neutralizing casein preparations (sodium caseinate), and removal of toxic constituents such as aflatoxin (Whitaker, 2009). Therefore, pigment from the alfalfa could be efficiently and safety also achieved by this method.

In acid and alkaline groups, the levels of carotenoids were dependent to normality of acid and alkaline. When the normality of acid and alkaline was decreased from 0.1 to 0.01 N, the slight increase in the extraction yield observed (Table 1).

This plant is an inexpensive animal feed rich in minerals, vitamins, carotenoids, and protein. Their carotenoids are vital nutrient for healthy growth and tissue color in fish. Alfalfa also utilize in pigmentation of the egg yolk (Yanar, 2008). Furthermore, carotenoids can apply in food such as margarine, ice cream, cheese, etc to obtain better health benefit. Its dye can be easily used as natural component for application in feed and food throughout the world. This matter could be considered by food industries. These products have functional and active ingredients. Carotenoids are considered to be critical in protection against oxidative damages. Carotenoids are soluble in the polar solvents including edible fats and oils. They are usually mixed with food; this method will boost the antioxidant content of food. Fatty foods such as oils are very sensitive to oxidation. Carotenoids inhibit lipid per-oxidation. They can delay oxidative rancidity (Antone, 2012). Therefore, carotenoids can keep foods for longer time.

### CONCLUSION

In conclusion, this study showed that enzymatic extraction is more effective as compared with other methods and the extraction of carotenoids from the plant could be efficiently and economically achieved by this method.

### ACKNOWLEDGEMENTS

Authors are grateful from Department of Environmental Health Engineer, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

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