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Extraction and characterization of lutein from plant sources

Shanza Arbab[1], Afshan Shafi[1], Umar Farooq[1], Shabbir Ahmad[1], Khizar Hayat[1], Ehtisham Raza[2], Zarnela Arbab[2]

[1]Department of Food Science & Technology, MNS University of Agriculture, 66000, Multan, Pakistan.
[2]Department of Microbiology, University of Agriculture, Faisalabad, 38000, Faisalabad, Pakistan.

Abstract

Lutein is yellow color hydroxyl-carotenoid compound, as well as a fat-soluble antioxidant which is helpful in brain and eye development. The research was planned for extraction and characterization of lutein from various plant sources. Extracted lutein was also utilized in food products. Specific objective of research was purification, quantification and characterization of lutein. Lutein was extracted from two different sources (marigold and spinach), through extraction process, saponification and HPLC method. Analysis of lutein color was performed through colorimeter by using L* a* b* parameters. Extracted lutein yield from spinach and marigold was 0.7±0.04% and 1.67±0.02% and intensity of color shows L* (25.01±2.40 and 42.79±0.88), a* (0.33±0.22 and 24.43±0.50) and b* (1.87±0.96 and 36.27±0.96) from spinach and marigold. Cupcakes were prepared by using various concentrations (0%, 0.5%, 1% and 1.5%) of extracted lutein. After product preparation the physico-chemical and sensory evaluation of cakes were carried out. The results indicated that 1% concentration of lutein was observed to be suitable for the development of food product as well as organoleptic results also indicated that 1% concentration of lutein was acceptable by the consumer. It is concluded that the extracted lutein was used in the development of cupcake as a natural color because of natural color and may help to prevent the oxidation, age-related molecular degeneration (AMD) and eye disease. Lutein rich food may use for many diseased like AMD, heart and cancer diseases. Extracted lutein also may use as a natural color in food items (candies and cupcake).

Keywords: Lutein, Extraction, AMD, Eye disease.

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**Corresponding Author:*
afshan.shafi@mnsuam.edu.pk

1. INTRODUCTION

Lutein is yellow color hydroxyl-carotenoid compound. It is fat soluble antioxidant. It belongs to class carotenoid which is called Xanthophyll. It is one of the major Carotenoids present in serum of most populations[1].

Lutein has to be taken as dietary sources because human being does not have the capacity to synthesis the lutein. Lutein is oxidation product of carotenoids in plants. Vegetables, fruits and egg yolks are the most important food sources of lutein. In various foods like egg yolk, spinach, kale, broccoli, green peas, green beans, corn, and green leafy vegetables lutein is present as a soluble pigment like lipid. Yellow carrots could increase lutein consumption because it is novel food source of lutein[2].

It is widely present in marigold flower, spinach, kale, eggs and marigold flowers. Egg yolk is rich source of lutein but it is high in saturated fat and also high in cholesterol. Lutein is also present in spinach but it is unpopular vegetable. Carrots are mostly consumed vegetable[3].

Carrots and maize are rich source of lutein. Egg yolk and maize (corn) consist of 85% of lutein. Maize consists of highest quantity of lutein (60% of total). Lutein is present in significant amount (30-50%) in different squash like orange juice, spinach, Zucchini, grapes and kiwi fruit. The statement shows that lutein present in different colors of fruits and vegetables. Dark leafy vegetables are recommended for intake because leafy vegetable are rich source of lutein. These leafy vegetables consist of 15-17% lutein content[4].

Concentration of lutein is maximum in all leafy green vegetables. Highest concentration of lutein was recorded in Basellaalba (504 ppm purple variety), Zaleyadecondra (309.28 ppm green variety and Brassica oleracea var. capitata (314.9 ppm green variety). Zaleyadecondra (red variety) consist of lowest concentration of lutein which is 60.91. Lutein is also present in high amount in purple and green colored varieties[5].

