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BLEACHING OF COLOR FIXED COTTONSEED OIL

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By
K. S. M. Hammad, S. A. Hallabo and S. M. Galal

Food Technology Department, Faculty of Agriculture, Cairo University, Giza, Egypt

ABSTRACT

Six varieties of bleaching agents: Commercial, Engelhard and Tonsil bleaching earth, magnesium oxide, aluminum oxide and charcoal were selected to evaluate their abilities on reducing color and gossypol content of color fixed neutralized cottonseed oil. Color of this oil was higher than 50 red units / 20 yellow using Lovibond Tintometer and 1 inch cell and its gossypol content was 200 ppm . The bleaching of the color fixed cottonseed oil was carried out by using two methods, conventional method (stirring oil with bleaching earth), and passing the oil on column filled with bleaching adsorbents. The aluminum oxide showed a superior adsorption capacity in reducing color to less than 1.4 red/20 yellow and reducing the gossypol content to 21 ppm, by using the column system. The conventional method failed to reduce the color of the treated samples therefore, alkaline pretreatments were conducted. The treatment of color fixed oil with alkaline solution (sodium hydroxide solution 14.5 Be^{o} or sodium silicate at 45 Be^{o}) followed by conventional Engelhard bleaching earth treatment reduced the color of oil to values lower than 1.4 Red/20Yellow.

Key words: *bleaching, color fixation, cottonseed oil and gossypol*

1. INTRODUCTION

The amount of cottonseed oil produced in Egypt in 2007 reached 46500 tons, while the imported quantity of cottonseed oil represented 5976 tons in 2006 (FAO, 2010).

Cottonseed oil obtained by conventional pressing process or solvent extraction contains gossypol pigments .Bleaching is an important step in the refining of vegetable/animal fats and oils for industrial applications. In edible oil processing, bleaching is responsible for clarified oil that is more stable and also more attractive to the consumer. Clarification is usually performed by an adsorption process which preferentially uses acid-treated clays to remove undesirable oil components .The well known problem of darkening solvent extracted cottonseed oil has been generally known as color fixation. This color problem has been accentuated because of difficulties in removing the fixed pigments from the dark colored oils by the current methods of alkali refining and bleaching. Color fixation occurs when the crude oil or the oilseeds are overheated during processing and it may also occur during their storage prior to processing (Zaher *et al*.,1991).

Several methods have been developed to remove gossypol pigments from cottonseed oil. Different chemicals were used in these methods such as ferric chloride (Yatsu *et al.*,1969), sodium silicate and ethanol amine (Attia *et al.*,1981) and sodium silicate followed by sodium hydroxide (Taha *et al.,* 1987).

The objectives of this work were to develop several applicable methods to remove pigments, particularly gossypol, from solvent extracted neutralized color fixed cottonseed oil. Color and gossypol content were followed. Besides, oxidative status of oil samples resulted from the succeeded treatments were determined.

2. MATERIALS AND METHODS

2.1. Materials

Ten kilograms of solvent extracted alkali-refined cottonseed oil were obtained from El-Badrashin Factory, Cairo Oil and Soap Company, Egypt. Sodium silicate solution (45 Be^{o}) (SiO_2 45.5 % and Na_2O 9 %) was obtained from Ghamra Factory, Cairo Oil and Soap Company, Egypt. Three types of bleaching earth were used in this study: commercial bleaching earth was procured by Nile Valley Vegetable Oil Company, Egypt, Engelhard bleaching earth (0-Basf) grade-F 160 a product of a Chemical Company (U.S.A.)and Tonsil bleaching earth of Sud-Cherie, Mexico were obtained from Cairo Oil and

Soap Company, Egypt. Besides activated charcoal (obtained from Cairo Oil and Soap Company, Egypt), basic aluminum oxide for analysis (Chromatography grade) (obtained from Hopkin and Williams, Ltd, London) and magnesium oxide (obtained from Hayashi Pure Chemical Ind. Ltd., Osaka, Japan) were also used as bleaching agents. Standards: Gossypol acetic acid obtained from Sigma- Aldrich Company, USA. and Fatty acids of C10,C12 ,C14 ,C16 ,C18,C18:1 ,C18:2,C18:3 and C20 (purity >99%by GLC)were obtained from Koch Light Laboratories , Ltd. England.

