Research Article

Stability of Lutein Content in Color Extracted from Marigold Flower and its Application in Candies

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Abstract | The current research aims to extract edible yellow color from Marigold (*Tagetes erecta*) flower by using cheap and safe techniques and making it stable for long period of time in food products as well as in extracted form by using preservatives and antioxidants like citric acid and determining its usefulness by applying on food products. Natural edible color was extracted from *T. erecta* gave lemon yellow color shade which when applied on candies increased the attractiveness. The spectrophotometric analysis at 474nm of extracted color both in crude form and in candies was found to be stable at 4°C and showed decay in mean lutein concentration at higher temperatures i.e. 25°C and 45°C. The microbiological study of the extracted color and candies prepared in laboratory and dyed with *T. erecta* extract color showed that the color extracted from *T.erecta* itself do not promote microbial growth rather it has antibacterial activity. The lethal dose and toxicity determination of the color extracted from marigold showed that it is safe for consumption as no clinical symptoms were observed in rabbits after giving the maximum dose of 1000ml of color.

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Introduction

Food additives, which include preservatives, food dyes, antioxidants, artificial sweeteners, are being used for thousands of years in food items for enhancing taste, color or smell (Carocho, 2014). Among a large number of food additives, synthetic or artificial food dyes are topic of debate nowadays as they have been found responsible for serious health issues in human race and therefore legislative actions are being taken. Less than 35 dyes are approved by U.S. Food and Drug Administration (FDA). The maximum amount of color that can be used in any food product is 0.1g/kg but mostly it is more than the allowable limit (Yadav et al., 2016). Use of synthetic food dyes that are being made from coal tar and petroleum are the emerging cause of various cancers, behavioral disorders, various food intolerances and mental disabilities (Kobylewski et al., 2010; Zahra et al., 2017). Children are found to be the most affected by the use of such food additives. Children who used diet prevalent by food additives showed hyperkinesis (hypersensitivity) and other behavioral disorders (Nigg and Holton, 2014).

Many food colors have been banned by FDA but it has been found that those nine food color that are approved for use in food items are somehow raising

health issues (Kobylewski et al., 2010). In developed countries a lot of work has been done on the production and use of natural colors. Natural colors are not only safe for health but also have various pharmacological benefits. The chemical compound present in them help to combat many diseases. ß-Carotene, a carotenoid, is orange-yellow in color. It is used as a nutritional supplement (provitamin A) along as a coloring agent (Rymbai et al., 2011; Sigurdson et al., 2017). Similarly Lutein, a carotenoid, is used as natural source for yellow color and along with that it has medicinal properties too (Calvo, 2005). It has been suggested that eating the food rich in lutein and zeaxanthin can decrease the chances of age related macular degeneration (Scripsemaet al., 2015; Abdel-Aal et al., 2013).

Figure 1: *Tageteserecta.*

Tagetes erecta (Marigold Flower) (Figure 1) commonly called genda can be used to extract edible yellow color. Moreover it is easily available and is a cheaper source of extracting natural color. *Tagetes erecta* belongs to the genus Tagetes, family Asteraceae and kingdom Plantae, has many traditional as well as medicinal uses (Dixit et al., 2013). The chemical constituent of this flower is lutein. Lutein (3R, 3'R, 6'R-βε-carotene-3,3'-diol) (Figure 2) belongs to a group of pigments called as xanthophylls and is an oxycarotenoid, and has no provitamin A activity. It is second most prevalent carotenoid in human body. Some studies shows that *Tagetes erecta* contain lutein and zeaxanthin (88-92%) predominantly (Gupta, 2014). Lutein can be used as coloring agent as well as nutrient supplement. Lutein is being used for coloring baked and non-baked items, food products made from wheat, candy floss, sauces, toddler and infant foods, processed foods such as fruits and fruits products, desserts, ice-creams, bubble gums in range from 2 to 330mg/kg. Lutein is currently an authorized natural food color in the EU (Cantrill, 2004).

