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EXTRACTION OF LUTEIN FROM SOME PLANT SOURCE IN DIFFERENT CONDITIONS AND APPLICATION IN FOOD SYSTEM

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Abstract

Carotenoids was extracted from plant included lemon peel (*Citrus limonum*), carrot (*Daucus carota*) end chard leaves (*Beta vulgaris*), which are divided in tow groups the first one was fresh samples, the second samples were frozen for tow month to a extract pigments by using many solvents (water, hexane, ethanol, acetone 85% and isopropanol) at 40 and 60 C and different period from 1-4 hours. The results showed that Isopropanol was the beast when compared with other solvent at 60 C and 3 hours. The concentration of pigments in frozen chard leaves was 57mg/g while the lutein at both lemon peel and carrot was 0.64 and 1.1 mg/g respectively for the same period. Lutein pigment was isolated for other pigments by using mixture 1:2 methanol : petroleum ether, petroleum ether: methanol, diethyl ether: methanol, we notice from the results that mixture diethyl ether: methanol was the best in isolation lutein from other pigments a 50mg/100g compared with mention solvent 38 and 33mg/100g respectively from chard leaves freeze. The lutein pigment was used in manufacturing sweets at 0.05 and 0.07% and study its sensory evaluation by specialist at food science department, the results showed that sweet contained 0.07% was the best.

Key words: Extraction, lutein, Food system, carotenoids, plant source.

Introduction

Since the early civilizations an in the beginning of food industry, pigment natural nor synthetic, were used to give an attractive presentation, perception of freshness and quality of food. The plant species were used to provide characteristic color and flavor in food (Nelson and Trout, 1964). Color plays a major role in determining the appeal of most food and is often used as index of freshness and whole sameness. Color may be changed during processing storage or preparation in ways that affect food appealing. Thus controlling changing and or stabilizing the color of foods is a major objective for food scientists. The quality of food related to many characteristic which are color, flavor and textural, but color is the most important a manly them, because its attractive appearance. The color considered as a key to determine the criteria of food safety its quality and characteristic appearance-like undesirable color in meat, fruit and vegetable because it gives an idea about probably danger or at least the existence of undesirable flavor that's why the food factories are interesting in getting more information about color sources of many factory processes (Mohsen *et al*., 2010; Nelson and Trout, 1964). Manufactured food represent 165-60 from the whole foods and because of the need of this food to additional foods to improve it and increase its storage age, there are 2500 chemical materials were used as food additional although a need the natural pigment to human consumer are very united the agreement about new pigment is very difficult because the FAD considered the pigment as food additional so that it put very hard instruction for allowing whey it (Delgado-Vargas *et al*., 2000). Carotenoids natural pigments in lipid-soluble include more than 700 kinds including xanthophylls and carotenes (Amorim-Carrilho *et al*., 2014). Xanthophylls such as zeaxanthin and lutein containing oxygen in this molecules, while carotenes *e.g.* lycopene and α-carotene contain hydrocarbon without any oxygen (Wills *et al.,* 1984). Carotenoids includes group of color material that are wide spread in plants leaves, flower and fruit its color are between light yellow and dark red, that absent fat and oil, the cause of this color is the conjugated duple bound exchanged. Its specials as antioxidant which reduced age and heart disease also it used as carotenoids have a grate range of application

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that benefit to human health for its specially characteristic it has been used in wilds application at many factory food. Lutein is member of xanthophyll's family of carotenoids color of food at wide period (Seddon *et al.*, 1994; Lorenz and Cysewski, 2000). The aim of this study was to extract natural pigments from plants in different solvents and temperature and use it in food application.

Materials and Methods

Materials

The Fresh material which include (*Citrus limonum, Daucus carota* and *Beta vulgaris)* were brought from the local market of Basra, washed, cleaned with tape water and dried after that the peel lemon and carrot were minced separately to get small slides. While the leaves of chard were cut to small peace's 0.5 cm in both length and then samples were divided into to groups the first group was extracted while it was fresh the second group was kept in polyethylene pages in freezing (-18^{o}C) for two months.