2.2. Methods

2.2.1.Physical and chemical properties of cottonseed oil

Color of refined and bleached cottonseed oil was measured by Lovibond Tintometer using 1 inch cell at 20 yellow , photometric index, refractive index, iodine value, saponification value, soap, unsaponifiable matter, anisidine value, peroxide value, acid value, and total gossypol content were determined according to AOCS official methods (1993). Absorption spectra of 4 % CCl_4 solutions of neutralized cottonseed oil (color fixed) and of the bleached oil was determined using Shimadzu spectrophotometer according to Yousef (1998)

2.2.2. Silicon content

Silicon content in cottonseed oil was determined before and after silicate treatment by ICP-OC using plasma emission at 288 nm after ashing according to Masson *et al*. (2007)

2.2.3. Gas chromatographic analysis of the fatty acids

2.2.3.1.Extraction of fatty acids

The fatty acid were extracted from the oil (after saponification and acidification) according to the method described by the AOAC (2000)

2.2.3.2.Preparation of fatty acids methyl esters

The methyl esters of the different fatty acids were prepared using a mixture of methanol: concentrated sulfuric acid at 99:1 v:v and the fatty acids methyl esters were extracted with hexane, according to the method described by Morsi *et al* (2008).

2.2.3.3. Identification of the fatty acids methyl esters by GLC

The fatty acid methyl esters were analyzed by a Hewlett Packard gas chromatograph apparatus 5890 equipped with a flame ionization detector. The chromatograph apparatus was fitted with FFAP 30m ×0.32mm×0.25mm capillary column coated with polyethylene glycol-TPA modified. The column oven temperature was programmed at 7 ^oC/min from 50 ^oC to 240 ^oC, whereas, it was kept for 30 min .Injector and detector temperatures were 260 and 300 ^oC, respectively. Gas flow rates were 33,30, 330, ml/min for N_2 , H_2 and air ,respectively and the split ratio was 100:1 .Peak identification was performed by at comparison of the relative retention time (RRT) for each peak with those of the standard fatty acids, using the relative retention time of oleic acid as 1.0 . The peak areas and the relative areas of the identified fatty acids were measured using HP Chemstation software .

2.2.4. Bleaching treatments

Pretreatment with alkaline solution was conducted before bleaching with conventional method.

2.2.4.1. Sodium silicate treatment

Neutralized color fixed cottonseed oil was treated with 10 and 20 % of sodium silicate (45 Be^o) at 65 ^oC for 30 min. using magnetic stirrer. The oil layer was separated by centrifugation at 3000 rpm for 15 min. The treated oil was washed by equal volume of hot water for three times. The washed water was discarded and oil was dried at 105 ^oC (Helmy *et al.*,1994) .The treated oil was subjected to bleaching with the most efficient bleaching earth (Engelhard) at (4 and 5 %) at 100 ^oC with mechanical stirring for 30 min. The oil was recovered by passing through a medium fast filter paper.

2.2.4.2. Sodium hydroxide solution treatment

A 100 ml sample of the neutralized color fixed cottonseed oil was treated with 2.5 and 10 ml sodium hydroxide solution (14.5 Be^o). The mixture was stirred mechanically for 30 min. at 65 ^oC. The resulting mixture was centrifuged for 15 min. at 3000 r.p.m (Deacon *et al*., 1957). The separated oil layer was washed with water till its soap content reached <300 ppm. The oil layer was dried and bleached with the most efficient bleaching earth (Engelhard) at 1.5, 2, 2.5 and 3 % levels and filtered through fast filter paper.

2.2.5. Bleaching earth treatments

2.2.5.1. Conventional method

Neutralized color fixed cottonseed oil was bleached with 5% Engelhard, Tonsil, commercial, Charcoal, Al_2O_3 or MgO, at 100°C for 30 min. and filtrated through fast filter paper. (Megahad and El-Kinawy, 2001).

2.2.5.2. Column system

Neutralized color fixed cottonseed oil either free from solvent or in the form of miscella (20% oil in hexane) was bleached by passing over column (30cm/1cm) of Al_2O_3, MgO, or best efficient bleaching earth (Engelhard) only since, using other bleaching agents no oil passed through the column. The total quantity of oil that passed through the column with color < 1.4 red units /20 yellow using Lovibond Tintometer and 1″ cell per 1 part of the adsorbent agent was recorded and this treatment was terminated when color of the treated oil exceeded 1.4 red units/20 yellow.