Constituents of Marigold flower have pharmacological importance such as anti-oxidant, antimycotic and analgesic activities (Gutierrez et al., 2006; Samra et al., 2008; Gopi et al., 2012). The antioxidant property of the lutein crafts it application even in making organic tea, which claims great medicinal value (Pratheesh et al., 2009). A diet rich in lutein contributes to elevated plasma lutein levels in pregnant women without a negative impact upon their health or pregnancy (Alexander et al., 2013). The anti-bacterial activity of different mixtures of *T. erecta*due to the presence of flavonoid has been reported (Motamedi et al., 2015).

Based on all the studies, color extraction will be done from marigold flower using water as solvent. Moreover the extracted color will be applied to candiesto determine the appealing effect of food. The stability of Lutein at different temperatures (4°C, 25°C, 45°C), lethal dose and microbiological analysis were performed to determine that whether it's safe for human consumption or not. Color extraction from *T. erecta* will lead to find new ways to reduce health risks and will also help to replace synthetic dyes with natural food dyes.

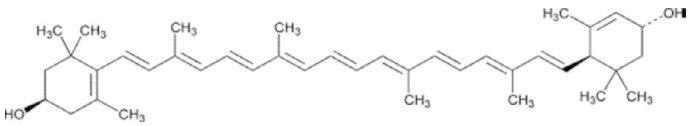

Figure 2: *Chemical structure of Lutein.*

Materials and Methods

Sample of Fully blown dark yellow marigold flowers were obtained from Botanical garden of Forman Christian College Lahore. All the chemicals used were of analytical grade.

Extraction of Color from flowers

Petals of marigold flowers were separated from receptacle, thoroughly washed and were placed in hot air oven for drying. The dried petals were grinded in to fine powder and weighed. Flower powder was blended in a blender with distilled water along with citric acid and NaCl. 2 drops of 5% KOH was added. The solution was filtered with the help of cotton cloth again and again. Filtrate was kept in hot air oven overnight. After drying paste form of extract was obtained. Small quantity of extract was dissolved in distilled water. The extract was further used in appli-

cation on food products, microbiological (antibacterial, antifungal) examination and LD50 (Lethal dose) testing to determine any possible toxicity and lethal dose of the extracted color.

Applications on food product (Candies)

Extract containing lutein, a xanthophyll responsible for yellow color was applied on candiesto analyze it from commercial point of view.

Preparation of the candies

Sugar and water was heated for a long time until a thick solution was formed. Glucose syrup was added and the solution was allowed to cool. The extract which was mixed in water, was added to the solution when its temperature was cooled down to 50°C. (Figure 3 and 4).

Figure 3: *Extract of T.erecta and lemon yellow dye shade.*

Shelf-life study of lutein at different temperatures in food products through Spectrophotometry

The candies that were dyed using Marigold extract color were used to determine the shelf life of lutein, xanthophyll responsible for yellow color in marigold flowers. Candies were dyed using color extracted from *Tagetes erecta*. Candies were packed in sterilized polythene bags and placed at different temperatures (4°C, 25°C, 45°C) in a freezer and incubator along with the extracts for 2 months. Spectrophotometric readings of both candy and extract stored at different temperatures were taken after interval of 7 days.

Preparation of the Sample for Spectrophotometric analysis

Extraction of lutein from candy: 10g of the candy stored at 4°C was dissolved in extractant (hexane10ml+ toluene7ml+ Alcohol6ml+Acetone7ml). 2ml of 5% KOH was also added and was shaken for 2 minutes. The flask was then placed on water bath at 56°C for 20 minutes. It was then allowed to cool in dark.

Figure 4: *Candies dyed with yellowcolor from T.erecta.*

Extraction of lutein from extract: 5g of the extract stored at 4°C was dissolve in extractant and same procedure was followed as mentioned above. Same protocol was followed for rest of the samples (Candies and extract) placed at 25°C and 45°C. The supernatant of the sample (candies and extract) was collected.

Preparation of Lutein standard: Standard of lutein (Sigma Aldrich) was prepared by dissolving 1mg in 50ml ethanol which is 20ppm. Further dilutions of standard were made from this stock solution. The reading was taken at 474nm using water as a blankon spectrophotometer (Model# UV-1800 240V.SHIMADZU Corporation Kyoto Japan) (Sivel et al., 2014) and a standard calibration curve was obtained. Similarly the reading of candies and extract samples were measured. The quantity of lutein in candies sample and extract sample were calculated using following formula:

$$Quantity\ of\ Lutein = As/Astandard \times Conc.\ of\ Standard$$

Where;

As: Absorbance of sample; Astandard: Absorbance of standard solution; Conc. of standard: 20ppm.

