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Novel single-solvent purification method of lutein from *tageteserecta* oleoresin and its parametric study

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ABSTRACT

Lutein is a xanthophyllobtained from Marigoldflowers whichis known to be a potent antioxidant and a safe food colorant. Owing to its potential use in the food and nutraceutical industry, a method was developed using ethanol as the only organic solvent in the process, to obtain lutein at purities above 90% with chemical recoveries >80%. The degradation profile of the lutein so obtained was studied under temperatures of 0°C, 20°C and 40°C over a 215 day period from which, an equation was arrived at, which could help predict the lutein content of such a purified product based on the time and temperature of its incubation. Lastly, the lutein was suspended in clear sunflower oil to a concentration of 15% (w/w) which was then tested for particle size, viscosity and colour value (L*a*b value) in vegetable margarine at 0.1% dosage.

KEYWORDS

Lutein;
Purification;
Degradation curve;
Marigold;
Colour value.

INTRODUCTION

Lutein isahigh-value commercial xanthophyllwith an empirical molecular formula of $C_{40}H_{56}O_2$ and a molecular weight of about 568.87 Da. At low purities, it is used in animal feed; particularly for poultry, to help impart colour to the egg-yolk and consequently increase the visual appeal of the egg to the end consumer. At purities above 90%, it is used as a food colour additive and is recognised as E161b by the European Food Safety Authority[9]

It is a well-known antioxidant and its consumption has been advocated by a number of nutritionists for maintaining good eye health. Although lutein does not show pro-vitamin A activity, it is thought to accumulate in the cells around the iris and absorb most of the incident U.V. light, thereby, protecting the eye from the harmful effects of such radiation[6, 2, 8, 10]

In nature however, much of the lutein is obtained as an ester with other plant lipids. Saponification is required to release the lutein from its ester form. A method was developed which could effectively extract and purify lutein from the Marigold flower oleoresin using ethanol as the only organic solvent. The product so obtained was then subjected to three temperature conditions of 0°C, 20°C and 40°C to study its keeping behaviour.

Being a commercially important product, many researchers have also developed methods to bring about its extraction and purification. Khachik F.

(2009) described a method for isolating lutein from a commercially available saponified marigold oleoresin. The method entails the sequential washing of the saponified mass using distilled water, ethanol and hexane which was followed by recrystallization in 1:1::hexane:dichloromethane. This method however may not be desirable due to the large number and quantity of solvents used and the inclusion of dichloromethane in its major purification step as it is a known neurotoxin and carcinogen.

A more simplistic silica gel based chromatographic approach for lutein purification has been described by Boonnoun P., et al (2012) wherein a product yield of 60% with 97% purity has been reported along with a detailed study of such a systems adsorption isotherm. However, the commercial feasibility of the process described may be doubtful due to the volume of solvents used. Moreover, the method does not render itself easy for routine production.

Mehta (2011) describes a purification method which involves the simultaneous saponification of the marigold oleoresin along with hexane fractionation. However, exact details of the yield and purity of the product are not clearly mentioned. Moreover, the requirement of flammable higher alkanes renders this process undesirable.

A method has been described by Swaminathan S. and Madavalappil K.P (2009) which brings about the extraction of oleoresin from the marigold flower using copious amounts of hexane which was then saponified using alkali ethanol strictly for 30 minutes post which it was sequentially washed with ethanol and water to obtain a product with 92% all trans lutein as its major constituent with a product yield of about 7.6 %. The short period of 30 minutes specified for saponification may have been possible due to the method adopted to extract the oleoresin from the marigold flowers. Moreover, the quick transition from extraction to the saponification may have helped maintain the working quality of the oleoresin; allowing for a reduced alkali processing. Furthermore, since the neutralisation of excess alkali in the saponified mass was not carried out using an acid and was instead simply washed with water, a large quantity of effluent was expected. This may also have contributed to the relatively lowered product yield.

Lutein at very high purities is generally not used for any product formulation as it is cumbersome to work with and has a poor bioavailability. The purified lutein obtained using the developed method was suspended in sunflower oil to a concentration of 15% (w/w) and was tested for viscosity, particle size and colour value in vegetable margarine at a low dosage of 0.1%. Such an oil suspension could potentially be a commercial product.

EXPERIMENTAL

Marigold oleoresin was procured from Yunaan Rainbow Biotech Co. Ltd. (China) and stored at 15°C under dry conditions and used *pro re nata*.

