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DETECTION OF CARMINE COCHINEAL (E120) AS A NATURAL COLORING MATTER IN MILK, YOGHURT AND ICE CREAM

By

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ABSTRACT

The addition of colors to food has continued and still occurs today in the manufacture of dairy products. The present of cochineal insects pigment (food additives) in daily intake has influence on human immune system and provoke an adverse reaction in some individuals. The given study was planned to detect carmine cochineal extract, (E120) as a natural coloring matter in milk, yoghurt and ice cream with strawberry flavor as mention on labels by manufacturer from supermarkets and shops in Tanta district at EL-Gharbia governorate in Egypt in 2015. Forty five (45) samples were collected from heat-treated milk, yoghurt and ice cream with strawberry flavor from supermarket constitutes different dairy companies production (n=15 to each one). Each sample undergoes the analysis of cochineal insects pigment, carminic acid (CA) with high performance liquid chromatographic (HPLC) technique. The results indicated that presence of CA (E 120) in examined milk; yoghurts and ice cream were 20%, 13.3% and 40% with a mean value of 153±31.9, 93.5± 22.8 and 185.1± 21.2 ppm respectively. The obtained data were evaluated according to maximum permissible limit for standardization and quality control. The public health significance and suggested precautions to protect consumers' health were discussed.

INTRODUCTION

Modern food manufacturers have become greatly concerned with conserving the aspect of foods, which may have lost their natural colors during processing. However, synthetic colorants are currently perceived as undesirable or harmful **(Noonan, 1995; Downham and Collins, 2000 and Sloan, 2002).** Moreover, the European Union and the United States have restrictedthe use of synthetic colorants as food additives **(FAO/OMS,2000)**.These restrictions have increased the use of natural colorants in the food industry **(Chaitanya Lakshmi, 2014)**.

Carminic acid (CA) is a natural colorant that is extracted from the bodies of Dactylopiidae female Insects i.e. cochineal. These insects are found in Chile, Spain and Mexico **(Sáenz *et al.*, 2004 and Ummihan *et al*., 2014)**. The major usage of this colorant today lies in cosmetics, foods, and pharmaceuticals **(Ke *et al.*, 2012)**. The color of (CA) is good light and heat stable and varies from orange to red, depending on pH. The cochineal extract finds application as food colorant in yogurts, ice cream, soft drinks and alcoholic beverages **(FDA, 1999).** Carmine corresponds to the lake of carminic acid, prepared by precipitation with Al2+ or Ca2+ **(Perez, 1992)**. Chemical structure of carminic acid is the 7 - α - D - Glucopyranosyl - 9,10 - dihydro -3, 5, 6, 8 tetrahydroxy-1-methyl-9,10-dioxo- 2-anthracene-carboxylic acid**, (Favaro, 2002)**. Its identification code as a food additive is E-120, and molecular structure consists of anthraquinone chromophore, a sugar residue and a carboxyl group. Thus, carminic acid has good solubility in water **(Favaro *et al.*, 1996).** Cases of adverse reactions to cochineal products were reported after occupational exposure, dermal contact, or consumption. Allergic reactions were demonstrated by urticaria accompanied by vomiting, diarrhea and dyspnea **(Beaudouin *et al.*, 1995 and Chung *et al.*, 2001).** Furthermore, the prick tests and the scratch tests with cochineal dye and carminic acid were also positive. Thereafter, avoided the foods containing a cochineal dye and showed a complete clinical remission. **(Kotobuki, 2007**). Insect-derived proteins (possibly complexes with carminic acid) might be responsible for IgE-mediated allergy to carmine **(Sugimoto, 2002).** Several analytical methods have been reported for the determination of CA using fluorometric, spectrophotometric,and chromatographic methods **(Carvalho and Collins, 1997 and Balakina *et al.*, 2006)**. Various high performance liquid chromatographic methods (HPLC) have been frequently used for the separation and quantitative determination of a wide variety of natural pigments. **(Méndez - Gallegos *et al.*, 2010).** The present study was undertaken to determine the CA in some dairy products by HPLC to know the extent of using the cochineal dyes in dairy industry and the extent of fraud in our nutrients.

