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An efficient condition of Saponification of Lutein ester from marigold flower

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ABSTRACT

Saponification of carotenoid esters leads to decomposition at high temperature and high concentration of alkali. Lutein ester is collected from marigold flower. The saponification of purified lutein ester is studied at different parameters using UV-visible spectrophotometer, HPLC. Findings showed efficient saponification in 0.5 M KOH at 50^{0}C for 30minutes.

Keywords: Lutein ester, Saponification, HPLC.

INTRODUCTION

Carotenoids are lipid- soluble, yellow-orange-red pigments found in all higher plants, algae, fungi and some animals either in free state (carotenes) or in the form of esters (xanthophylls). Saponification of carotenoid esters is carried out to remove the chlorophyll and to get free xanthophylls. There are numbers of saponification methods used in order to improve both xanthophylls extraction and purification efficiency [1,2,3]. Saponification of lutein esters using 40%KOH at 75°C was also reported [4]. But in most of these methods, high temperature and long processing times are required which can result in the degradation and formation of unwanted isomeric compounds [5,6]. Enzymatic saponification are also used to enhance xanthophylls extraction from marigold flower [7,8] . But it too had practical limitations, due to the use of expensive commercial enzyme. The extract with only the purified form with a lutein content of known concentration and a pure crystalline lutein isolated from marigold flower is allowed for food use. As the high concentration of alkali and high temperature provokes the degradation of carotenoids, so there is a need of an efficient and optimal condition for saponification.

MATERIALS AND METHODS

Materials

Marigold flower (*Calendula officinilalis*) was collected from local nursery. All the chemicals and solvents used for extraction were of analytical grade and solvents used for HPLC were of HPLC grade purchased from Qualigens Fine Chemicals, Mumbai, India. Lutein standard was purchased from Sigma Aldrich, USA.

Table1 Different reaction parameters for Saponification of Lutein ester

Sets	Temperature (^{0}C)	Time (minutes)	Concentration of KOH (M)	Sets	Temperature (^{0}C)	Time (minutes)	Concentration of KOH(M)
S1	25	Over night	0.1	**S34**	50	5	1.0
S2	25	Over night	0.5	**S35**	50	15	1.0
S3	25	Over night	1.0	**S36**	50	30	1.0
S4	25	Over night	2.0	**S37**	50	50	1.0
S5	25	Over night	5.0	**S38**	50	5	2.0
S6	40	5	0.1	**S39**	50	15	2.0
S7	40	15	0.1	**S40**	50	30	2.0
S8	40	30	0.1	**S41**	50	50	2.0
S9	40	50	0.1	**S42**	50	5	5.0
S10	40	5	0.5	**S43**	50	15	5.0
S11	40	15	0.5	**S44**	50	30	5.0
S12	40	30	0.5	**S45**	50	50	5.0
S13	40	50	0.5	**S46**	60	5	0.1
S14	40	5	1.0	**S47**	60	15	0.1
S15	40	15	1.0	**S48**	60	30	0.1
S16	40	30	1.0	**S49**	60	50	0.1
S17	40	50	1.0	**S50**	60	5	0.5
S18	40	5	2.0	**S51**	60	15	0.5
S19	40	15	2.0	**S52**	60	30	0.5
S20	40	30	2.0	**S53**	60	50	0.5
S21	40	50	2.0	**S54**	60	5	1.0
S22	40	5	5.0	**S55**	60	15	1.0
S23	40	15	5.0	**S56**	60	30	1.0
S24	40	30	5.0	**S57**	60	50	1.0
S25	40	50	5.0	**S58**	60	5	2.0
S26	50	5	0.1	**S59**	60	15	2.0
S27	50	15	0.1	**S60**	60	30	2.0
S28	50	30	0.1	**S61**	60	50	2.0
S29	50	50	0.1	**S62**	60	5	5.0
S30	50	5	0.5	**S63**	60	15	5.0
S31	50	15	0.5	**S64**	60	30	5.0
S32	50	30	0.5	**S65**	60	50	5.0
S33	50	50	0.5				

Methods

Extraction- Lutein ester is extracted from marigold flower. The flower petals were dried, grinded with anhydrous Na_2SO_4 and extracted with hexane. The extract is purified by column chromatography and TLC. The purity of the lutein ester was confirmed by UV-visible spectrophotometer and HPLC. The Lutein ester content was measured by UV-visible

spectrophotometer (UV-1601 SHIMADZU) at 446nm in hexane with an extinction coefficient of 2671 for 1% solution. All analysis were performed under dimlight and N_2 atmosphere.