2.2.5.3. Regeneration of adsorbent

The abused adsorbent (aluminum oxide) was washed several times with acetone (1:1), and then the treated adsorbent was ashed at 550 ^{o}C according to Kuk and Tetlow (2005) before reusing for bleaching.

Bleachability was calculated using the following formula:

Bleachability = (Photometric index for color fixed oil-Photometric index for bleached oil)/ Photometric index for color fixed oil) *100 according to Helmy (1985).

3. RESULTS AND DISCUSSION

3.1. Physical and chemical properties of cottonseed oil

The results in Table (1) show that refractive index at 20/20 ^{o}C, saponification value, iodine value of the neutralized color fixed cottonseed oil are 1.4692 ,184 mg KOH/g oil and 106 g I_2 /100g oil, respectively. The same results indicated that acid value ,peroxide value, oxidation value (Totox), soap, unsaponifable matter % are 0.4 mg KOH/g oil ,1.87 milliequivalents peroxide /kg oil, ,4.05, 626.2 ppm and 2.4%, respectively. These results are in accordance with those of the Egyptian standard for semi refined cottonseed oil No.1837. (Anon, 2005).

The obtained results in Table (1) show that the total gossypol content and color of the investigated color fixed oil are 200 ppm and more than 50 red at 20 yellow using Lovibond Tintometer and 1″ cell. The fatty acid composition of the investigated oil is in accordance with that of the FAO/WHO food standards (1999). Boki et al. (1992) reported that acid value, iodine value and saponification value of alkali refined cottonseed oil were 0.04mg KOH/g oil,110 g I2 /100g and 189.1 mg KOH/g oil, respectively. The results in the same Table, (1) demonstrate that the saturated and polyunsaturated fatty acids of the investigated oil reached 29.86% and 47.11%, respectively. Jones and King (1996) reported that the saturated and polyunsaturated fatty acids of cottonseed oil ranged from 28.8 % to 30 % and from 50.5% to 52.6%, respectively.

Table (1): Physical and chemical properties of cottonseed oil.

Tested parameter	Neutralized color of Fixed oil
Refractive index at 20 /20°C	1.4692
Saponification value (mg KOH/gm oil)	184
Iodine value g I_2/100g oil	106
Acid value (mg KOH/gm oil)	0.4
Peroxide value(m.equiv. /1Kg oil) Anisidine value	1.87 0.318
Oxidation value (Totox)	4.05
Soap(ppm)	626.2
Unsaponifiable matter%.	2.4
Color at 1 in. cell 20 yellow*Photometric index	Out of range >50 red 53.34
Gossypol content (ppm)	200
Silicon content (ppm)	14
Fatty acids	Percent by weight (%)
Myristic	0.79
Palmitic	25.8
Stearic	2.83
Arachidic	0.44
Palmitoleic	0.8
Oleic	20.9
Linoleic	46.76
Linolenic	0.35
Total saturated fatty acids Total unsaturated fatty acids	29.86 68.81
Un known	1.33

*Lovibond Tintometer using 1 inch cell .

3.2. Effect of adsorbents on the color and total gossypol content of color fixed cottonseed oil by conventional bleaching

The results in Table (2) indicate that bleaching of refined color fixed cottonseed oil with different bleaching earths at 5% level did not succeed in reducing either the color of the bleached oil to <5.5 R/20Y using Lovibond Tintometer (1″ cell) a reducing the photometric index to <12.03. The highest bleachability >75% was recorded for the bleaching earth types: Engelhard and Tonsil. The gossypol content of the Engelhard and Tonsil treated oil samples were 39 and 55 ppm, respectively. Comparing the photometric index of color fixed cottonseed oil treated with Engelhard with that of the oil samples treated with other investigated adsorbents indicated that the efficiency of the commercial bleaching earth, aluminum oxide and magnesium oxide in reducing pigments from the oil was <50% of that of the Engelhard. The same results showed that the efficiency of activated charcoal represented 85% of that of the Engelhard bleaching earth. These results are in agreement with those of Zaher *et al*., (1986). who used 3% Tonsil clay (neutral) to bleach refined cottonseed oil after fixation (crude oil was heated at 60 ^{o}C for one month). Their results showed that the conversion of crude oil pigments to other types of pigments through fixation caused an increase in the bleached oil color, with more marked absorption at 380 nm.