Lutein is organic tetraterpenoid pigment which is nutritionally beneficial. Its molecular formula is $C_{40}H5_6O_2$. Lutein has 568.87144 g/mol weight[6]. In plant kingdom, lutein is one of naturally known carotenoid among 600 carotenoids. Lutein structure is linked to yellow color and the result is that it gives Yellow color to human eye retinal macula, egg Yolk and animal fat. Lutein consists of 40 carbon atoms and these carbon atoms are recognized as tetraterpenoid. An alternate conjugated double bond is present in the biochemical structure of lutein. Single bond is present with polyene chain. This polyene chain ends by ring which contain on either sides. Lutein is dehydroxy- carotenoids. These dehydroxy-carotenoids which consist of ionone ring system being substituted at both the 3 and 3´ carbons. If the conjugated polyene chain is present then Chemical reactivity of lutein is attributed. This is electron rich and highly reactive system. π-electrons is most striking characteristic[7].

Carotenoids can act as antioxidant in human diet. The mechanism of these antioxidant properties takes place through free radical, oxidizing spices and quenching singlet oxygen. Lutein is natural dihydroxy-carotenoid. In plant cells it can be esterified with fatty acid. Hydroxyl group are present at each ionone ring. These rings are present to produce mono and diacylated derivatives. It can be esterfied due to these hydroxyl groups. Lutein is also present in microalgae. It is present in microalgae in its free form. Lutein consists of long chain of conjugated carbon–carbon double bonds and lutein structure is related to heat, oxygen and light degradation reactions. Oxygen, light and temperature can be affected during processing[5]. In nature, around 700 carotenoids have been characterized. Human blood consists 20 or 30 carotenoids but lutein is found in eye[1].

Lutein is only carotenoids stored in our body in the retina and lens eyes[8]. Lutein accumulates in neural tissue. It accumulates in retina which is central region of eye. In retina lutein came from macular pigment (MP). MP plays an important role in the eye health and prevents the retinal disease like age-related macular degeneration (AMD)[9].

According to scientific study lutein is active compound and it is present in human body. It is present in eye and other tissues of body. 3 Age-related macular degeneration (AMD) can be prevented by intake lutein. Lutein is responsible for increasing macular pigment density[10].

AMD is disease of eyes. Lutein plays an important role to prevent the AMD disease. Lutein filters the sun light and also filters the blue lights which damage the eyes. It is considered that AMD is linked in retina with long-term oxidative damage. According to recent studies lutein is very powerful antioxidant. Lutein acts as antioxidant so that it protects the lens from oxidative damage by absorbing the ultraviolet light[11].

In developed countries, blindness increases in older adults. Due to AMD blindness disease takes place. AMD disease is mostly present in man than women because AMD increase with increasing age[1].

Lutein also protects the skin from UV damage in infants. It also prevents the coronary heart disease, cancer and cardiovascular[10].

According to recent papers lutein accumulated in brain. Lutein helps to improve the cognitive function in elder persons. Recent studies shows that intake of lutein in man is 0.8mg to 2.4mg per day. Lutein also protects the ocular tissues[12].

2. MATERIALS AND METHODS

2.1 Sample preparation

Sample was prepared from the petals of marigold and leaves of spinach. After it, sample were dried, grind into fine powder and store at 4°C in a domestic refrigerator until use[10].

2.2 Separation and purification of lutein

To isolate the lutein from marigold (genus; *Tagetes* & family; *Asteraceae*) flowers and spinach (*Spinacia oleracea*) leaves, several steps were necessary. Purification and separation procedure consist three main steps: extraction, saponification and crystallization[10].

2.2.1 Extraction of oleoresin

100 grams of fine powder was extracted with 500 ml of hexane at 40°C for 4 hours. By using the rotary vacuum evaporator at 40°C for 15 minutes the extract was evaporated. After the vacuum evaporation, drying process was started. Drying process takes place in vacuum oven at 30°C for 2 hours. 100g of marigold flowers and leaves of spinach sample were used from which only 10g of dried oleoresin obtained. The prepared oleoresins were used for further investigation on lutein separation and purification process[10].

2.2.2 Saponification of lutein fatty acid esters

1 gram of marigold and spinach extract was dissolved in ethanol. 2 ml of ethanol was used for saponification at the temperature 75°C. After it, 0.5ml solution of potassium hydroxide (KOH) was used.