Statistical analysis

The data thus obtained was subjected to statistical analysis through Mixed Anova Test (SPSS ver. 8.0). This test was run to determine that whether the mean

lutein concentration decay at high temperature storage conditions and increasing time (days) shows significant results or insignificant results. Level of significance used was 0.05 (5%) (Steel et al., 1997).

Microbiological analysis

Microbiological analysis of the candies dyed with color from marigold and marigold extract was done for the period of 2-months to detect the bacterial, yeast and fungal load. Fresh candies were prepared and half of them were stored at 4°C along with extract and other half was stored at room temperature along with extract. Microbiological analysis of extract and candies stored at different temperatures (4°C, 25°C) was done according to the methods provided in the Manual of Food Quality control (Andrew, 1992).

Dilutions preparation

Steam sterilization of all media and equipment was done at 15 psi for 20 minutes in an autoclave at 121°C. 10g of sample was aseptically weighed and diluted in ratio of 1:10 i.e 10gm in 90 ml Butter Field Phosphate Buffer (BFB). Serial dilutions were prepared by taking 1ml of the first dilution and dissolving in to 9ml BFB. To prepare third dilution 1ml of the second dilution was mixed in 9ml of BFB. The dilutions were stored in test tubes for further use.

Total Plate Count (TPC): Total Plate count was done to detect total bacterial colonies. In TPC, plate count agar (PCA) media 20-25ml/plate were used. 1ml from each dilution prepared earlier was poured on three plates of PCA and were allowed to incubate at 35°C for 24-48 hours.

Detection of total Coliforms: Load of total coliforms is measured in units of most probable number/gram. Lauryl Tryptose Broth Single Strength (LTSS) is used for the detection of total coliforms. Nine test tubes of 10ml LTSS were taken and Durham tubes were placed inside them. Then the nine test tubes were divided in set of three for three dilutions. 1ml from each dilution was taken and poured in these test tubes. Tubes were then incubated at 35°C for 24-48 hours. Gas production in Durham tubes is the indicator of presence of coliforms in sample. For enumerating the total coliforms in the sample a sterilized loop from each gas producing tube was taken and inoculated it in Brilliant Green Broth (BGB) tubes (10ml each with Durham tube). Again gas production will show that sample contains >1100 coliforms.

Detection of fecal coliforms: Fecal coliforms are measured in units of most probable number per gram. Lauryl Tryptose Broth Single Strength (LTSS) was used for the detection of fecal coliforms is and the procedure was same as mentioned above.

For detection of fecal coliforms, EC media is used. Nine test tubes containing EC media were taken and were divided in set of three for all three dilutions. Loop was taken from each gas producing tubes of LTSS and was inoculated in EC media tubes. These tubes were placed in water bath at 44.5oC for 24-48 hours. Gas production shows presence of fecal coliforms.

Detection of E.coli: The media used for detection of E. coli is Eosin Methylene Blue (EMB) Agar. Its colonies are measured in units of Most Probable Number (MPN/g).

Step 1: Three plates with 20-25ml/plate EMB media were prepared. A loop from each EC positive tube were taken and streaked on plates containing EMB media. The plates were then incubated at 35°C for 24-48 hours. Green colonies on EMB media plates will show the presence of E. coli.

Step 2: Nine tubes of Tryptone media were prepared with 5ml/tube. Loop from each EC positive tube was taken and inoculated in Tryptone media tubes. Tubes were then allowed to incubate in water bath at 44-45°C for 16-18 hours. After 16-18 hours 0.2ml Kovac's reagent was added to all tubes. Formation of a purple ring will show the presence of E. coli. Number of tubes with purple ring would be noted down.

Detection of Salmonella: Step 1: 10g of sample was taken and mixed in 90ml of Lactose Broth (LB) media. It was then incubated at 35°C for 24 hours.

Step 2: Took 10ml of Tetra thionate Broth (TTB) and 200µl iodine, mixed it and added 1ml from mixture from step 1 then incubated it at 35°C for 24 hours.