Saponification- 1ml of methanolic extract (containing 0.01% BHT) of purified Lutein ester was treated with different concentration of methanolic KOH at different temperature for different reaction time as shown in Table.1. The saponified extract is diluted with water followed by extraction with hexane until colorless. The hexane extract was then washed with excess of water to remove alkali. The saponified extract was evaporated to dryness in rotavapour and redissolved in 1ml of methanol. The lutein content was estimated using HPLC (SPD-M10AVP, SHIMADZU). The estimations were performed on Supel Cosil-LC8 (25cmX 4.6mm, 5µm) column. The solvent system used were for pump A CH_3CN: H_2O (97.5:2.5) and for pump B CH_3CN: DCM (70:30).

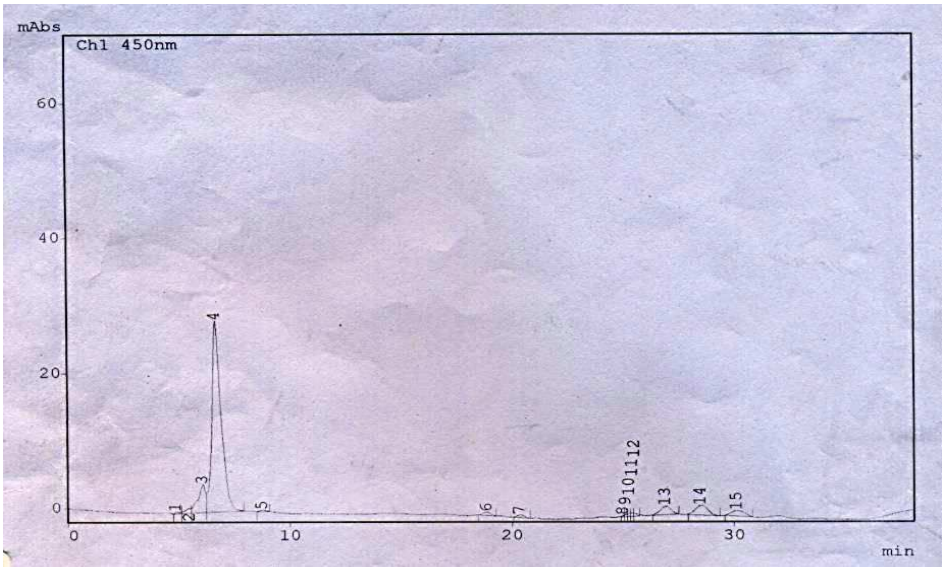
RESULTS AND DISCUSSION

A. Preparation of lutein standard curve

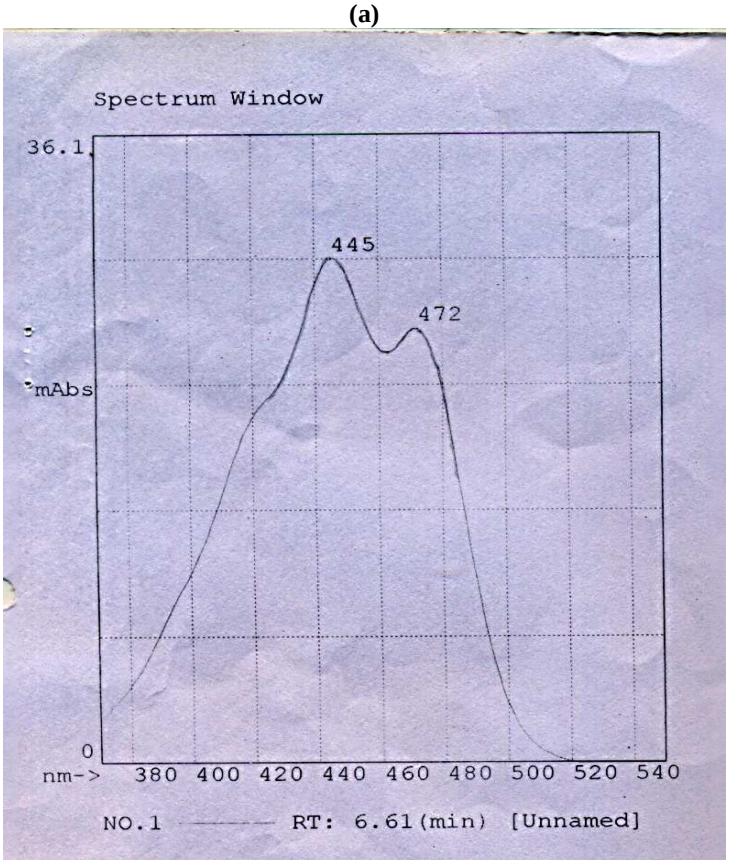
Standard curve of lutein were prepared with known concentration and peak area. The equation of the curve Y= 15251.37 X + 5158.71 and R^2 value (0.99) showed good linearity of peak area and concentration.

Table2: Mean lutein recovery concentration(%) in different set conditions

Sets	Mean Lutein recovery concentration (%)	Sets	Mean Lutein recovery concentration (%)	Sets	Mean Lutein recovery concentration (%)
S1	3.55	**S23**	3.29	**S45**	3.10
S2	8.26	**S24**	4.19	**S46**	2.49
S3	7.67	**S25**	3.28	**S47**	3.94
S4	6.54	**S26**	2.63	**S48**	5.89
S5	3.81	**S27**	3.89	**S49**	4.48
S6	2.41	**S28**	5.63	**S50**	4.12
S7	2.63	**S29**	4.26	**S51**	5.65
S8	2.93	**S30**	3.87	**S52**	5.67
S9	2.62	**S31**	4.56	**S53**	4.02
S10	3.46	**S32**	9.53	**S54**	3.93
S11	6.21	**S33**	4.36	**S55**	4.28
S12	6.26	**S34**	3.91	**S56**	4.21
S13	5.26	**S35**	4.33	**S57**	3.51