KOH was added in mixture slowly. Mixture of oleoresin was placed for 4 hours for saponification process. After this, cool the mixture at 65°C. pH of solution was adjusted to about 7.0 with aqueous solution of hydrochloric acid. This step was considered as a completion step for saponification. The resulted mixture was subjected to crystallization to further purify the free lutein product[13].

2.2.3 Crystallization of lutein

After neutralization, Saponified oleoresin sample was mixed in a specified crystallization solvent mixture of H_2O : EtOH (ethanol) in 2:0.5 at 65°C. After it, mixture was cooled at 65°C for 30 min and yellow precipitate was obtained. The precipitate obtained was filtered by Whatman filter paper No.5 under suction until dried. Weigh the dried precipitates and analyze for the amount of free lutein to determine the yield (amount of free lutein in precipitate per the total free lutein) and the purity (amount of free lutein per weight of dry precipitate) of the resulted product[14].

2.3 Analysis of lutein

2.3.1 Color estimation through colorimeter

Color is important parameter in lutein. Colorimeter is used to detect the color of marigold and spinach extract. Color of extracted lutein from both sources was measured using Minolta Chroma meter. Data was reported in the L*, a* and b* color notation system determined with standard protocol given by[15].

2.3.2 Analysis of free lutein by HPLC

In order to analyze the lutein content in the final product (precipitate), the precipitate was dissolved in 50 ml of ethanol under sonication at 30°C ± 5°C for 15 minutes. A small amount of insoluble impurity suspended in the solution was separated from the soluble portion by centrifugation. The solid impurity was extract again with 50 ml of ethanol to recover all the lutein. After it solutions was combined and analyze for the concentration of free lutein with HPLC. The free lutein fatty acid esters from saponification and crystallization steps were analyzed by HPLC method. The sample solution was injected to Lichrocart C-18 column, a Diode Array Detector Module 335 and an automatic injector. A 5 µm reversed-phase was used. Chromatographic separation was obtained with a gradient solvent system of acetonitrile:methanol (9:1, v:v) (A) and ethylacetate (B), from 0% to 100% of B using a linear gradient over 30 min, at a flow rate of 1 ml/min and detection at 450 nm[12].

2.4 Product development

Lutein supplemented cakes were prepared by using lutein in different concentration 0%, 0.5%, 1% and 1.5% respectively, as a natural color[12]. Recipe and treatment plan for the preparation of lutein supplemented cakes is given in Table 1 respectively.

Table 1: Treatment plan for the preparation of lutein enriched cupcake.

Treatments	Ingredients						
	Lutein extracts	flour	Sugar	Butter	Salt	Eggs	Milk
T_0	0%	250g	250g	250g	1g	4	4tps
T_1	0.5	250g	250g	250g	1g	4	4tps
T_2	1.0%	250g	250g	250g	1g	4	4tps
T_3	1.5%	250g	250g	250g	1g	4	4tps

2.5 Product analysis

2.5.1 Physico-chemical analysis

The prepared cake was analyzed for physico-chemical parameters like crude fat, crude protein, Ash and moisture by following the method of[16].

2.5.2 Analysis of moisture

The cake's moisture content was measured in a hot air oven using the method described by[17]. Place a 5g sample of cake in a china dish that has already been weighed, and then bake it at 105°C for 24 hours to dry it out. The sample was weighed once more after drying, and the final reading a percentage of the actual moisture was recorded using the formula below.

$$\text{Moisture}\% = \frac{weight\ of\ actual\ sample - weight\ of\ dried\ sample}{\text{actual sample weight}} \times 100$$

2.5.3 Crude fat

The cake sample was assessed using the soxhlet apparatus method, as stated in[17]. Up to 5g of cake sample was obtained and placed in an extraction thimble. The thimble was then sealed in a Soxhlet device using hexane for two to three hours. The apparatus's tube contained fumes that were concentrated in the upper part due to the passage of cold water. To capture these vapors, a conical flask containing a solvent was placed on the heater. Next, a thimble containing a sample was taken, and these condensation-dipped drops were placed inside of it. The previously heated flask was disassembled, dried, and cooled before being weighed. The receiving flask containing the fat was then dried in an oven, cooled in a desiccator, and weighed again. After that, the crude fat percentage was determined using the formula below.