MATERIAL AND METHODS

1. Collection of samples:

A total of 45 random samples of heat treated milk with strawberry flavor obtained from different dairy companies, yoghurt and ice cream with strawberry as mentioned on label (n=15 to each one) were collected from different supermarkets in Tanta city, Gharbia

Governorate, Egypt. All collected samples were preserved in an icebox then transferred to the laboratory to be examined for their content of cochineal carmine.

2. Quantitative estimation of cochineal carmine:

2.1. Apparatus and Chemicals:

The high performance liquid chromatography (HPLC) was an Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254nm wavelength. In addition, auto sampler (model G1329A VP-ODS) and Shim pack (150× 4.6 mm) column (Shimadzu, Kyoto, Japan) were used. HPLC-grade acetonitrile and formic acid, citric acid, hydrochloric acid and ammonium hydroxide solutions as well as HPLC-grade distilled water obtained from Fisher Chemicals (United Kingdom) were used for analysis. Working standard solutions of CA (Sigma Aldrich, Germany) were prepared by dissolving about 0.001 g of standard additives in 10 mL of methanol.

2.2. Procedures (Sugimoto *et al.*, 2002 and Sabatino *et al.*, 2013):

Accurately, 50 mL of sample was weighed and dissolved in 1000 mL HPLC-grade distilled water. The solutions were filtered with 0.45-mm PTFE filter (Pall Corporation, USA) and directly analysed **(Harris, 2006)**. Eluent flow rate was 200 mL/min, column temperature 30^{o} C and the injection volume 10 mL. A binary gradient of 0.3% formic acid in water (A) and 0.3% formic acid in acetonitrile (B) was used. The mobile-phase gradient program was as follows: 0 min, 5% B; 50 min, 28% B; 60 min, 43% B; 60 - 65 min, 43% B; 70-80 min, 5% B. The wavelength range examined by the photodiode-array detector was 200–500 nm and the retention time for estimation of carminic acid was 4.28 minutes.

2.3. Method validation (Sabatino *et al.*, 2013):

Specificity was confirmed by analyses of 10 blanks and 10 positive samples, blanks being additives and red-colored samples that did not contain carminic acid. Instrumental precision was checked by repeated measurement of the same solution of carminic acid and calculating the Relative Standard Deviation (RSD). Four point calibration curves were obtained by diluting stock solutions over the range 10-100 mg/L, making two replicates for each concentration. Regression equations with linearity range, slope, y-intercept and coefficient of correlation (r2) were evaluated for carminic acid at 490 and 525 nm, respectively. Statistical tests (Mandel and residual analysis with normal distribution of the calibration

points) were performed to prove linearity of the regression. Seven replicates of blank red-colored beverages spiked with carminic acid at levels close to the limit of detection (LOD) were measured and the Standard Deviations (SD) was determined. LOD and limit of quantification (LOQ) were calculated. Accuracy and precision of the method were ascertained by spiking blank samples of to obtain a final concentration of 50 mg/L of carminic acid and (six replicates each). The average percentage of recovery and the RSD (repeatability) were evaluated. Values of mean and standard deviation (SD) were calculated to verify the statistical significance of all parameters.

RESULTS AND DISCUSSION

Table (1): Determination of Cochineal carmine (ppm) in heat-treated milk, yoghurt and ice cream with strawberry flavor from different dairy companies.

Type of sample	No. of examined samples	Positive samples		Concentration ppm (mg/kg or L)		
		No.	%	Min.	Max.	Mean± SE
Strawberry milk	15	3	20	84.9	217.8	153±31.9
Strawberry Yoghurt	15	2	13.3	61.4	125.6	93.5± 22.8
Strawberry Ice cream	15	6	40	104.4	257.2	185.1± 21.2

(Table 2): results of positive samples of CA with related to declared on labels.