Methods

• Carotenoid extraction

5g of samples was mixed with different solvent 4:1 included (water, hexane, ethanol, acetone 85% and isopropanol) the mixture put in water path at 40-50^{o}C for (1, 2, 3, 4 hours) then the samples were filtered and absorbency measured at the 447nm. (Fikselov *et al*., 2008). The concentration of lutein calculated using the response factor as follow:

$$C = A / E^{1\%}_{1cm} \times 100$$

C = concentration, E = specific extraction, A = absorbance

• UV-VIS analysis of pigment extraction

UV-Visible absorption of carotenoids were measured from 400-700nm (Thermo fisher sciatic U.S.A) of the freshly extracted peel lemon, carrot and chard by isopropanol at 60^{o}C.

• The isolation of lutein pigment

The method of Vatsala and Rekha, (2013) was followed in isolation lutein from the other pigment by taking 50gm from the previous extracting samples and mixing them with different solvent (2:1) which (methanol: petroleum ether, petroleum ether: methanol, diethyl ether: methanol) volume 150 ml for 30 minutes with shaking each 5 minutes, than a saturated sodium chloride solution was added in rate 1:1 and put in separating funnel to form two layer light yellow at the bottom and dark green at the tap, the light yellow layer was ignored because it wasn't have any lutein, the dark green layer was taken and added to KOH 10% saponification and left over night. The saponified mixture were transferred sparely to separating funnel and equal volume of petroleum ether was\added and left for 5 minutes at room temperature followed by addition of equal volume distilled water to the saponified mixture, shaken rigorously and allowed to stand for 15-20 minutes, two layers were observed the bottom layer was removed. The tap layer was washed repeatedly by distilled water for complete removal of alkaline left to remove the solvent and to concentrate the pigment to its lowest volume. The absorption was read by spectrophotometer from 447nm then the pigment was left to be dried completely. The addition of pigment in food applications lutein was added in manufacture sweets according to Nelson and Trout, (1964) which Bused at concentration 0.05 and 0.07% with control the sensory evaluation was made by specialists at food science department.

• Isolation by Thin layer Chromatography TLC

The lutien extracted was first dissolved in a little amount of hexane. The lutein was separated and isolated from the plant extracts by preparative TLC on silica gel 60 (20×20 cm) aluminum gel. The TLC chamber was content hexane acetone (3:2) the mobile phase. The TLC plate activated by drying at 110^{o}C for 20-30 minutes. Plant extracts were obtained by a spots capillary tube and the plate was placed in chamber. Afterward the spots were identified by means of a pencil, Rf values were compared to that reported in the literature, then each unit was scraped separately and then dissolved in hexane for further analysis.

• Spectral Analysis Of lutien

The UV-vis absorption spectra of the lutein samples were acquired by using a UV-vis spectrophotometer (UK). The spot of the samples were dissolved in 5ml of hexane and measured in the range between 420-662 nm. All measurements were per farmed with three replicates (Sasugun *et al*., 2018).

Results and Discussion

The result at fig. 1, showed that the extractions temperature effects on the concentration of lutein which gained from fresh chard leaves. The yield of extraction at 60^{o}C was higher (2.3mg/g) was higher compared to that at 40^{o}C (2.1 gm/g), the results were agreed with Fikselova *et al.,* (2008) when they study the effect of temperature at the concentration of carotene from carrot showed that the increased of temperature in helps in releaser carotene from the plant tissue More over the results show isopropanol is higher in obtain lutein (4.3

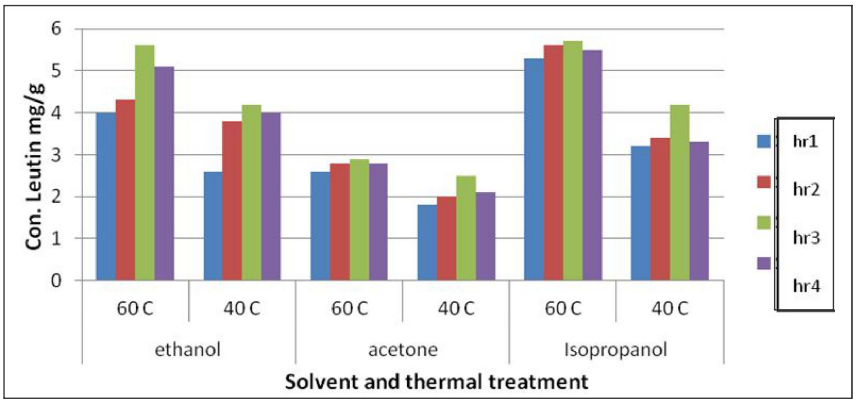

Fig. 1: Effect of solvents and thermal treatments on concentration of lutein in fresh chard leaves.

