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Carotenoids Extraction Optimization of Lutein-Based Banana Peel

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ABSTRACT

Carotenoids are among pigments with antioxidant properties and healthy effects on human body. Lutein is one of the most important carotenoids that is abundantly found in banana peel and belongs to xanthophyll's family. The present study aims to banana extract in order to use the applied properties of its carotenoids specially lutein. The banana peels were dried in vacuum oven and changed to powder, three solvents of ethanol 96%, methanol 99.5% and ethyl acetate 80% were used in 27 and 35 centigrade to extract its juice and study the amount of lutein. HPLC, UV-VIS spectrophotometer and FRAP test were respectively used to determine the amount of lutein and the antioxidant power of the extracts. The findings showed that considering the inhibition time of pure lutein with HPLC that took about 2.311 minutes, the amount of lutein gained by ethyl acetate solvent was more as compared to other extracts and its peak area was about 400.Similarly, through UV-VIS colorimetry and considering the amount of light absorbance of pure lutein, the amount of light absorbance in ethyl acetate solvent in 27°C was more as compared to other samples and its amount of absorbance was about 2.204.The antioxidant power of ethyl acetate in 27°C in FRAP method showed the higher amount.

KEYWORDS: Antioxidant, Ethyl acetate, Colorimetry, Carotenoids, Lutein

1. INTRODUCTION

Nowadays, the waste materials of food industries are of significant importance. The extractions of major and useful compounds such as antioxidants, enzymes, antimicrobial compounds and the like from these materials looking to be useless add to their values. One of these waste materials is banana peels with significant carotenoids compounds including lutein.

Carotenoids including beta-carotene, alpha carotene and lycopene play a part in light absorption by the plants. Beta- carotene and alpha- carotene are responsible for the orange color of carrot, lycopene is responsible for the red color of tomato and Astaxanthin is responsible for the pink or red color in crabs and salmon fish [20].

Thanks to their wide distribution in animal and plant resources, and their antioxidant and bioactive properties, Carotenoid shaves so many applications in coloring food materials. Among the carotenoids that have more usage as strainers are beta- carotene, lutein, zeaxanthin and lycopene [8].

For the customer, the desired color in different edible products is a sign of suitable production process and favorable quality of the product. That is why using synthesized or natural pigments in food industry has become widespread. However, nowadays, the concern about using synthesized colors and the customers' tendency to use natural colored materials, has directed the attention toward using natural pigments [7].

Carotenoids as antioxidants have an effective role in preventing oxidation reactions.

In a large number of fruit wastes, the antioxidant properties are available because of the presence of carotenoids. For instance, the remaining of star fruit, pressed grapes, citric fruit peels, pomegranate wastes and banana peels are ranked as cheap sources of antioxidants [1, 2, 3, 4, 5].

The skin and pericarp layer of ripe tomato almost contain 80% of the total lycopene existing in ripe tomato [10].

The fruit peels are rich source of colored and antioxidant compounds .By extracting different isomers of carotene and tocopherol from the peels and suitable processing, the risk of contamination caused by wastes removal can be reduced and the productivity of production in agriculture and food sector can increase [6].

2. MATERIALS AND METHODS

Here we are studying some carotenoid compounds in lutein-based banana peels. In extracting carotenoid extract from banana peels, the solvents ethanol 96%, methanol 99.5% and ethyl acetate 80% have been used, all the three was made by Merck Company in Germany.

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2.1. Banana peels preparation

After providing banana with Dwarf Cavendish variety (preferably a little more yellow) and complete wash, they were peeled and were put in vacuumed VO400 oven made in Germany in 40°C temperature for 14 hours to dry. The dried banana peel was then powdered by mill (made in Germany) and was placed in a dry place.