S14	3.85	**S36**	5.56	**S58**	3.70
S15	4.16	**S37**	4.29	**S59**	4.28
S16	5.47	**S38**	3.89	**S60**	4.12
S17	3.67	**S39**	4.30	**S61**	2.96
S18	3.63	**S40**	5.31	**S62**	3.48
S19	4.14	**S41**	3.28	**S63**	4.15
S20	4.13	**S42**	4.98	**S64**	3.86
S21	3.36	**S43**	4.28	**S65**	2.71
S22	2.23	**S44**	4.08	-	-



(a)



(b)

Fig1.(a) HPLC Chromatogram of saponified purified Lutein ester sample showing the Lutein peak at 6.61 min and (b) its UV absorption spectra

B. Effect of KOH concentration

Saponifications of lutein ester with different KOH concentration were investigated. It was found that the lutein concentration increases as the KOH concentration increases from 0.1M to 0.5M

and decreases on further increase of KOH concentration. This may be due to the degradation of lutein at high concentration of KOH. Therefore 0.5M is the optimum KOH concentration to get maximum yield of lutein.

C. **Effect of temperature:**
Saponification of Lutein ester was carried out at 40°c, 50°c and at 60°c. At 50^0C the lutein concentration was found to be maximum. As the temperature increases lutein concentration decreases. It may be due to degradation at high temperature.

E. Effect of Reaction time:
The lutein ester Saponification was investigated for a reaction time of 5min,15min,30min and for overnight. Though the conversion of lutein ester in overnight condition was found to be good but it is not convenient as it is time consuming. As the reaction time increases from 5 to 30 minutes the mean lutein concentration was increased and then decreased with increase of reaction time. For 5M KOH concentration the mean lutein concentration was decreased with increase of reaction time. This may be due to degradation of lutein at higher KOH concentration for longer time.