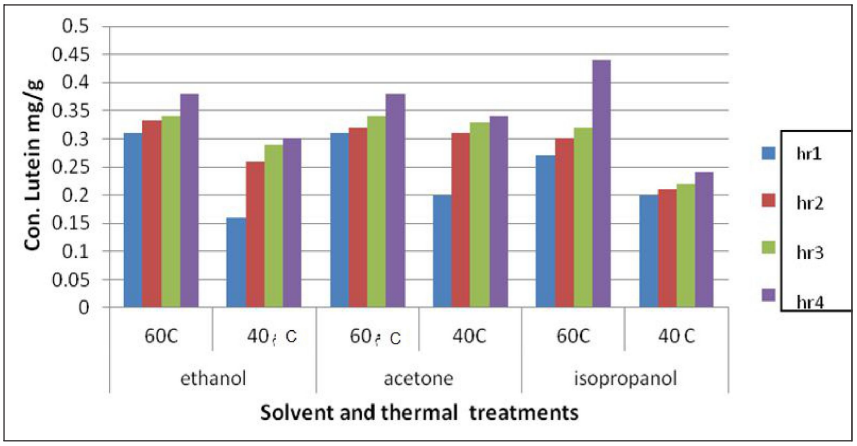

Fig. 2: Effect of solvents and thermal treatments on concentration of lutein in frozen chard leaves.

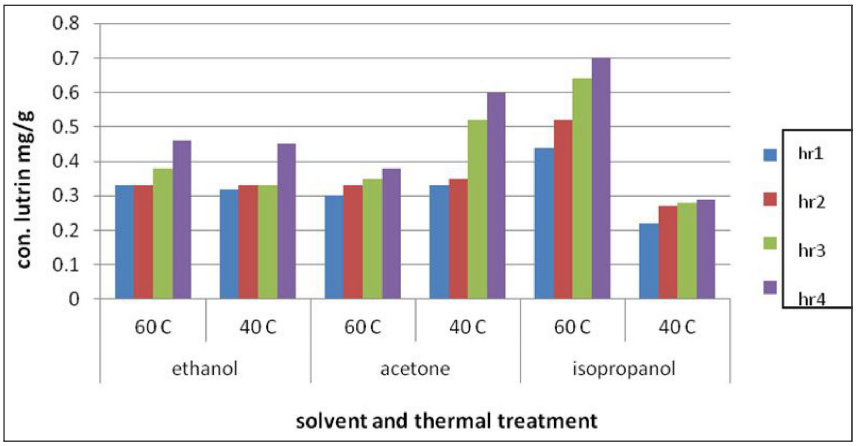

Fig. 3: Effect of solvents and thermal treatments on concentration of lutein in fresh lemon peel.

mg/g) compared with acetone 85% and ethanol (2.3, 2.3 mg/g) respectively, the addition of isopropanol in solvents and analyzing fatty acid and other compound which belong to its wide polarity (Nelson and Trout, 1964). Carotenoids are soluble in polar solvent, including edible fat and oil.Because. Carotenoids lip soluble, they are usually extracted from plant sources with organic solvent such as chloroform, hexane, acetone etc (Belitz *et al.,* 2004).

The lutein concentration was increase with the increase of heating processing. The yield of lutein after 3 hours was the highest (2.6mg/g) at the first hour was 2.1 mg/g then increased to reached at second hour 2.2 (mg/g) then it decrease after four hours to be 2.4 mg/g (Fig.1). This my be due to the period of 3 hour is fairly enough to extract all pigments in food material, this results were agreed with (Fikseloba *et al.,* 2008). They explain the increased of heating process has appositive effect an extraction process, this increase due to changes happen in the cell wall and on the other side the increasing of degree of heating extraction result interlayer actions. While estimated Chondora-Hioe, (2017) the compound of lutein and ß-carotene in the leaves of some Asian vegetables with HPLC technology, the contained a compound of lutein with 694-5919 µg/ 100g. Both Li and Engelberth, (2018) found that ethanol gave the highest extraction rate for the lutein compound 77.03 mg/L compared to other solvent used in the study.