Five trials were carried out, each using 500g of oleoresin. The oleoresin was first dissolved in 500 ml ethanol by stirring at 45°C for 20 minutes. 150 ml of 45% KOH of 1:3:: water : ethanol was then gradually added and allowed to stir at 70°C for 2 hours in a sealed container with appropriate condenser attachments to minimize solvent loss. The sample was then filtered through a 2µ filter cloth and the retentate was recovered and suspended in 500 ml of water and was gradually neutralised using 2M citric acid. The solution was stirredfurther for 20 minutes and was then filtered through a 2µ filter cloth. The retentate was recovered and stirred in 1L of 40% ethanol at 45°C for 60 minutes and filtered once again through a 2µ filter cloth. This step was repeated one more time. The retentate was finally stirred at 45°C in 1.2L of 70% ethanol for 60 minutes and filtered through a 2µ filter cloth to obtain a retentate which was dried at 40°C under vacuum for a period of 3 hours.

10g of the product obtained from all thetrials was pooled and divided into three portions which were incubated either at 0°C, 25°C or 40°C in dark. Samplesand were tested every 5 days for a period of 35 days post which, they were tested every 10 days until a period 215 days. Graphing of the data sets to produce a degradation curve, along with the generation of respective trend-lines, equations and regression analysis values for each temperature condition, was carried out using Microsoft Excel.

Lutein estimationwas carried out using U.V/Vis Spectrophotometry at 445 nm in 9:1:: ethanol:chloroform, considering $E_{1cm}^{1\%} = 2550$[9]

The remainder of the lutein sample from each batch was suspended in clear refined sunflower oil to a lutein content of 15%(w/w) using a high-speed shear mixer. Particle size determination was carried out using Microscopy (Motic BA – 210 using Motic particle size detection software) at 10x magnification. Viscosity was determined using Brookfield Viscometer LVDV- II +Pro programmable rheometer. Measurements were made at 20°C across an r.p.m. range of 10 to 100, with an interval of 10, in an ascending and descending order, keeping a 60 second gap between each reading. Colour value (L*a*b value) was determined using X-rite Color i5 (Michigan, U.S.A) at a dosage of 0.1% in vegetable margarine.

RESULTS AND DISCUSSION

The process required around 7.5 – 8 hours to yield the final purified lutein. The process showed an average yield of 15.69% with an average chemical recovery of 82.49%; both of which were obtained quite consistently, as indicated by their respective variance values of 0.1 and 4.84. The average purity of the samples generated was 91.34% as shown in the table below:

Stability of the lutein samples under different temperature conditions was assessed; the results for which are represented in the table below and depicted in the supplementing graph.

The equations obtained for the temperature based exponential degradation curves were:

$$\%\ Lutein = 90.25 \times e^{-0.004 \times Time\ (in\ days)}$$

For 0°C with an R^2 value of 0.9868

$$\%\ Lutein = 90.25 \times e^{-0.007 \times Time\ (in\ days)}$$

For 20°C with an R^2 value of 0.9769

$$\%\ Lutein = 90.25 \times e^{-0.032 \times Time\ (in\ days)}$$

For 40°C with an R^2 value of 0.8551

Thus, for temperature conditions of 0°C, 20°C and 40°C, rate constants of 0.004 day^{-1}, 0.007 day^{-1} and 0.032 day^{-1} were observed respectively. Hence,

TABLE 1: A trial wise detail of the parameters measured during the production of the purified lutein. Chemical recovery is the percentage measure of the lutein estimated in the purified sample obtained against the lutein estimated in the Saponified oleoresin. Product yield is the percentage measure of the weight of the purified lutein product obtained from the unsaponified oleoresin

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	Standard Deviation	Variance
Weight of unsaponified oleoresin (g)	250	250	250	250	250	250.00	0.00	0.00
Lutein purity in unsaponified oleoresin (%)	16.70	17.30	16.70	15.90	17.10	16.74	0.54	0.29
Total lutein in unsaponified oleoresin (g)	41.75	43.25	41.75	39.75	42.75	41.85	1.34	1.80
Weight of Saponified mass (g)	203.93	202.64	208.07	201.31	204.53	204.09	2.55	6.48
Lutein purity of Saponified mass (%)	21.65	20.87	21.56	21.42	20.95	21.29	0.36	0.13
Total Lutein content in Saponified mass (g)	44.15	42.29	44.86	43.12	42.85	43.45	1.04	1.07
Weight of purified Lutein mass (g)	39.63	40.02	39.68	38.04	38.80	39.23	0.80	0.65
Purity of purified Lutein mass (%)	90.56	90.89	91.48	91.76	92.00	91.34	0.60	0.36
Total Lutein content in purified Lutein mass (g)	35.89	36.37	36.30	34.91	35.70	35.83	0.59	0.35
Chemical Recovery (%)	81.29	86.01	80.92	80.95	83.30	82.49	2.20	4.84
Product Yield (%)	15.85	16.01	15.87	15.22	15.52	15.69	0.32	0.10