$$fat\% = \frac{\text{Actual sample weight} - \text{free fat sample}}{\text{actual sample weight}}$$

2.5.4 Crude protein

The Kjeldhal technique was used to assess the cake's crude protein content, as shown in[17]. A 2 gram sample of cake was put in a digestion tube along with 20 milliliters of 98% concentrated sulfuric acid (H2SO4) and two catalyst tablets as the digestion combination. The translucent leftovers were obtained after the digesting process lasted for three to four hours. Next, add 70 milliliters of 40% NaOH solution to the mixture to neutralize it while also releasing the ammonia gas. Kjeldahl's distillation technique was utilized to accomplish the distillation of the neutralized solution. The liberated NH4 gas was held in a 4% boric acid solution that contained indicators. The titration was done against 0.1N Sulphuric acid of the ammonia which was collected to end point which showed purple color. The similar procedure was repeated without the sample for getting the blank reading. Then the percentage protein of the cake was estimated by using the following formula below.

$$Nitrogen\% = \frac{0.1\text{N vol. of H2SO4} \times \text{vol. of dillution prepared} \times 0.0014}{\text{actual weight} \times \text{dillution vol.}} \times 100$$

2.5.5 Ash

The procedure outlined in[17] was used to assess the sample's ash percentage. Weigh out a 10-gram sample and place it in a crucible. Next, place it in a muffle furnace set at 5500C for five hours. The material turned into a grayish whitish residue after five hours. The following equation was used to estimate the percentage of ash. The following equation was used to estimate the percentage of ash.

$$ASH\% = \frac{-weightofgrayishresidues}{\text{weight of actual sample}} \times 100$$

2.5.6 Crude fiber

The fiber content of the cake was assessed using the method described in[17]. 10g of flour should be weighed and then digested in 200 ml of 1.25 percent H2SO4. Following digestion and filtering, the material underwent three ethanol washes. Following a second washing with ethanol, the sample was boiled in 200 ml of NaOH for 30 minutes, after which it was filtered once more and then washed three times with ethanol. Following sample ignition at 600ºC for two to four hours, crude fiber was computed using the formula below.

$$fiber\% = \frac{\text{weight loss on ignition}}{\text{weight of actual sample}} \times 100$$

2.5.7 Sensory assessment

The cake prepared were evaluated organoleptically for sensory attributes including taste, texture, color, aroma and acceptability by a panel of experts according to the protocol adopted by the[18].

2.5.8 Color estimation through colorimeter

Color of lutein enriched cakes will be measured using Minolta Chroma meter and data will be reported in the L*, a* and b* color notation system determined with standard protocol given by[15].

2.6 Statistical analysis

The data which was found from research was subjected to analysis of variance (ANOVA) by using STATISTIX (Version 8.1) software as recommended by[19].

3. RESULTS AND DISCUSSIONS

Extraction and color estimation of lutein

Lutein was extracted from two different sources (marigold and spinach). The results indicated that yield of lutein from marigold were observed 1.67±0.02% whereas the yield from spinach were 0.7±0.04% as shown in (Table 2).

Whereas the lutein color indicated that it contain L* (25.01±2.40 and 42.79±0.88), a* (0.33±0.22 and 24.43±0.50) and b* (1.87±0.96 and 36.27±0.96) from spinach and marigold.

Color estimation

Color of marigold and spinach extract was measured through colorimeter. The results indicated that color of marigold extract contain L*, a*, b* with mean value of 42.79±0.88, 24.43±0.50, 36.27±0.96. Further results of spinach extract revealed that the mean value of L*, a*, b* extract was 25.01±2.40, 0.33±0.22, 1.87±0.96 as shown in (Table 2). The result further revealed that L* value indicated the lightness and b* value indicates the intensity of yellow color whereas a* value represent green color. Sometime L*, a*, b* also known as Lab or Color space.