Fig. 2, showed that lutein concentration of chard leaves which are freezing for two months, was increased in all samples and this may be due to storage period increase in carotenoids extraction. Increased carotenes yield due to storing and freezing of the samples were shown as compared to the fresh samples. 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.

The processing of food involves changes in the structural integrity of the martials which produces both negative loss of carotenoids du to oxidation and positive increased bioavailability effect (Livny *et al.,* 2003). Fig. 3 and 4 show the of lutein of fresh and froze lemon peels, in fresh the concentration of lutein was 0.16mg/g after one hour 40°C, while it was 0.31 mg/g after one at 60°C. At the freezing lemon peels the concentration of lutein in both heat treatment were 0.45,

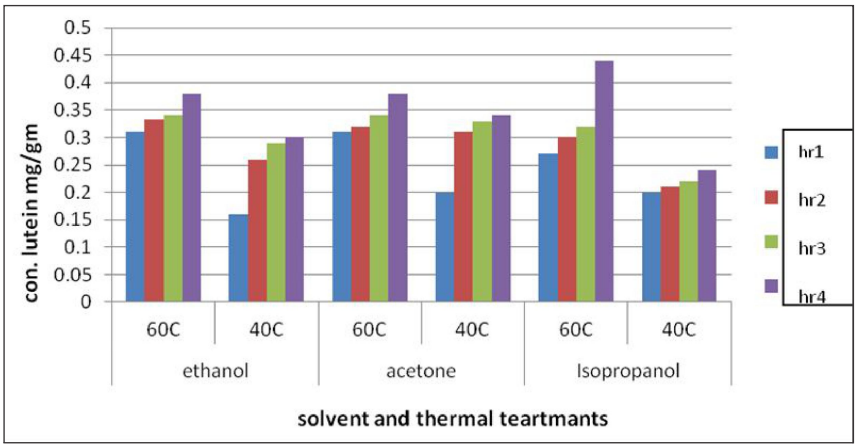

Fig. 4: Effect solvent and thermal treatment on concentration lutein in frozen lemon peel.

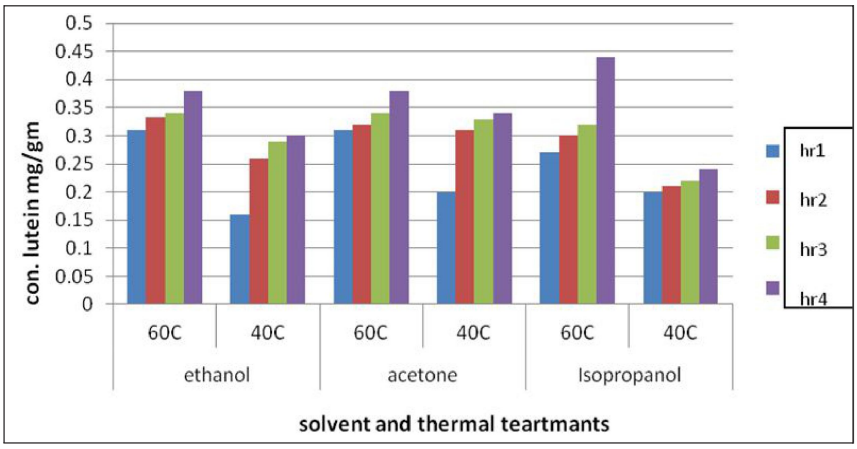

Fig. 5: Effect of solvents and thermal treatments on concentration of lutein in fresh carrot