3.3. Effect of adsorbents on the color and gossypol content of color fixed cottonseed oil by column system

Accumulative data in Table (3) obtained from the bleaching process through column show that using 100g of each of magnesium oxide, Engelhard bleaching earth and aluminum oxide (AL_2O_3) is efficient to bleach 170 ; 200 and 800g, respectively, of the investigated cottonseed oil to less than 1.4 red /20 yellow using 1″ cell . The same results are obtained by passing 400 g of oil in the form of miscella (20:80) over column of AL_2O_3. The obtained results indicate that this successful treatment decreased acid value and soap content of cottonseed oil from 0.4 to 0.33 mg KOH/g oil and from 626.2 to 179 ppm, respectively. Also, the results in the same table prove that the efficiency of the regenerated AL_2O_3 is 62 % of that of the fresh AL_2O_3.

3.4. Effect of sodium hydroxide solution (14.5 Be^{o}) on the reduction of color and gossypol content of color fixed cottonseed oil

The data in Table (4) reveal that the treated oil with NaOH solution (14.5%) at 2.5 % level followed by bleaching with the most efficient bleaching agent (Engelhard bleaching earth) at 2% level decreased gossypol content of the oil from 200 ppm to 70.8 ppm and color (red units) from > 50 to 1.4 .On the other hand, the results show that increasing bleaching earth (Engelhard) level from 2 to 2.5 and 3 % after alkali treatment , (2.5%NaOH) caused a clear reduction in color (red units) of the oil to 1.3 and 1.1 and gossypol content to 32.9 and 35.7 ppm, respectively .

Increasing bleaching earth level from 2 to 3% caused a decrease in the photometric index from 2.7 to 2.1 and increased the bleachability from 94.93 to 96.06%. The same results illustrated that increasing NaOH (14.5 %) :oil ratio used from 2.5:100 to 10:100 followed by bleaching with lower level of bleaching earth (1.5 %) caused a remarkable decrease in gossypol content to 43.7 ppm and caused a sharp decrease in color of oil measured by Lovibond Tintometer to less than 0.1 red/20yellow using 1 inch cell or spectrophotometrically (photometric index) to 0.562 .Though color value of the treated oil (2.5%NaOH followed by bleaching with 2% Engelhard) was lower than that obtained by 2.5%NaOH followed by bleaching with 3% Engelhard. The total gossypol content of the treated oil resulted from 2.5%NaOH +2% Engelhard was higher than that of oil resulted from 2.5%NaOH+3% Engelhard treatment .This contradiction could be due to gossypol derivatives content rather than total gossypol content.

Taha *et al.* (1987) found that gossypol content and refined oil color (photometric index) of fresh extracted neutralized cottonseed oil (agitation with air) and that stored for one year before refining (mechanical agitation) were 0% ; 62.9 and 0.1%,25.4, respectively . Yousef (1998) alkali treated cottonseed oil contained 5% sodium oleate or 10% sodium stearate followed by bleaching using Tonsil at 110 ^{o}C for 10 min. He found that gossypol content and color index of 5% sodium oleate and 10% sodium stearate oil samples were 0.006%, 55.9 and 0.01 % and 18.3, respectively.

3.5. Effect of sodium silicate solution (45 Be^{o}) and combination of Engelhard and charcoal on reducing color and gossypol content of color fixed cottonseed oil

The results in Table (5) illustrate that treating color fixed cottonseed oil with sodium silicate solution (45 Baume) at 10 and 20% level for 30 min. at 65^{o}C led to a remarkable decrease in its color to 13 and 3.4 red units at 20 yellow using Lovibond Tintometer and 1 inch cell .

Increasing sodium silicate concentration from 10 to 20 % decreased sharply photometric index of the cotton seed oil under investigation from 18.23 to 8.5 and increased bleachability to 84.06 %. However, the color of the silicate treated cottonseed oil was the higher than 1.4 R/20Y as recommended by the Egyptian standard for edible cotton seed oil No. 49-8. (Anon, 1986).