Table 2: Lutein Yield and color estimation from marigold and spinach sources.

	Marigold	**Spinach**
Yield	1.67±0.02%	0.7±0.04%
Color estimation of lutein		
L*	42.79±0.88	25.01±2.40
a*	24.43±0.50	0.33±0.22
b*	36.27±0.96	1.87±0.96

Mean±S.E

Similar findings were also documented by (15) during his research experiment which was conducted on lutein extraction and measured L*, a*, b* value from marigold. It was observed in this experiment that the result of L*, a*, b* was 47.9±0.50 25.2±0.65 50.5±0.24 from marigold flowers. Another research finding reported by (11) also well agrees with current study results regarding L*, a*, b* values of spinach.

HPLC of lutein

After the extraction of lutein, residues of lutein were collected for further identification and characterization process on HPLC. For this lutein esters were prepared using hexane. An HPLC system consisting of two LC-20AT pumps, SPD-M20A diode array detector was used. The chromatographic data were recorded. Standard lutein with 92% purity was used in this process to compare the extracted lutein esters. Lutein ester showed the same absorption comparable with standard lutein spectrum. The identification of lutein was confirmed by comparison with the retention time of a standard lutein. Quantitative determination of lutein and lutein esters were based on analytical curve of lutein. The results showed that chromatogram peak area (A) was of standard lutein, B area was showed the chromatogram of spinach and C area was represented the lutein which was extracted from marigold.

HPLC results further revealed that lutein esters extracted from marigold showed longer peak resemble to standard peak with yield 1.67% as shown in (Fig 1). The absence of shorter peaks in the Chromatogram (C) of lutein esters extracted from marigold represent the purity of lutein in marigold similar to the standard lutein chromatogram. Whereas chromatogram of lutein esters extracted from spinach (B) indicated a resembled peak with standard lutein peak with multiple shorter peaks. These shorter peaks indicated the presence of zeaxanthin and other carotenoids in lutein esters extracted from spinach as compared to lutein which was extracted from marigold and standard lutein.

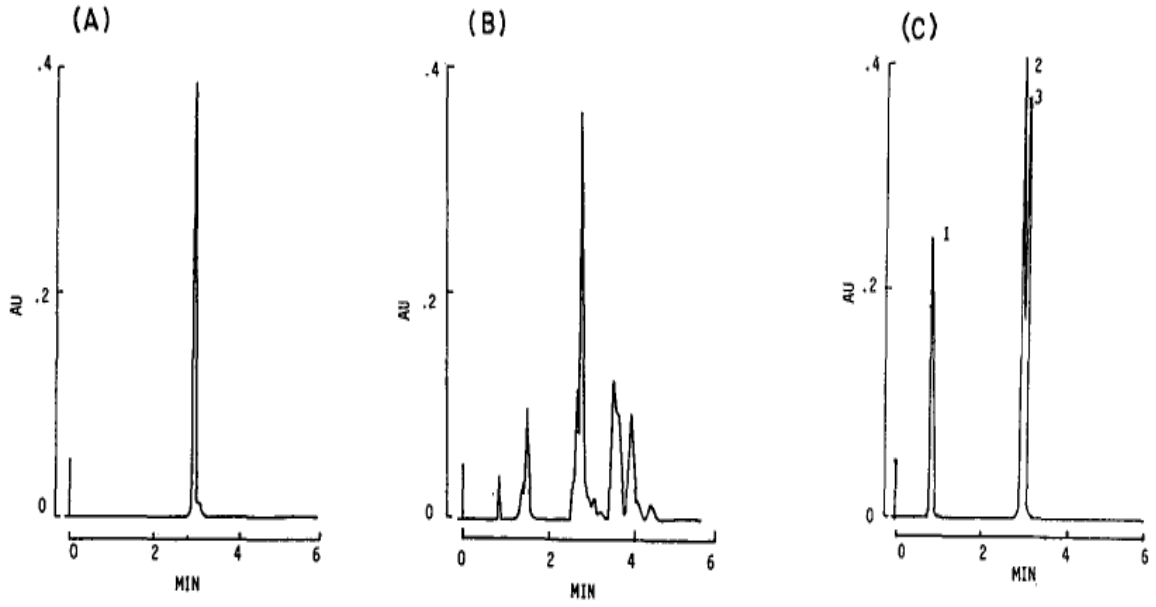

Figure 1: (A) = chromatogram of Standard lutein with 92% purity, (B) = chromatogram of lutein from spinach, C = Chromatogram of lutein from marigold.