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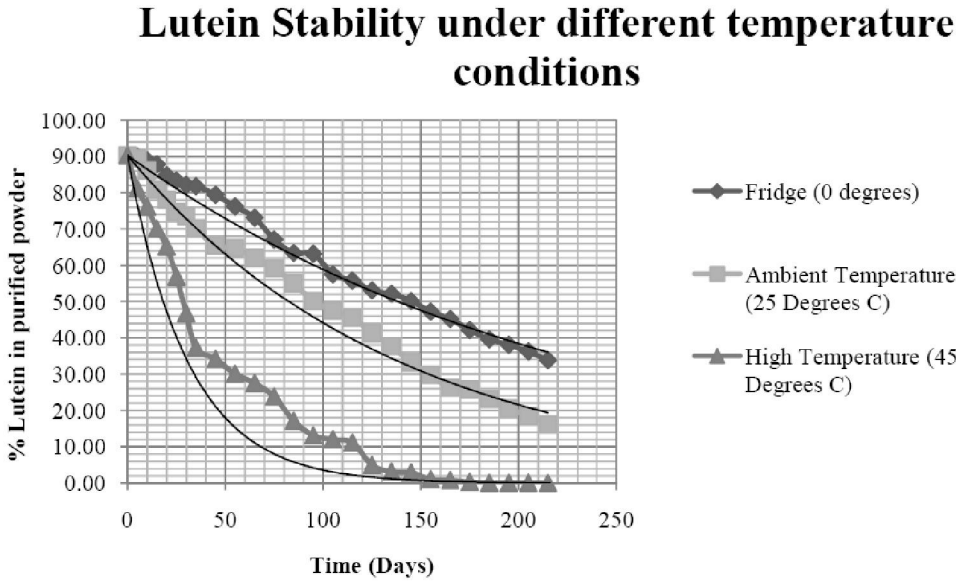
TABLE 2 : The % lutein content of the purified product observed across a 215 day period for a corresponding temperature condition

Time (Days)	% Lutein of purified product			Time (Days)	% Lutein of purified product		
	0°C	20°C	40°C		0°C	20°C	40°
0	90.25	90.25	90.25	95	63.18	50.12	13.10
5	90.02	89.50	81.15	105	57.50	47.50	12.02
10	88.85	86.10	76.15	115	55.80	45.50	11.12
15	87.80	80.80	70.10	125	53.08	41.25	5.00
20	84.80	78.10	65.20	135	52.14	37.72	3.12
25	83.35	74.75	56.90	145	50.12	33.17	2.87
30	82.10	73.50	46.70	155	47.18	29.80	1.09
35	81.75	70.20	37.25	165	45.12	26.20	0.83
45	79.40	65.40	34.12	175	42.08	25.80	0.30
55	76.20	64.80	30.08	185	39.50	23.12	0.03
65	73.10	62.08	27.50	195	38.02	20.50	0.03
75	67.08	59.20	23.80	205	36.17	18.50	0.05
85	63.40	55.10	17.12	215	33.78	16.14	0.01



Graph 1 : The degradation profile of the purified lutein product under temperature conditions of 0°C, 20°C and 40°C

TABLE 3 : Values of the parametric studies made on the 15% lutein oil suspension

Trial	Viscosity (cP)	Particle Size (μ)	L	a	b	ΔE
1	2378	5.8	73.565	22.733	11.452	77.844
3	2305	6.3	79.351	21.736	11.154	83.027
2	2588	4.6	74.848	25.843	15.226	80.634
4	2692	4.6	73.895	24.441	13.284	78.958
5	2555	4.6	75.023	24.989	14.985	80.483
Average	2504	5.2	75.336	23.948	13.220	80.189

the rate constants as a function of temperature may be approximately expressed as $2^{(0.05t-7.966)}$ (Where 't' is the temperature in °C).

An equation to predict the lutein content may thus

be constructed as:

$$\%\ Lutein = (Initial\ \%\ Lutein) \times e^{-(2^{(0.05t-7.988)} \times Time)}$$

While the accurate determination of such temperature – time – rate-constant relations may require multiple data sets from a wider range of temperatures, the equation so constructed may serve as a good cursory reference.

The viscosity, particle size and colour value of the 15% oil suspension given in the table below:

The sunflower oil suspension of lutein was analysed for particle size, viscosity and colour value (L*a*b value). An average particle size of 5.2µ with a viscosity of 2504cP for the oil suspended product was observed suggesting ease of handling and dispensability. Visually, the colour appeared as a saffron-orange liquid with an average L*a*b value of 75.336, 23.948 and 13.220 respectively when used at a 0.1% dose in vegetable margarine.

CONCLUSION

A method to obtain lutein at purities above 90% fromMarigold oleoresin was developed with product yields of about 15% and chemical recoveries of ~82%. Thelutein content of a given sample under known temperature and time conditions may be approximately predicted using the formula:

$$\%\ Lutein = (Initial\ \%\ Lutein) \times e^{-(2^{(0.05t-7.988)} \times Time)},$$

where 't' is the temperature in ^{o}C and time is in days. Furthermore, the lutein so obtained may be used to produce a low-viscosity oil suspension product with a desirable saffron-orange colour.

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