Type of sample	No. of positive samples	Samples declared on labels	
		No.	%
Strawberry milk	3	2	66.6
Strawberry Yoghurt	2	1	50
Strawberry Ice cream	6	2	33.3

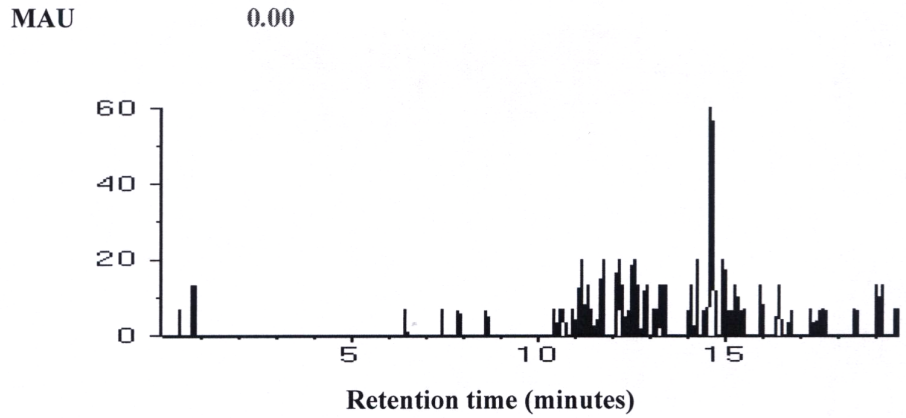

Fig. (1): HPLC chromatogram of carmine concentration in the Examined yoghurt sample (Negative).

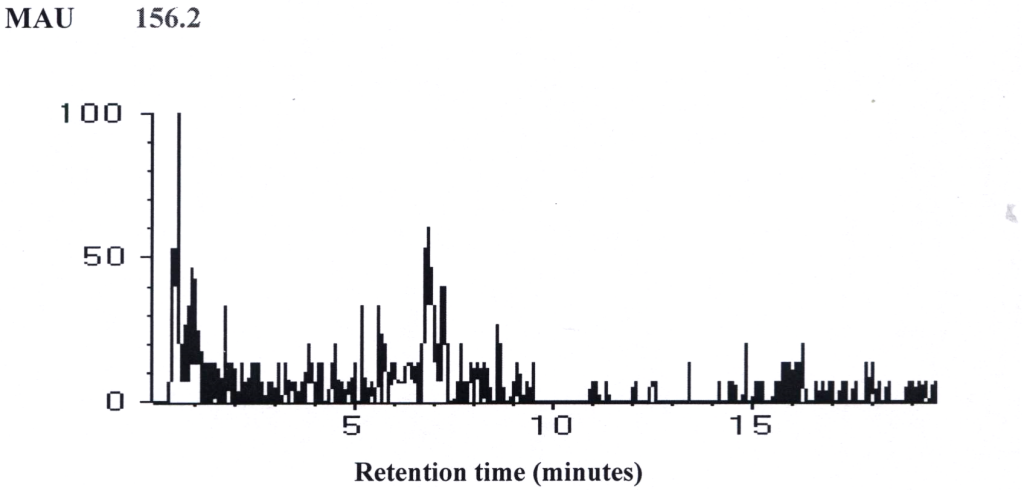

Fig. (2): HPLC chromatogram of carmine concentration in the examined milk sample (Positive).