Step 3: After incubation period loops from these mixtures were taken and streaked them on Bismuth Sulphite Agar (BSA), Xylose Lysine Deoxycholate Agar (XLD) agar and Hektoen Enteric Agar (HEA) plates. Then the plates were incubated at 35°C for 24-48 hours. Salmonella presence will be indicated by the formation of black colonies in BSA media. XLD media will show the presence of salmonella if dark black colonies would be appeared in it while HEA will show black/dark green colonies of salmonella.

Detection of staphylococcus aureus

Baird Parker Agar (BPA) and Egg Yolk Agar (EYA) were used to detect staphylococcus aureus in the sample. 0.4, 0.3, 0.3ml of first, second and third dilutions were poured in 20-25ml BPA and 400ml EYA and were mixed well. The plates were then incubated at 35˚C for 24-48 hours. Formation of black colonies will indicate presence of Staphylococcus aureus.

Detection of yeast and mold: Media used for the detection of yeast and molds in the sample is Petro dextrose Agar (PDA). 1ml of all the three dilutions prepared before was poured to 1, 2 and 3 PDA plates respectively. The plates were incubated at 25˚C for 5 days. After incubation the plates were counted.

Toxicological analysis

For the determination of toxic effects of the marigold extract for yellow color, toxicological analysis on healthy rabbits was conducted. 6-8 weeks old 12 rabbits were bought. 6 were in experimental group and 6 were in control group. Rabbits were housed in animal house at temperature 25°C and 12 hour day/light cycle with food and water. All rabbits were weighed. 100ml-500ml doses were prepared andgiven to rabbits of experimental group with the help of dropper, after every14 days interval dose quantity was increased. Physical examination, clinical signs, diet intake and weight of rabbits were examined regularly for a period of two months.

Results and Discussion

Extraction of color from natural sources and making them stable for use in food items is the dire need of today. A lot of work has been done on the extraction of yellow color form Marigold flower (*T. erecta*). Many researches have been done on the extraction of color from marigold but using organic solvents. Pratheesh et al. (2009) extracted lutein using hexane as solvent. The stability of lutein in water is more as compared to Ascorbic acid solution. One possible reason for this can be the neutral pH of water. It has been studied that lutein decay occurs more in acidic pH. Stability and attractiveness of color on candies remain same till 3 months.

Shelf life study of lutein

Lutein is not only known for its nutritive value but also have crucial role in treatment of diseases like age related macular degeneration. By consuming 6mg of lutein per day can reduce the risk of AMD to about 43% (Seddon et al., 1994). So a lot of work is being done for addition of lutein in food samples and making it stable in food. The stability of lutein was analyzed at three different temperatures (4°C, 25°C and 45°C) for a period of two months in candies and extract samples. Minimum decay in lutein quantity was observed in candies stored at 4°C during 63 days of storage period. This decay didn't lower the attractiveness of candies. They were giving same tempting look till 63rd day. Lutein when subjected to heat or light starts degrading. So food items such as frozen products can be dyed with *T. erecta* extract. Some studies showed reduction (10% of initial concentration) in carotenoid concentration when added in foods after the storage for 5 weeks (Aryana et al., 2006). Domingos et al. (2014) added lutein as a dye in yoghurt and determined its stability and also oxidative stability of yogurt. They determined lutein content in yogurt stored in refrigerators using spectrophotometer. They found that lutein content remain same in samples stored in dark and in light. In another study shelf life stability of lutein content fortified in drinking yoghurts and vegetable oils was assessed. A kinetic analysis of the data showed lutein degradation in the various foodstuffs. In drinking yoghurts maximum percentage reductions in the carotenoid content after 5 weeks at 4°C were around 20% and 12% and 37% in vegetable oils at 25 °C for up to 4 months (Lavecchiaand Zuorro, 2008; Zuorro andLavecchia, 2010).