Development of lutein supplemented cakes

Lutein supplemented cupcakes were prepared by adding lutein extract which was extracted from spinach leaves and marigold flowers with different concentrations as 0%, 0.5%, 1% and 1.5% as shown in Table 1 (mentioned above).

Physico-chemical analysis of lutein supplemented cake

Moisture

The statistical results relating the moisture % of cake supplemented with lutein showed that the effects of treatments and storage time were found to be very high. On the other hand, the combined effect of the treatments and the length of storage on the product's moisture % was shown to be insignificant. Due to differences in treatments and storage times, the moisture contents varied from 18.83±0.06% to 17.53±0.01%. The highest moisture content (18.83±0.06) was observed in cake with T_1 whereas the lowest moisture contents (17.53±0.01) were observed in T_3 (Table 3). Moisture contents were ranged from 21.28±0.07 to 15.53±0.07 in storage periods. The highest moisture content (21.28±0.07) was observed in cake at 1st day whereas the lowest moisture content (15.53±0.07) at six day of storage (Table 4). It's possible that moisture from the surroundings was absorbed, which is why the moisture contents decreased during storage.

Crude fat

The statistical results relating the fat percentage of cake supplemented with lutein showed that the effects of treatments and storage time were found to be very high. On the other hand, the combined effect of the treatments and the length of storage on the product's fat content was shown to be insignificant. The range in fat contents was caused by differences in treatments and storage times, ranging from 12.39±0.03% to 12.43±0.03%. The highest fat content (12.43±0.03) was observed in cake

with T_3 whereas the lowest fat contents (12.39±0.03) were observed in T_1 (Table 3). Fat contents were ranged from12.32±0.04% to 12.54±0.04% in storage periods. The highest fat content (12.32±0.04%) was observed in cake at 1st day whereas the lowest fat content (12.54±0.04%) at six day of storage (Table 4). It is possible that fat from the surroundings is absorbed, which would explain the drop in fat amounts during storage.

Crude fiber

The results of treatments and the time of storage had a substantial impact on the fiber percentage of the cake, according to the statistical analysis of lutein-supplemented cakes. On the other hand, the combined effect of the treatments and the length of storage on the product's fiber % was shown to be insignificant. Because of differences in treatments and storage times, the fiber contents varied from 1.70±0.04% to 1.79±0.02%. The highest fiber content (1.79±0.02%) was observed in cake with T_3 whereas the lowest fiber contents (1.70±0.04) were observed in T_1 (Table 3). Fiber contents were ranged from1.61±0.01% to 1.86±0.01% in storage periods. The highest fiber content (1.86±0.01%) was observed in cake at 6th day whereas the lowest fiber content (1.61±0.01%) at first day of storage (Table 4). Fiber from the environment may have been absorbed, which would explain the increase in fiber contents during storage.

Ash

The results of treatments and the time of storage had a substantial impact on the ash percentage of the cake, according to the statistical analysis of the lutein-supplemented cake. On the other hand, the combined effect of the treatments and the length of storage on the product's ash % was shown to be insignificant. The ash contents varied between 0.98±0.01% and 1.02±0.02% as a result of different treatments and storage times. The highest ash content (1.02±0.02%) was observed in cake with T_3 whereas the lowest ash contents (0.98±0.01%) were observed in T_1 (Table 3). Ash contents were ranged from 0.88±0.01% to 1.11±0.01% in storage periods. The highest ash content (1.11±0.01%) was observed in cake at 6th day whereas the lowest ash content (0.88±0.01%) at first day of storage (Table 4). The absorption of ash from the surroundings may be the cause of the increase in ash contents during storage.