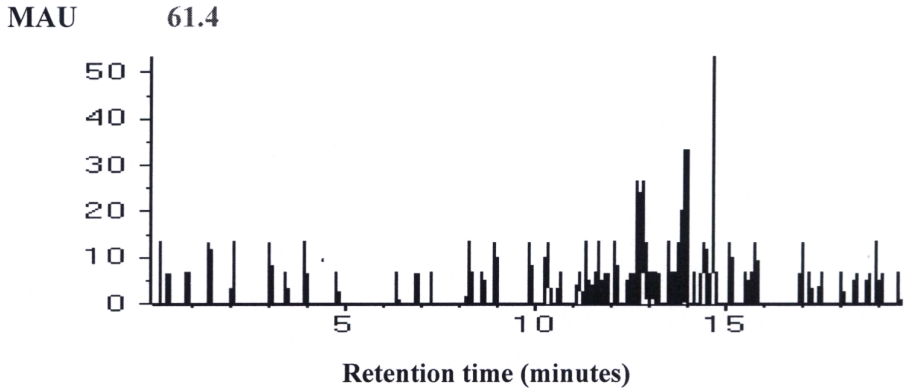

Fig. (3): HPLC chromatogram of carmine concentration in the examined yoghurt sample (Positive).

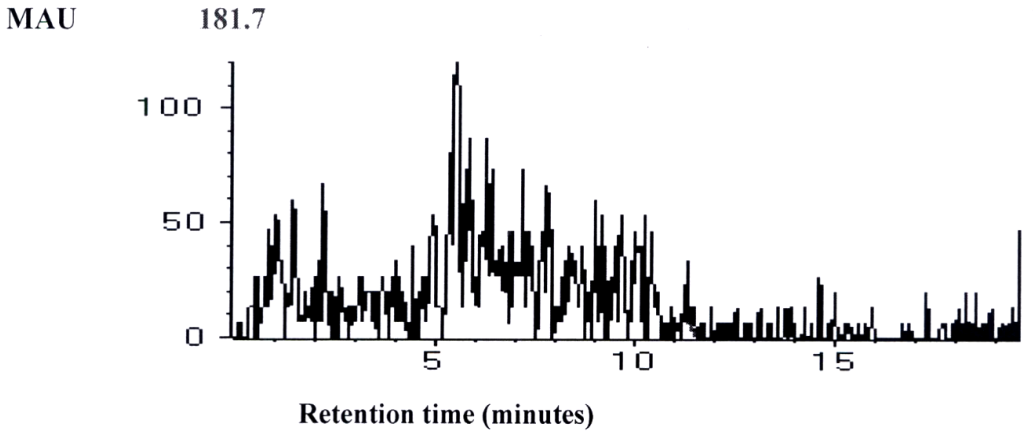

Fig. (4): HPLC chromatogram of carmine concentration in the examined ice cream sample (Positive).

Cochineal extract is obtained from the dried bodies of female *Dactylopius coccus* Costa insects (cochineal). The extract is used directly in food and is also processed further to carmines. Specifications exist for cochineal extract and carmines, both of which contain carminic acid (CA) as the coloring principle **(Ke *et al.,* 2012)**. The most common pigment widely used as red coloring agent (E120). The composition of carminic acid was proposed to be C22 H20 O13 **(Lloyd, 1990)**. Colors were generally added to serve as a visual cue for quality, to induce the perception of flavor and to meet consumer expectations. The cochineal extract must comply with the following specifications: pH 5.0-5.5; carminic acid content: min.1.8%, total solids: 5.7- 6.3%, total protein: 2.2% max., lead: 10 ppm max., and arsenic max. 1ppm **(Sugimoto, 2002)**. The addition of colors to food and beverages has continued and still occurs today in the manufacture of products such as biscuits, pastry products, cakes, processed meats, cheese, margarine, yoghurt, confectionery, ice cream, cordials and soft drink, and serves a number of technological purposes. **(Ghorpade *et al.,* 1995)**. The results revealed in (Table 1) , Fig.(2) indicated that CA percentage in heat treated milk samples containing strawberry flavor were three samples out of fifteen represented 20%, and mean values were (153±31.9 ppm). Furthermore, the results in (Table2) indicated that two samples mentioned E-120 on label, while third positive sample not mentioned E-120 on label. Carmine is used in foods such as strawberry milk drinks, popsicles, and candy products **(Ke *et al.*, 2012).** Moreover, the samples without declared the color additives with usual and common name on labels were disagreed with that reported by **(FDA, 2011).** That published a final rule amending its color additive regulations for cochineal extract and carmine to require