2.2. Extraction with solvent

At first, 15 gr Banana peel was mixed with50mL of each solvent and the mixture was poured in Erlenmeyer flask and was covered by an aluminum sheet. Then it was placed into shaker incubator (Fanavarn Sahand model made in Iran) in 27 and 35°C for 150 rounds per minute for 12 hours and was then filtered by Whatman paper number 2.It was finally put in rotary machine (LABOROTA 4003 – control) in 40°C with 70 rounds in suitable vacuum so that the remained solvent is omitted and was finally filtered by Buchner funnel (filtration set made by commonwealth market) and was moved to 100mLvolumetric flask. The flasks were covered by aluminum sheet in 5°C inside the fridge in order to keep away from sun.

2.3. High Performance Liquid Chromatography (HPLC) preparation

At first, a mixture of methanol (80%), distilled water (5%) and methyl-tert-butyl-alcohol (15%) was added in solvent container (mobile phase) of HPLC (Agilent Technologies 1200 series) until the machine reaches its base state [12].Then the extracts were injected and twenty minutes were given for each one until the peaks appear. This is the time that after 4.1 minutes, the first and highest peak of extracts appears and the peak area was read.

2.4. UV-VIS Spectrophotometric Determination

At first, UV-VIS Spectrophotometer (Agilent Technologies Cary 8454 made in USA) in 245 nm wavelength was calibrated by pure methanol. Then the pure lutein absorption with it was read .This method was used to measure lutein-based colorimetry of the extracts [13].

2.5. Measuring antioxidant power of the extracts by Ferric reducing Antioxidant power (FRAP) method

FRAP solution was primarily prepared as follows: Buffer acetate, TPTZ reagent and 20 mL solution of iron (III) chloride hexahydrate were mixed in 1:1:10 volume ratio and were kept in a dark place. Then spectrophotometer was calibrated by TPTZ solution and the amount of absorption accompanied with iron power of inhibition against control in 595 nm was read [14].

In the presence of antioxidants, the intensity of color increases. By measuring the color intensity, antioxidant capacity can be gained.

The results of FRAP test is strictly related to the time of analysis .Therefore FRAP method will be appropriate for comparative studies such as the impact of one treatment on antioxidant capacity.

3. FINDINGS AND DISCUSSION

3.1. Quality Identification of lutein

In HPLC, the peak of different extracts with 5µL in 446nm wavelength was compared with pure standard lutein peak .The summary of which is shown in table 1.

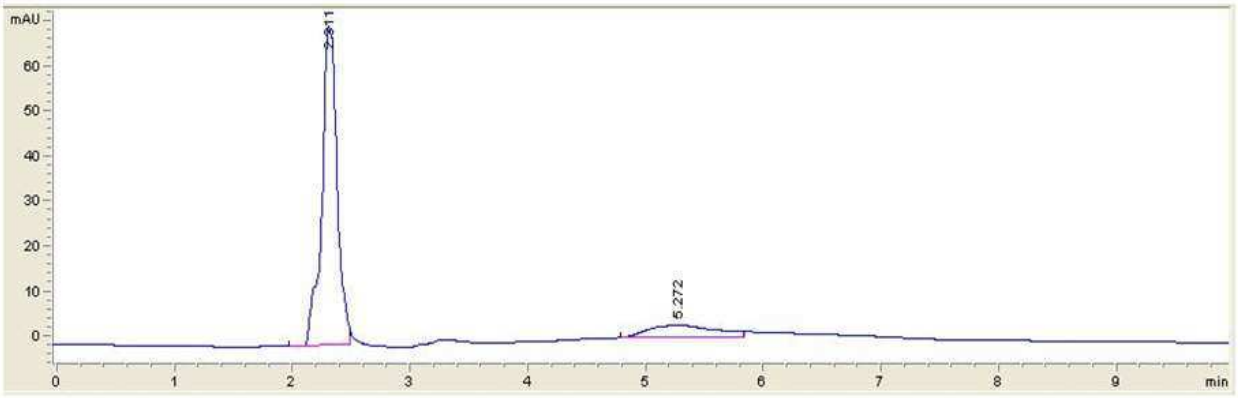

Fig.1 Standard pure Lutein with HPLC

According to fig.1, the inhibition time of pure lutein with HPLC was about 2.311 minutes and lutein level below the peak was read as 15614. (The time was constant as 4.1in all extracts)