Crude protein

The results of treatments and the time of storage had a substantial impact on the protein percentage of the cake, as demonstrated by the statistical implications about the protein percentage of cake enhanced with lutein. On the other hand, the combined effect of the treatments and the length of storage on the product's protein % was shown to be insignificant. The protein levels varied depending on the treatments and storage times, ranging from 8.27±0.09% to 8.20±0.03%. The highest protein content (8.27±0.09) was observed in cake with T_1 whereas the lowest protein contents (8.20±0.03) were observed in T_3 (Table 3). Protein contents were ranged from 8.46±0.04 to 7.94±0.04 in storage periods. The highest protein content (8.46±0.04) was observed in the cake at 1st day whereas the lowest protein content (7.94±0.04) at six day of storage (Table 4). Protein absorption from the surroundings may be the cause of the drop in protein levels during storage.

Sensory evaluation of lutein supplemented cake

Color

The impact of several treatments on the color of the cake supplemented with lutein revealed that T0 had the greatest color score, with a mean score of 6.67±0.02, appropriately. As shown in (Table 3), the T3 substantially had the lowest color score, with an average score of 6.61±0.03. The highest color content (7.94±0.03) was observed in cake at 0 day whereas the lowest color content (5.79±0.05) at six day of storage (Table 4).

Texture

The effects of the treatments on the texture of the lutein-supplemented cake showed that T0 (the cake without lutein supplementation) had the considerably highest texture score, with a mean score of 8.14±0.02, in accordance. However, as seen in (Table 3), T3's texture score, with a mean score of 7.95±0.02, was substantially lower. The highest value of texture (8.39±0.06) was observed in cake at 0 day whereas the lowest value of texture (7.58±0.03) at six day of storage (Table 4).

Taste

The study examined how different treatments affected the taste of cake supplemented with lutein. The results showed that T0 (cake supplemented with lutein) had the highest texture score, with a mean score of 7.37±0.03, indicating a significant difference. However, as seen in (Table 3), T3's texture score, with a mean score of 7.00±0.02, was substantially lower. The highest taste value (7.59±0.04) was observed in cake at 0 day whereas the lowest taste value (6.27±0.03) at six day of storage (Table 4).

Volume

The impact of several treatments on the lutein-supplemented cake volume revealed that T0 (the cake without lutein supplementation) had the greatest volume score, with a mean score of 7.67±0.01 in relation to it. Nevertheless, as shown in (Table 3), T3's texture score, with a mean score of 7.63±0.07, was substantially lower. The highest volume (7.77±0.07) was observed in cake at 0 day whereas the lowest value of volume (7.53±0.07) at six day of storage (Table 4).

Overall acceptability

The impact of treatments on the overall acceptability of the lutein-supplemented cake revealed that T0 (the cake without lutein supplementation) received the greatest overall acceptability score, with a mean score of 7.11±0.01 in accordance. However, as seen in (Table 3), T3's texture score, with a mean score of 6.93±0.03, was substantially lower. The highest value of overall acceptability (7.61±0.02) was observed in cake at 0 day whereas the lowest value of overall acceptability (6.76±0.04) at six day of storage (Table 4).

Color estimation through colorimeter

L* value of cake:

The effects of treatments on the L*value of the cake enriched with lutein showed that the color score had the maximum L* value in T3, 48.21±0.09, and the lowest value in T0, 47.97±0.09 (Table 3). The highest value of L* (48.89±0.09) was observed in cake at 6^{th} day whereas the lowest value of L* observed (47.32±0.09) at 0 day of storage (Table 4).

a* value of cake:

The lutein supplemented cake's a* value showed that the highest color score (15.54±0.05) in T3 and the lowest value (19.13±0.04) in T0 were the results of treatment effects (Table 3). The highest value of a* (16.00±0.04) was observed in cake at 6th day whereas the lowest value of a* observed (14.74±0.04) at 0 day of storage (Table 4).

b* value of cake

Treatments' effects on the b* value of the lutein-supplemented cake showed that T3 had the highest b* value of color score (19.48±0.07), while T0 had the lowest value (19.13±0.04) (Table 3). The highest value of b* (20.03±0.05) was observed in cake at 6th day whereas the lowest value of b* observed (18.61±0.05) at 0 day of storage (Table 4).