that these additives be declared by their common or usual names on the labels of all food and cosmetic products that contain them. The purpose of the rule is to enable individuals with sensitivities to cochineal extract and carmine to avoid products containing these additives. On the other hand, the positive result of CA in examined yoghurt samples (Table 1), Fig. (3) was two out of fifteen samples representing 13.3% and the mean values were (93.5± 22.8ppm). Such result agreed with **those of Perez and Kosztarab, 1992 and Allevi *et al.,* 1998)**. They stated that Cochineal dye is one of the most light and heat stable of all the colorants and can withstand sterilization and baking temperatures more than many synthetic food colors. It also can be detected in yoghurt, candy, fruit drinks and some processed foods. Carmine also binds strongly to protein and therefore works well in meat, milk and fish products, where it binds into the structure, and recommended that polarography method was successfully applied to strawberry flavored milk and candy samples **(Carvalho and Collins, 1997; Gonzalez *et al.,* 2002 and Ummihan *et al.,* 2014)**. From the obtained results, it could be concluded that high performance liquid chromatographic method was suitable, sensitive and the most accurate method for detection of CA in foodstuff. Results in (Table 1), Fig.(4) indicated that ice cream with strawberry flavor as mentioned on label were contained CA, six samples out of fifteen represented by 40%, and the mean values were (185.1± 21.2 ppm). On the other hand, result in (Table 2) revealed that two samples mentioned E-120 on their label and other four samples without declared CA on labels. This fraud was common in dairy industry and may effect on public health. This result was agreed with **(Shunck and Marchlewski 1994)**. CA has been widely used to color foods, beverages, textiles, pharmaceuticals and cosmetics products due to its relatively high chemical and biological stability. Many cases of hypersensitivity to carmine, carminic acid and cochineal extract were recorded.Hypersensitivity reaction included contact dermatitis, urticaria, angioedema, occupational asthma and systemic anaphylaxis **(Sampson, 2003)**. More than half of these reports declared the involvement of IgE - mediated diagnostic response to carmine and its derivatives **(James *et al.,* 1997 and Chung *et al.,* 2001).**

(Table 3): results of positive samples of CA with related to permissible limit.

Type of samples	No. of positive samples	Samples exceeding permissible limit		Maximum permissible limit ppm *
		No.	%	
Strawberry milk	3	2	66.6	100
strawberry Yoghurt	2	1	50	
strawberry Ice cream	6	6	100	

***according to (EC, 2008 and EU, 2012)**

The results obtain in (Table 3) , Fig.(2, 4) indicated that samples exceeding permissible limit of CA in heat treated milk, yoghurt and ice cream with strawberry flavor as declared on labels were 66.6%, 50%, and 100% respectively. These results explained the extent of fraud in some dairy companies in Egypt and ensured that greatly bad effect on public health of consumers. The European Parliament and the Council of the European Union **(EC, 2008)** specifies the main use and limits for each color additives to foodstuff. Cochineal extract, carmine and carminic acid (soft drinks 100 mg\L and in solid food 50-500 mg\kg) **(EU, 2012)**. Carmine cochineal coloring agents is toxic particularly in high dose. It is recommended limiting its use and declaring its presence and source (insect) of its origin in the product. It also requires scientific reviews to determine the specific allergen of CA and whether it could be eliminated from the coloring agents **(Zeinab and EL-Fetouh, 2010)**. Furthermore, adverse reactions to cochineal colors after occupational exposure, dermal contact, or consumption of colored food and drinks have been the subject of case reports. The reported effects were the consequence of allergic reactions, and the involvement of an immunologically mediated mechanism has been demonstrated. The nature of the adverse reactions, e.g. urticaria, rhinitis, diarrhea, and anaphylaxis, provides clear evidence that systemic reactions can follow exposure of a sensitized individual to cochineal colors. Some of the adverse reactions were severe and required emergency treatment **(Acero, 1998; Kotobuki, 2007and Mccann *et al.*, 2007). (CSPI, 2006)** supports the proposed rule to require the labeling of cochineal extract and carmine on foods and cosmetics to protect consumers who know they are allergic to these color additives. However, Food and Drug Administration **(FDA, 2011)** should ban the use of cochineal extract and carmine in food and cosmetics in order to protect people who do not know they are allergic to them.