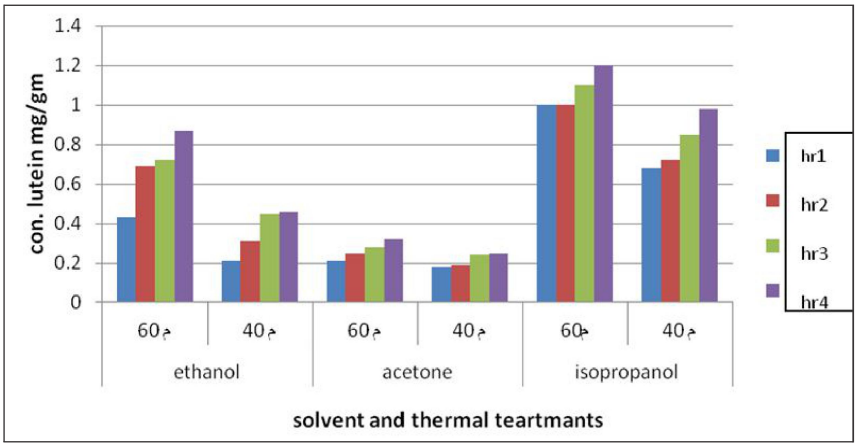

Fig. 6: Effect solvent and thermal treatment on concentration lutein in frozen carrot.

0.46 mg/g after 4 hours and the isopropanol was the highest among the all other solvent, the concentration of lutein was 0.44, 0.70 mg/g at fresh and frozen lemon peels after 4 hours at 60°C, respectively.

The results at fig. 5 and 6, shows the concentration of lutein that extract from fresh and frozen carrot we notice that the best results were at 60°C at 4 hours by using isopropanol in compared with other solvents for both treatment the lutein in fresh carrot was 0.44 mg/g and get higher to reach 1.2 mg/g in freezing carrot. These results were agreement with Milicua *et al.,* (1991) to solubilize carrot with the yield of 80µg/g. On other hand isopropanol was the most efficient solvent in extracting lutein from carrot.

Effect solvent on extraction the lutein

The results in fig. 7, showed the concentration of lutein in using, the mixture of diethyl ether: methanol (2:1) gave the highest concentration 50mg/g compared with mixture of petroleum ether :methanol and methanol :petroleum ether 38 and 33mg/g respectively. This can be explain by that addition of a saturated NaCl and the saponification carotenoid overnight will help to get rid of and separated chlorophyll, methanol works on pull out water molecule while diethyl ether works on extract lutein pigment they secondary reaction where the pigment is concentration in organic solvent after its solvation in it (Cverkovic and Markovic, 2008; Vatsala and Rekha, 2013).

Carotenoids Characterization by UV-VIS Spectra

Fig. 8, shows the absorption spectrum of extracted carotenoids (lutein, chlorophyll a, chlorophyll b, capsorubin and ß-carotene. The absorption of extracted pigments strongly depend on the degree and time of extract, type of solvent and plants. extract carotenoids from Lem indicated the presence three peaks at 447, 451, 472 nm (lutein, ß-carotene and capsorubin respectively, while the extract carotenoid from char. showed two peaks lutein at 447nm and ß-carotene at 451nm. Furthermore the char. extract showed three peaks at 447, 644 and 662 (lutein, Chl a and Chl b respectively). The results were agreed with proposed by Mohsen *et al*., (2010) when estimating carotenoids in some plants residues such as mango and tomato peels where the lutein compound appeared at 447, ß-carotene 451 and

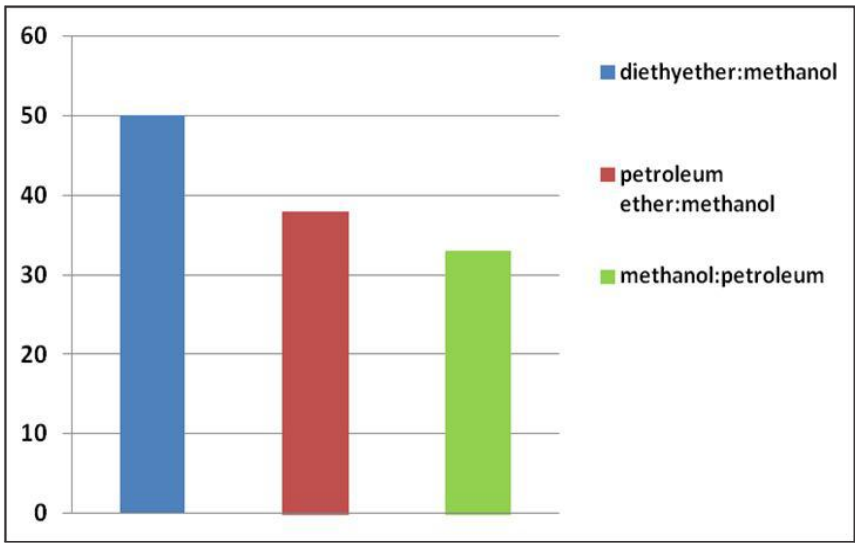

Fig. 7: Effect solvent on extraction the lutein.