Table1. The level below the peak of extracts with different solvents compared with the level below peak in pure lutein

Type of solvent	Time (min)	Temperature (°C)	Peak Area
Ethyl acetate	4.1	27	400
Ethyl acetate	4.1	35	394
Methanol	4.1	27	369
Methanol	4.1	35	342
Ethanol	4.1	27	201
Ethanol	4.1	35	152

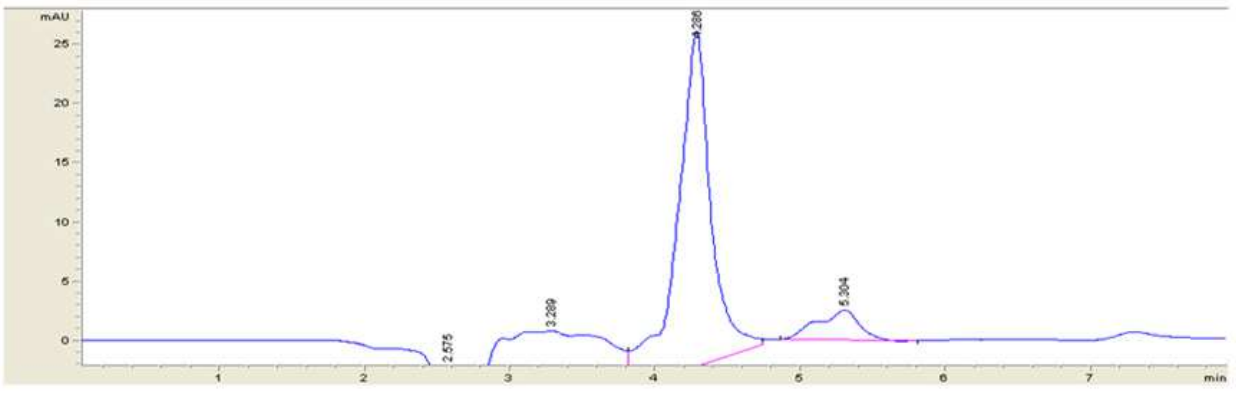

Fig. 2 The graph of sample lutein of powdered banana peel with ethyl acetate 80% in 27℃

According to table 1 and fig.2 the amount of lutein in the extract gained from ethyl acetate was higher as compared to other extracts and its level below peak was about 400.The temperature of extraction had no significant effect in gaining effective carotenoids .In this way, ethyl acetate 80%with a lower density and temperature was more appropriate as compared to other solvents.

According to our findings, Vernalis W and Jacobaea studied the amount of lutein on two species of Senecio by HPLC in 2009 with a similar laboratorial method .Their findings also showed that the best solvent was ethyl acetate and the peak area was read as 444 [19].

Mahagamasekara in 2010 also studied the amount of lutein on 13 species of vegetables with green color in Sri Lanka by HPLC .The most compound was related to lutein and by baking some percent of lutein in the laboratorial level, the level of lutein decreased .The findings also indicated that lutein is more hydrophilic [16,17].

2.3. Lutein colorimetry with UV-VIS spectrophotometer

After pure lutein is poured into cell, the amount of pure lutein absorbance in 245nm was read as 1.347.

Then Absorbance of samples was read in 245nm. Thus it was revealed that the amount of light absorbance in ethyl acetate solvent extract in 27°C was higher as compared to other extracts and the amount of its absorption was about 2.204.