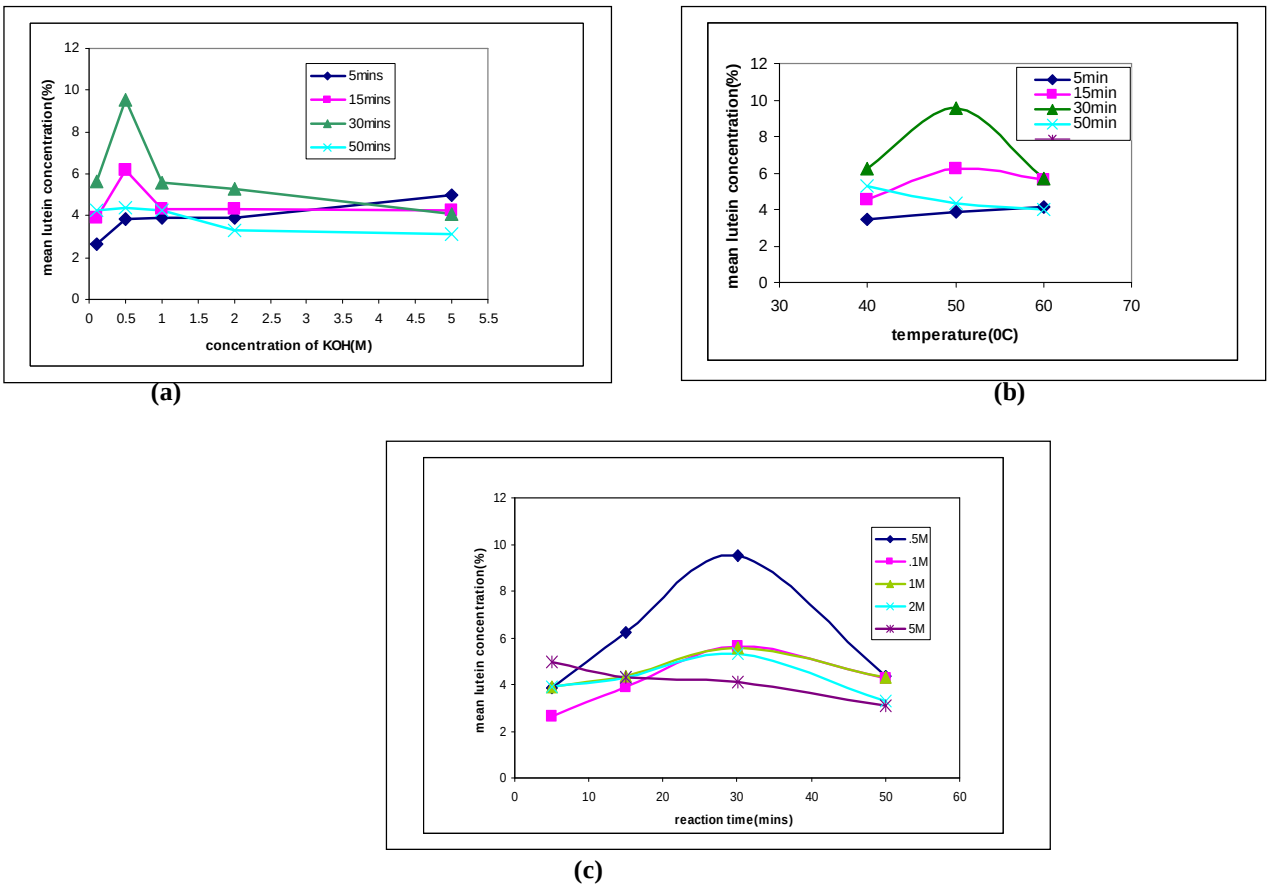


Fig2 Effect of (a) KOH concentration, (b) temperature and (c) reaction time on alkaline hydrolysis of Lutein esters

The Saponification of lutein ester in S_{32} (at 0.5M KOH and at 50°C for 30 min reaction time) gives the maximum conversion. So, this was found to be the best way to saponify lutein ester in alkaline condition

CONCLUSION

In conventional method saponification is usually done with higher alkali concentration and for longer time which leads to degradation of lutein. However the modified saponified method used in our study is found to be more efficient and thus may overcome the difficulties arises in conventional saponification method.

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