The graph of mean lutein concentrations in candies and extracts at different temperatures with time showed that there is a significant decay in mean lutein concentration of candies stored at different temperatures (Figure 5 and 6). Overall minimum decay was observed at 4°C while maximum decay at 45°C. The Mixed Anova test was run to determine significant or non-significant difference for decay in mean lutein concentration for both candies and extract at different temperatures for 63 days. At 0-day (Time 1) the pair wise comparison for different temperatures showed insignificant decay in mean lutein concentrations. At 7th day (Time 2) the pair of 4 degrees with 25 degrees showed insignificant decay but significant decay was observed between pair of 4 degrees with 45 degrees. Similarly the pair of 25 degrees with 4 degrees showed insignificant decay while there was significant decay of 25 degrees with 45 degrees. Significant decay was observed for the pair of 45 degrees with 4 degrees and 25 degrees. From 14th day

(Time 3) to 63rd day (Time 10) significant decay in mean lutein concentration was observed in all possible pairs. The food products (Candies) dyed with *T. erecta* extract color can be stored at 4°C and 25°C up to 14 days as the Mixed Anova test results indicated insignificant decay in mean lutein concentrations at both of these temperatures. But after 14th day significant decay was observed between the candies at 4°C with 25°C and 45°C. The Extract showed significant decay in pair-wise comparison in all possible pairs indicating that the Extract is more sensitive to storage conditions. Lutein is a temperature and light sensitive compound that can be stored for long period of time under specific conditions. Low temperature storage can retain maximum percentage of lutein. Light also play role in decay of lutein. Proper protection from light can retain maximum amount of lutein. PH also play vital role in preventing the decay of lutein. Acidic pH can cause decay in lutein quantity (Shi and Chen, 1997). 85-87% of lutein was retained in candies stored at 4°C. Quite similar results were cited by Shi and Chen (1997). They studied stability of lutein at different storage conditions including temperature, light and solvent. 88% of lutein was retained in AA+-KOH solution stored at -12°C whereas 80% of lutein was retained in water solution at -30°C.

Microbiological analysis

The total viable count for bacterial and fungal load for both extract and candies samples stored at different temperatures is shown in Table 1 for 2 months. The aerobic plate count for candies stored at 4°C and 25°C was detected as $1.7x10^2$ and $1.3x10^3$ respectively for 1st month. The TPC for extract stored at 4°C and 25°C was found to be $1.0x10^3$ and $2.1x10^3$ respectively for 1st month. The aerobic plate count for 2nd month of candies stored at 4°C and 25°C showed $1.6x10^2$ and $1.2x10^3$ cfu/gm respectively. The extract at 4°C and 25°C showed count of $1.0x10^3$ and $2.0x10^3$ cfu/gm respectively for 2nd month. Total plate count of candies and extract increased as temperature increased from 4°C and 25°C because cooler conditions prevent the growth of microbes. Total coliforms and fecal coliforms and E. coli were found negative in samples of both candies and extract stored at 4°C and 25°C. *Staphylococcus aureus* was not found in candies stored at 4°C and 25°C and was also not present in extract stored at 4°C. The extract stored at 25°C showed count of $2.0x10^1$ cfu/gm *Staph. aureus* in 1st month and count of $1.9x\ 10^1$ cfu/gm *Staph. aureus* in 2nd month (Table 1).

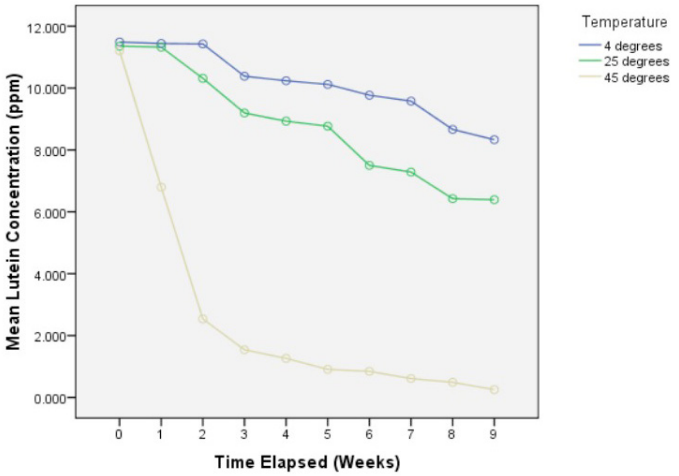

Figure 5: *Mean Lutein Concentration in candies with time at different temperatures.*