The results in the same table illustrate that treating color fixed cottonseed oil with Engelhard at 4 or 5 % level succeeded in reducing color (red units) of the silicate treated cottonseed oil to 2 and 1, respectively. Addition of charcoal to Engelhard at 10 % level of the Engelhard that was used in the bleaching process decreased color of the silicate treated cottonseed oil to ≤ 1 R/20Y using Lovibond Tintometer and 1 inch cell and decreased the photometric index to ≤ 2.3. The results showed that the combined treatments of cottonseed oil (silicate treatment at 10 % level followed by bleaching with 5% Engelhard + 0.5 %

Table (2). The color , Photometric index , Bleachability and gossypol content of color fixed cottonseed oil after different bleaching agents treatments.

Type of adsorbent at 5%	Color (red units) at 20 yellow	Photometric index	Bleachability	Gossypol (ppm)
Bleaching earth (Commercial)	18.7	23.9	55.19	88
Bleaching earth (Tonsil)	6.3	13. 30	75.06	55
Bleaching earth (Engelhard)	5.5	12.03	77.44	39
Activated Charcoal	7.9	14.11	73.54	117
Alumina (Al_2O_3)	47.9	32.3	39.6	133
Magnesium oxide	33.0	28.3	46.94	128

The color and gossypol content of color fixed cottonseed oil was >50 red unit /20 yellow using 1 inch cell and 200ppm, respectively

Table (3). The color and gossypol content of color fixed cottonseed oil after bleached with adsorbents through column system.

Type of adsorbent	Oil: adsorbent	Photometric index	Bleachability	Gossypol (ppm)
Aluminum oxide(AL_2O_3)	8:1	.4599	99.13	21.5
Aluminum oxide(AL_2O_3) oil with hexane (20:80)	4:1	.565	98.94	30
Engelhard bleaching earth	2:1	.535	98.99	8.68
Magnesium oxide	1.7:1	1.968	96.31	42
Al_2O_3 regenerated first time	5:1	.463	99.13	27.1
Al_2O_3 regenerated second time	4:1	.384	99.28	30

Color of bleached cottonseed oil for all treatments was <1.4 red unit/20yellow using 1 inch cell

Table (4): The color, gossypol content and loss % of color fixed cottonseed oil bleached with Engelhard bleaching earth after sodium hydroxide solution (14.5 Be^{o}) treatment.

Treatment	Color (red units) at 20 yellow	Photometric index	Bleach ability	Gossypol (ppm)	Loss (%)
10% NaOH +1.5% (Engelhard)	Less than 0.1	.562	98.94	43.7	16.9
2.5%NaOH +2% (Engelhard)	1.4	2.7	94.93	70.8	14.2
2.5%NaOH +2.5% (Engelhard)	1.3	2.4	95.50	32.9	14.3
2.5%NaOH+3% (Engelhard)	1.1	2.1	96.06	35.7	14.3

Lovibond Color of oil samples treated with 10 %NaOH or 2.5 %NaOH was 3.7 red units/20 yellow and 7 red units/20 yellow/ using 1 inch cell , respectively

Table (5): The color and gossypol content of color fixed cottonseed oil bleached with different adsorbents after sodium silicate solution (45 Beo) treatment.

Treatment	Color (red units) at 20 yellow	Photometric index	Bleach ability	Gossypol (ppm)
10% Silicate	**13**	**18.23**	**65.82**	**99.5**
10% silicate + 4% (Engelhard)	**2**	**5.005**	**90.61**	**31.9**
10% silicate + 4% (Engelhard) + 0.4% charcoal	**1**	**2.3**	**95.68**	**21.9**
10% silicate + 5% (Engelhard) +0.5%charcoal	**0.9**	**2**	**96.25**	**20**
10% Silicate + 5%charcoal	**5**	**11.71**	**78.04**	**68.8**
20% Silicate	**3.4**	**8.5**	**84.06**	**62**
Silicate 20% + 5% (Engelhard)	**0.6**	**1.077**	**97.98**	**5.7**

Lovibond Color using 1 inch cell

Table (6): Oxidative status of oil resulted from successful treatments.