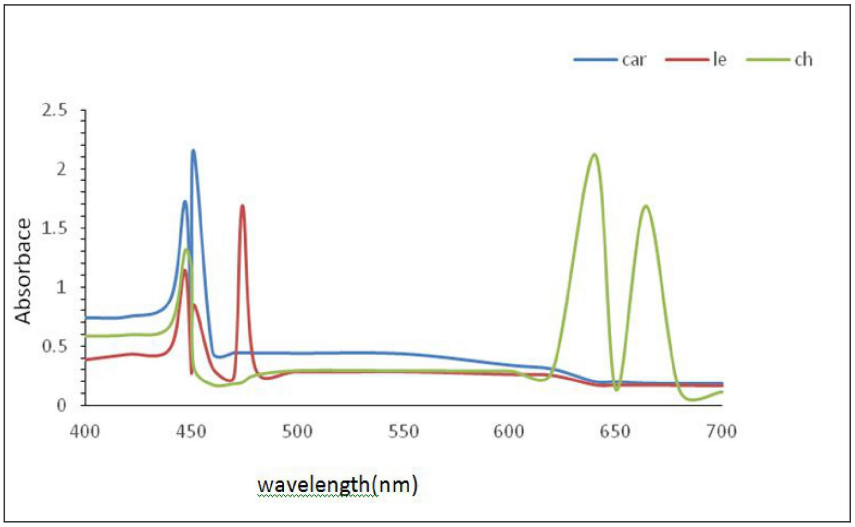

Fig. 8: UV-Vis absorption spectra of carotenoids.

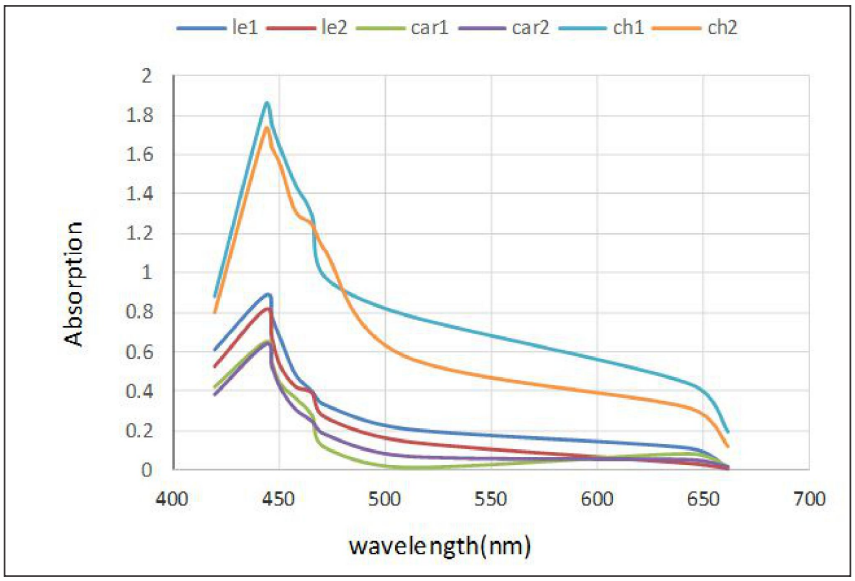

Fig. 9: VU- Visible absorption spectra of lutein.

capsorubin 474nm. Pigments responsible for photosynthesis are compounds of a very different chemical composition. They are present in isoprenoid carotenoids, flavones and anthocyanins. The chlorophyll which is necessary for photosynthesis. The amount and presence of pigments varies by species (Sumanta *et al*., 2014). The properties of carotenoids play an important role in maintaining the health of human, the last years showed the appears of large numbers in the market of carotenoids dietary supplements (Li *et al*., 2015).