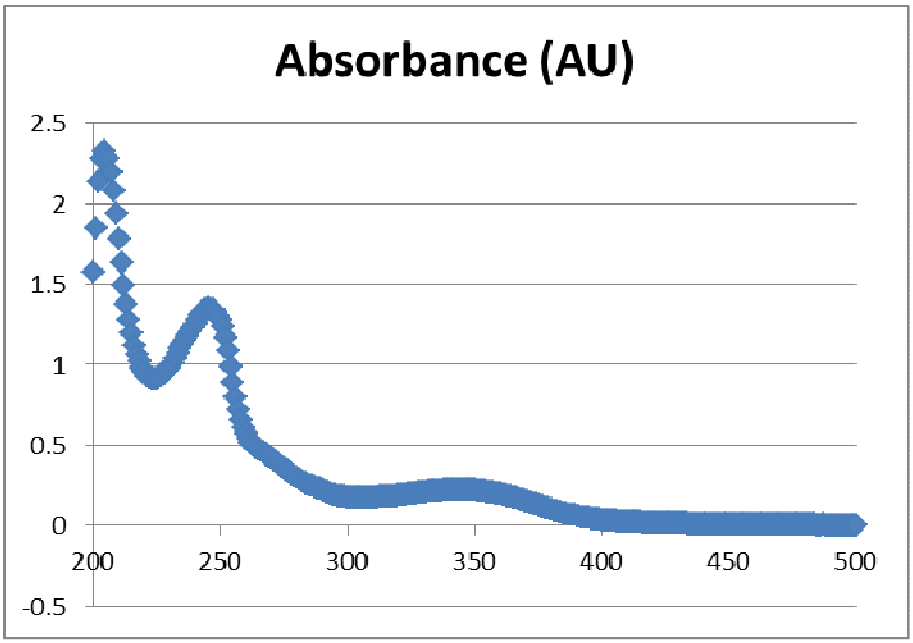

Fig.3: Lutein absorbance in UV-VIS Spectrophotometer

According to these findings, Andrea investigated the different methods of lutein preparation of supercritical fluid. Primarily the lutein absorbance was done using UV-VIS spectrophotometer and as the time passed, absorbance increased .Finally, the esters released from free lutein created a reversible system with free fatty acids in CALB system [18].

Similarly, Vernalis Waldst ., Kit and Jacobaea studied the amount of lutein on two species of Senecio by UV-VIS spectrophotometer with similar laboratorial method, their findings also indicated that the amount of absorbance read for lutein was higher as compared to other compounds and the amount of extract absorbance was read in 450 nm [19].

3.3. Determining the antioxidant power of the extracts

In determining the antioxidant power of the extracts, the light absorbance of the sample has a direct relationship with the power of reduction. This means that lighter absorbance means more reducing property. This test was used to determine the antioxidant power of the extracts. The antioxidant power of ethyl acetate extract in 27°C in FRAP method also showed the highest degree.

Ethyl acetate and ethanol had the highest and lowest antioxidant power in FRAP method. The extraction conditions which determine the maximum antioxidant power has indicated that the number of stages of extraction, temperature and time are among the effective factors in connection with antioxidant power of banana peel [9].

The powerful antioxidant property of ethyl acetate can be related to lutein existing in it .As lutein has antioxidant and reducing property and play a part in eye health as well as preventing cardio-vascular diseases, cerebral infraction and lung cancer.

Studies showed that antioxidant power of ethyl acetate was more than ethanol. Methanol 99.5% and ethanol 96% have the highest efficiency among common solvents for extracting herbal materials for polar and non-polar compounds .Therefore, any measuring with methanol extract can cause extraction of polar and non-polar compounds resulting to higher antioxidant power. The ferric reducing antioxidant power is a test which directly measures the antioxidants or reducers in the sample and has a direct relationship with their antioxidant density [11]. In these methods the extracts that have a high ferric reducing antioxidant activity can easily neutralize free radicals existing in the body.

In the test for measuring the reducing power of antioxidant activities made based on light absorbance, an increase in light absorbance of the reaction indicated an increase in antioxidant activity as light absorbance was used to intensify the reducing property.

Generally, the reducing power of ethyl acetate was gained as higher than methanol and methanol higher than ethanol.

4. Conclusion

Extraction of banana peel was done by three solvents (ethanol, ethyl acetate and methanol) and HPLC method was used to determine the amount of lutein and the best solvent was ethyl acetate. Peak area was about 400.UV-VIS spectrophotometer was used for lutein colorimetry. The best amount of light absorbance was for ethyl acetate that was read as 2.204. FRAP test was done to measure antioxidant power of the extracts .Ethyl acetate extract showed the most antioxidant power and the property of reduction.

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