Type of treatment	Peroxide value (m.equiv./1Kg oil)	Anisidine value	Totox
2.5% NaOH +2% (Engelhard)	1.9	0.069	3.86
Column of AL_2O_3	13.43	0.76	27.56
20% silicate + 5% (Engelhard)	15.39	0.037	30.824

Peroxide value, anisidine value and totox of color fixed oil were 1.87 milliequivalents /Kg oil, 0.318 and 4.05 respectively.

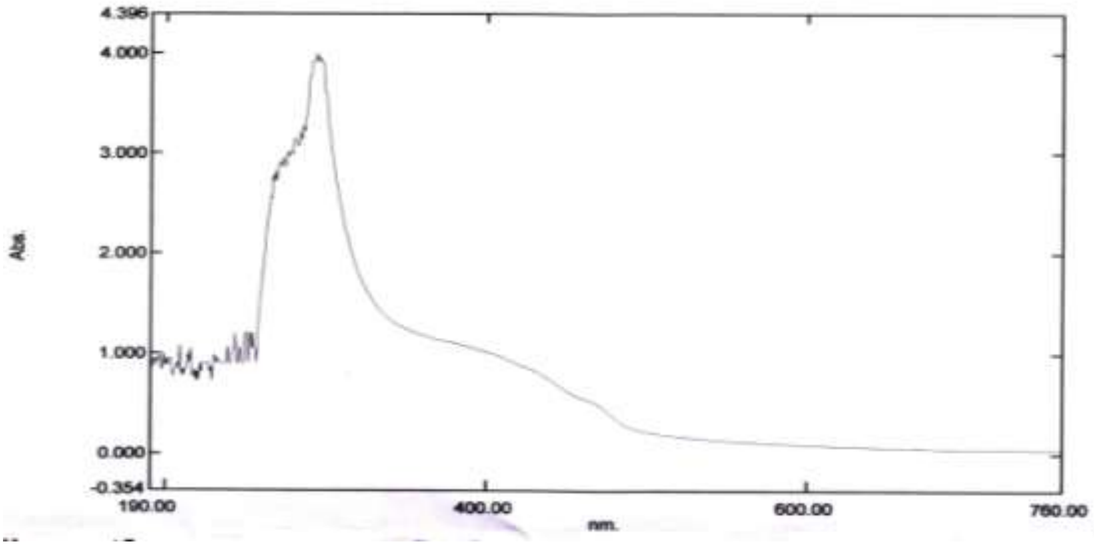

Fig. (1). Absorption spectrum of color fixed cottonseed oil.

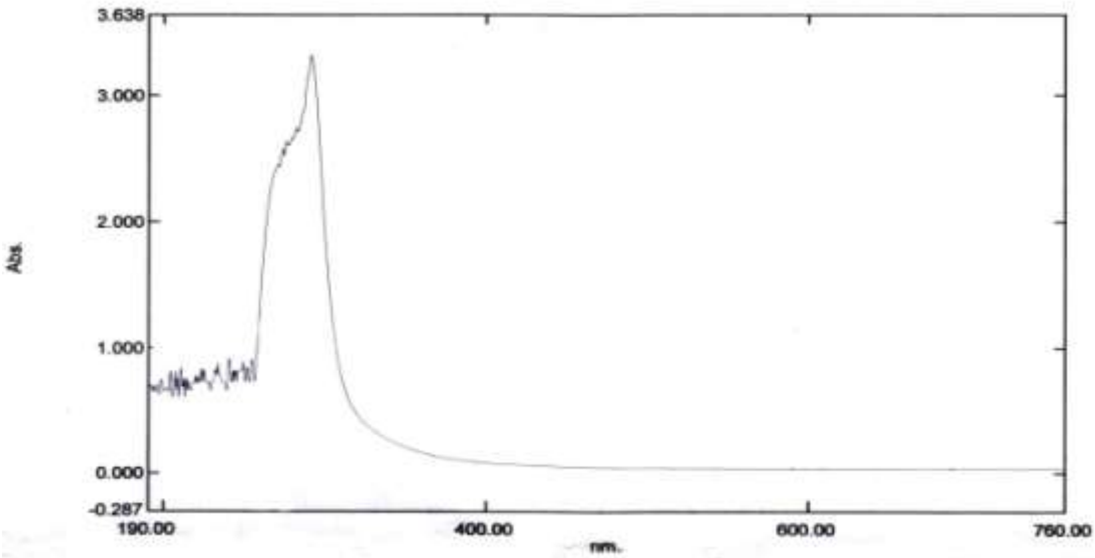

Fig. (2). Absorption spectrum of bleached cottonseed oil (Al_2O_3 treated oil using column system).