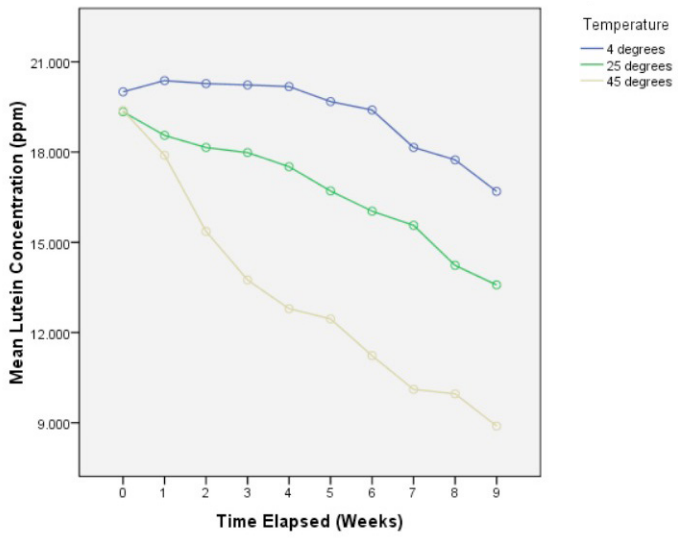

Figure 6: *Mean Lutein Concentration in extract with time at different temperatures.*

No Salmonella growth was observed in any of the samples in both months. Yeast load was positive in all samples with viable count of <10 cfu/gm. Mold was also present in candies stored at 4°C and 25°C and extract stored at 4°C with viable count of <10 cfu/gm. Mold count in extract at 25°C was found to be $6.0x10^1$ in 1st as well as 2nd month. The microbiological tests of the candies and *T.erecta* extract placed at different temperatures (Room temp and refrigeration temp) was done to study growth of different bacteria (*E. coli*, Salmonella, *Staph. aureus*, Coliforms and fecal coliforms) and fungi (yeast and molds). The microbiological results showed that the candies and extract stored at low temperature (4°C) inhibits growth of microbes. Extract samples stored at room temperature showed growth of microbes (*Staph. aureus* and mold). The FDA (Food and Drug Administration) permits maximum limits of 104 cfu/g for total aerobic

Table 1: *Microbiological analysis of candies and extract at different temperatures.*

Microbiological analysis of candies and extract at different temperatures								
	(1st month)				(2nd month)			
	4°C candy	25°C candy	4°C extract	25°C extract	4°C candy	25°C candy	4°C extract	25°C extract
Total Plate Count/g	$1.7x10^2$	$1.3x10^3$	$1.0x10^3$	$2.1x10^3$	$1.6x10^2$	$1.2x10^3$	$1.0x10^3$	$2.0x10^3$
Total Coliforms (MPN/g)	ND	ND	ND	ND	ND	ND	ND	ND
Fecal Coliforms (MPN/g)	ND	ND	ND	ND	ND	ND	ND	ND
E. coli (MPN/g)	ND	ND	ND	ND	ND	ND	ND	ND
Staph. aureus/g	ND	ND	ND	$2.0x10^1$	ND	ND	ND	$1.9x10^1$
Salmonella sp./25g	ND	ND	ND	ND	ND	ND	ND	ND
Yeast count/g	<10	<10	<10	<10	<10	<10	<10	<10
Mold count/g	<10	<10	<10	$6.0x10^1$	<10	<10	<10	$6.0x10^1$

***ND:** *Not detected.*

Table 2: *Toxicological studies of T. erecta extract on rabbits.*

Days interval	Control Group					Experimental Group				
	Dose ml/week	Weight (Kg)		Diet intake	Toxicity symptoms	Dose ml/week	Weight (Kg)		Diet intake	Toxicity symptoms
0-day	ND	Female 1,2,3)	Male (1,2,3)			0	Female 1,2,3)	Male (1,2,3)		
14th day	ND	507, 550, 540	685, 652, 625	Regular	Nil	100	503, 565, 545	688, 654, 630	Regular	Nil
28th day	ND	507, 550, 540	685, 652, 625	Regular	Nil	200	503, 565, 545	688, 654, 630	Regular	Nil
42nd day	ND	507, 550, 540	685, 652, 625	Regular	Nil	300	503, 565, 545	688, 654, 630	Regular	Nil
56th day	ND	507, 550, 540	685, 652, 625	Regular	Nil	400	503, 565, 545	688, 654, 630	Regular	Nil
70th day	ND	507, 550, 540	685, 652, 625	Regular	Nil	500	503, 565, 545	688, 654, 630	Regular	Nil