Table 3: Effect of treatment on lutein supplemented cake.

Parameters	**T0**	**T1**	**T2**	**T3**
Physico-chemical analysis of lutein supplemented cake				
Moisture (%)	18.83±0.06	18.43±0.05	17.93±0.02	17.53±0.01
Fat (%)	12.39±0.03	12.41±0.01	12.43±0.02	12.43±0.03
Fiber (%)	1.70±0.04	1.72±0.02	1.75±0.02	1.79±0.02
Ash (%)	0.98±0.01	0.99±0.01	1.01±0.03	1.02±0.02
Protein (%)	8.27±0.09	8.26±0.04	8.23±0.05	8.20±0.03
Sensory evaluation of lutein supplemented cake				
Color	6.67±0.02	6.65±0.02	6.62±0.03	6.61±0.03
Texture	8.14±0.02	8.08±0.01	7.99±0.01	7.95±0.02
Taste	7.37±0.03	7.18±0.03	7.07±0.02	7.00±0.02
Volume	7.67±0.01	7.66±0.03	7.65±0.05	7.63±0.07
Overall acceptability	7.11±0.01	7.08±0.04	7.07±0.03	6.93±0.03
Color estimation through colorimeter				
L*	47.97±0.09	47.98±0.09	48.04±0.07	48.21±0.09
a*	15.13±0.05	15.32±0.05	15.37±0.04	15.54±0.05
b*	19.13±0.04	19.24±0.02	19.37±0.04	19.48±0.07

Mean±S.E

Table 4: Effect of storage period on lutein supplemented cake.

Parameters	0 month	2 months	4 months	6 months
Physico-chemical analysis of lutein supplemented cake				
Moisture (%)	21.28±0.07	18.97±0.07	16.93±0.04	15.53±0.07
Fat (%)	12.32±0.04	12.37±0.03	12.44±0.04	12.54±0.04
Fiber (%)	1.61±0.01	1.71±0.01	1.77±0.02	1.86±0.01
Ash (%)	0.88±0.01	0.96±0.01	1.06±0.01	1.11±0.01
Protein (%)	8.46±0.04	8.30±0.02	8.17±0.04	7.94±0.04
Sensory evaluation of lutein supplemented cake				
Color	7.94±0.03	6.94±0.02	5.88±0.05	5.79±0.05
Texture	8.39±0.06	8.27±0.06	7.93±0.08	7.58±0.03
Taste	7.59±0.04	7.46±0.06	7.25±0.06	6.27±0.03
Volume	7.77±0.07	7.69±0.05	7.62±0.06	7.53±0.07
Overall acceptability	7.61±0.02	6.96±0.02	6.88±0.03	6.76±0.04
Color estimation through colorimeter				
L*	47.32±0.09	47.63±0.07	48.27±0.05	48.89±0.09
a*	14.74±0.04	15.08±0.02	15.53±0.05	16.00±0.04
b*	18.61±0.05	19.03±0.05	19.54±0.08	20.03±0.05

Mean±S.E

4. CONCLUSIONS AND RECOMMENDATIONS

Lutein was extracted from marigold and spinach with the ratio of 1.67±0.02 and 0.7±0.04. The color of extracted lutein was measured through calorimeter. Extracted lutein was used in the development of cupcake as a natural color because study proves that lutein has a positive impact on human health. Lutein rich food was recommended for many diseased like AMD, heart, cancer. Extracted lutein was also recommended as a natural color in food items (candies and cupcake).

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NOVELTY STATEMENT

This study offers valuable insights into the effective use of lutein with their health potentials and acceptability of lutein in bakery as well as other products.

AUTHOR'S CONTRIBUTION

Sahnza Arbab conducted all research work during his post-graduation, Afshan Shafi, Umar Farooq regarded the main research idea and management of the article, Shabbir Ahmad as helped us as a lab incharge, Khizar Hayat and Ehtisham raza helped in manuscript preparation, collection and analysis of data, Zarnela Arbab helped in experimental layout and designing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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