followed by bleaching with 5% Engelhard + 0.5 % charcoal), (silicate treatment at 10% level followed by bleaching with 4% Engelhard), (silicate treatment at 10% level followed by bleaching with 4% Engelhard + 0.4 % charcoal) and (silicate treatment at 20% level followed by bleaching with 5% Engelhard) increased bleachability to > 90.61% and decreased gossypol content to ≤ 31.9 ppm.

From the results in Tables 3,4 and 5 it could be noticed that the successful treatments in bleaching fixed color cottonseed oil were treating oil with 2.5%NaOH followed by bleaching with 2% (Engelhard), passing tested oil over a column of AL_2O_3 at a ratio of 8 parts to 1 part of AL_2O_3 and finally treating oil with sodium silicate (45^0Be) at 20 % level followed by bleaching with 5 % Engelhard. The oxidative status of the oil samples resulted from the successful treatments was evaluated. The obtained results in Table (6) indicate that the best decolorizing treatment that kept the oxidative status of oil from being deteriorated was the NaoH treatment followed by bleaching with 2 % Engelhard.

Figs. (1 & 2) illustrate a comparison between the absorption spectra of color fixed cottonseed oil and the bleached oil (Al_2O_3 treated oil using column system). These figures indicate that bleaching by column system caused a higher reduction in the oil absorption in a wavelength range from 340 to 500. The pigments which absorb at this range and that could be removed by bleaching. Gossypol, absorption band from 310 to 420 nm; gossyfulvin, an orange colored pigment adsorbs at 440 and gossypurpurin, a purple colored pigment which absorbs from 500 to 560. These results are in agreement with Zaher *et al*., (1990).

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تبيض زيت بذرة القطن ذو اللون الثابت

كريمة سعيد محمد حماد - سعد احمد حلابو - سامى محمد جلال

قسم تكنولوجيا الاغذية - كلية الزراعة-جامعة القاهرة – الجيزة – مصر

ملخص

تم اختيار ستة أنواع من تراب التبيض هى : تراب تبيض تجارى ، أنجلهارد ، تونسل ، أكسيد ماغنسيوم ، اكسيد ألومنيوم والفحم النشط لتقييم تأثير كلا منه ا على إزالة اللون وصبغة الجوسيبول فى زيت بذرة القطن المعادل ذو اللون الثابت. كانت درجة اللون فى الزيت ومحتواه من الجوسيبول فى البداية أكبر من 50 أحمر/20 أصفر (باستخدام جهاز Lovibond Tintometer وخلية 1 بوصة) و 200 جزء في المليون، على التوالي . تم تبيض زيت بذرة القطن ذو اللون الثابت بطريقتين هما الطريقة التقليدية (وهي عبارة عن تقليب الزيت مع تراب التبيض) او تمرير الزيت علي عمود ممتليء بمادة من مواد التبيض محل الدراسه. أوضحت النتائج ان أكسيد الألومنيوم هو اكثر مواد التبيض كفاءة في الإدمصاص حيث تم خفض لون الزيت الي اقل من 1,4 احمر/20 اصفر وخفض محتوي الزيت من الجوسيبول الي 21 جزء في المليون وذلك باستخدام طريقة التبيض من خلال العمود. تبين ايضا ان الطريقة التقليدية في التبيض لزيت بذرة القطن لم تؤد الغرض المطلوب من حيث خفض اللون ومحتوي الجوسيبول في الزيت ولهذا السبب تم ادخال معاملات اولية بمواد قلوية علي الزيت مثل (محلول هيدروكسيد صوديوم 14,5بومية او سليكات صوديوم 45 بومية) يتبعها التبيض بالطريقة التقليدية باستخدام تراب انجلهارد ، وقد نجح ذلك فى خفض لون الزيت الي اقل من 1,4 احمر/ 20 اصفر مما يجعله يطابق المواصفات القياسيه المصرية لزيت بذرة القطن المعد للطعام من حيث اللون.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (62) العدد الأول (يناير 2011):40-48.