***ND:** *Normal diet.*

plate count in candies. The present microbiological results of TPC lies within the safe range in accordance with the limit set by FDA. Yeast and mold count is also within the safe range according to Turkish Food Codex Regulation on Microbiological Criteria which permits the 102 /gm count of yeast and molds in confectionery products.

The *Staph. aureus* count should be zero in candies and confectionary products according to limits set by the Codex Alimentarius Commission of Joint FAO/ WHO Food Standards Programme (Codex Alimentarius, 2003).In present study *Staph. aureus* was found positive in extract sample stored at 25°C. The high temperature can be the one reason for its growth whereas the candies at 25°C didn't showed presence of *Staph. aureus*. The possible reason can be the low water content or extreme drying procedure involved in the manufacturing of candies. Drying and low water content can impair the growth of microbes. Products with higher water content are mostly deteriorate with microbial growth (Hasanuzzaman et al., 2014). The mold count in extract at 25°C also showed more growth than extract stored at 4°C. Extract contain more water content than candies which can be one possible reason but on other hand extract at 4°C showed less mold growth which indicates that low storage temperature is suitable. From test it is indicated that *T. erecta* itself do not promote bacterial or fungal growth at refrigeration temperature (Dasgupta et al., 2012). The negative result of many microbes like Salmonella and *E. coli* at both low and high temperatures shows the antimicrobial activity of *T. erecta* extract. For long term storage of *T. erecta* extract low temperatures are suitable.

Toxicological studies

The toxicological analysis of the marigold extract on healthy rabbits for 2 months was done (Table 2). The experimental group and control group showed no change in weight. Physical examination showed positive result. All rabbits remained healthy and active throughout 2 months which indicates that there are no side effects of consuming *T. erecta* extract in

food. Different doses of extract were given to healthy rabbits, while the control group was given the normal diet. Weekly physical examination was done to determine any effect of extract. No differences were studied in pathological features relative to controls. Various studies conducted on toxicity of lutein showed that it has no side effects if consumed. Instead it has more nutritive value. Kruger et al. (2002) firstly assess the safety of lutein and zeaxanthin. They determine by four week and thirteen week oral rat toxicology study. They observed no clinical signs, illness or toxicity related to test material in rats. Test material didn't cause any change in the overall eating habits of rabbits of experimental group.

Conclusion

The purpose of the current research was to extract edible yellow color from *Tagetes erecta* by using cheap yet safe solvent and making it stable for long period of time in food products as well as in extracted form and to determine its stability and usefulness in food products. Color extracted from *T. erecta* which gave lemon yellow color shade and when applied on food products increased the attractiveness of food by giving them lemon yellow color. The stability of the extract in crude form and in candies was determined through spectrophotometer at 474nm. The color both in crude form and in candies was found to be stable at 4°C and showed decay in mean lutein concentration, at higher temperatures i.e. 25°C and 45°C. The microbiological analysis of the extracted color in crude form and candies that were dyed with color from *T. erecta* showed that the color both in crude form and in candies do not promote microbial growth rather it has antibacterial activity. The lethal dose and toxicity determination of the color extracted from marigold showed that it is safe for consumption as no clinical symptoms were observed in rabbits after giving the maximum dose of 1000ml of extracted color.

Author's Contribution

Alim-un-Nisa: Concieved the idea and provided technical input at every step.
Sania Mazhar: Methedology and microbialogical assays.
Imran Kalim: Methedology and spectrphotometric analysis.
Ijaz Ahmad, Sajila Hina and Qurat-ul-Ain Syed: Provided technical input at every step
Naseem Zahra: Methedology and shelf life study.
Shahid Masood: Methedology and colour extraction.
Muhammad Khalid Saeed: Data collection, data entry and analysis
Maida Asif: Helped in writing the manuscript.

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