Thin Layer Chromatography (TLC)

The results of TLC shown in table 1, showed the Rf values of separated lutein compound from the plant extracts separated by the hexane acetone (3:2), the spots appeared dark yellow-yellow in all samples. The Rf values of the lutein ranged from 0.74-0.81, the result was similar to the obtained by Surendranath *et al*., (2016), from marigold petal powder, the value were obtained in Thin layer chromatography of the samples thus indicating that the orange yellow - dark yellow spot represented the presence of lutein in the samples extracted and the single spot. Saponification can be used to remove overlapping fats present in plant samples (Howe and Tanumihardjo, 2006). Saponification also decomposes the fatty acid esters of xanthophylls, such as lutein, ß-cryproxanthin and zeaxnyhin, which can facilitate chromatography separation. Saponification is usually done using a ethanolic, methamolic or aqueous solution of sodium or potassium hydroxide (Lee *et al*., 2001; Kurilich *et al*., 2003).

UV-VIS Spectra of lutein

Fig. 9, shows the appearance of a single peak along the 447nm of the chromatographic spots separated by TLC of carrot, lemon peel and chard leaves samples. The peaks refer to the lutein compound in these results are consistent agreement with El-Reay *et al*., (2013), they said that lutein gave the

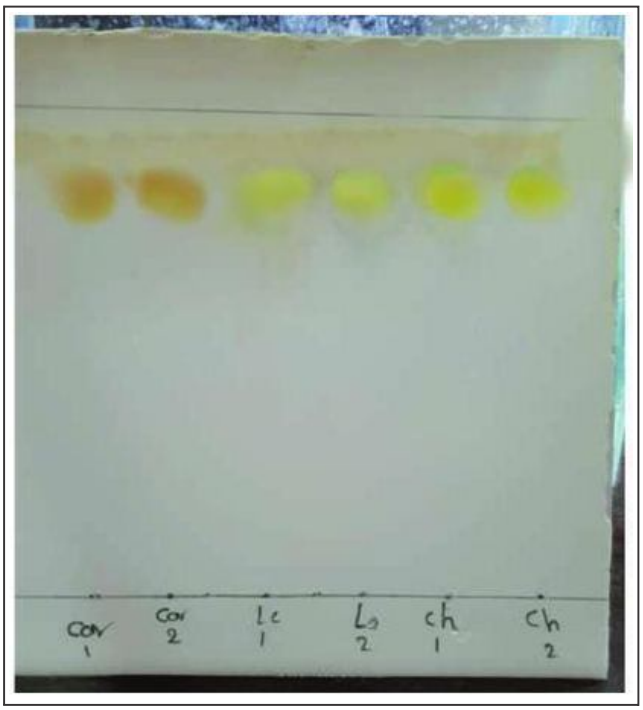

Fig. 9: TLC plate.

Table 1: Rf values for lutein samples

Sample No.	Rf value	Color of spots
Le 1	0.74	Yellow
Le 2	0.75	Yellow
Car 1	0.81	Orange Yellow
Car 2	0.79	Orange Yellow
Cha 1	0.76	Dark Yellow
Cha 2	0.75	Dark Yellow

Table 2: Sensory evaluation of the application of lutein in sweet.

Characteristic	Sample 1	Sample 2	Sample 3
Aroma	19	19	19
Test	19	19	20
Color	10	20	19
Textures	14	15	15
Overall acceptability	13	13	13
Total	75	86	97

highest peak at 443nm.

Food application

The result of sensory evaluation of sweet showed that the addition 0.07% lutein pigment Tap (2). Gave the highest degree which was 97% when compared with 0.05% concentration and control which was 86 and 75% respectively. The addition muddle at 0.07% pigment get 29, 20, 19 in color, test and flavor respectively, that indicate pigment give a dispersible color and don't effect on test and flavor.

Conclusion

It is a possible to conclude that plant source can be used to produce lutein pigment for using in food industry rather than chemical industrial pigment which is produced as a colored pigments. It was found isopropanol is than best isolation pigment than others from chard leaves. In addition, the frozen sample were the best as well as the pride of time (3 hours).

1. The results showed that Isopropanol was the beast when compared with other solvent at 60°C and 3 hours.

2. The concentration of pigments in frozen chard leaves was 57mg/g while the lutein at both lemon peel and carrot was 0.64 and 1.1 mg/g respectively for the same period.

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