CONCLUSIONS

Cochineal colors E120 were added in some dairy products with or without declared on labels. The presence of E120 in heat-treated milk, yoghurts and ice cream with strawberry flavor exceed than permissible limit in some dairy companies under study. The present of cochineal, colors (food additives) in daily intake influence on human immune system and provoke an adverse reaction in some individuals. It is recommended that consumer should be vigilant and always check labels when buying such food products. If there are any doubts, should discard such products since this is the only safe way to avoid allergens.

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REFERENCES

Acero, S. (1998): Occupational asthma and food allergy due to carmine. Allergy.53 (9): 897-901.

Allevi, P.; Anastacia, M.; Bingham, S.; Ciufferda, P.; Fiecchi, A.; Muir, M. and Tyman, J. (1998): Synthesis of carminic acid, the colourant principle of cochineal. J. Chem Soc. Perkin Trans. (1):575-582.

Balakina, G. G.; Vasiliev, V. G. and Karpovva, E. V. (2006): HPLC and molecular spectroscopic investigation of red dye obtained from an ancient pazyryk textile. Dies pigments 71: 54 - 60.

Beaudouin, E.; Kanny, G. and Lambert, H. (1995): Food anaphylaxis following ingestion of carmine .Ann. Allergy Asthma Immunol. 74: 427- 430.

Carvalho, R. N. and Collins, C. H. (1997): HPLC determination of carminic acid in foodstuff and beverages using diode array and fluorescence detection. Chromatographia 45: 63-66.

CSPI (Center for Science in the Public Interest) (2006): Nutrition Action Healthletter publishrr of Division of Dockets Management (HFA-305) FDA Room 1061 5630 Fishers Lane Rockville, MD 20852 Dnq 3 71 Fed. Reg. 4839 - 4851.

Chaitanya Lakshmi, G. (2014): Food Coloring. The Natural Way. Research Journal of Chemical Sciences 4: (2), 87-96,

Chung, K.; Baker, J.R. and Baldwin, J.L. (2001): Allergy, Ann. Allergy Asthma Immunol. 56: pp. 73 -77.

Downham, A.andCollins,P.(2000): Colouring our foods in the last and next millennium. International Journal of Food Science and Technology. 35 (1): p. 5-22.

(EC) European Commission, Commission Regulation (2008): the European Parliament and of the Council on food additives and in particular Article 10 (3) and Article 14, NO. 1333 2008R1333 - EN -02.12.2011 - 002.001- 70.

(EU) Commission Regulation (2012): cochineal, carminic acid, carmines (E 120) in certain food categories and Annex to Regulation (EU) No. 231.

FAO/OMS, (2000): Compendium of Food additive Specifications. Addendum 8. FAO Food and Nutrition Paper. WHO Technical Report Series No.52 Add. 8, Roma.

Favaro, G.; Malatesta, V. and Miliani, C. (1996): Photosensitization of photochromism of spiro-indolin-oxazines by camphorquinone. J. photochem photobio A. Chem 97 45-52.

Favaro, G. (2002): Role of protolytic interactions in photo-aging processes of carminic acid and carminic lake in solution and painted layers. Journal of the Chemical Society-Perkin Transactions 2, (1): p. 192-197**.**

FDA (Food and Drug Administration) (1999): Food Color Facts. http://www.cfsan.fda.gov/~ lrd/ colorfac.html.

FDA (Food and Drug Administration) (2011): Requires Label Declaration of Color Additives-Cochineal Extract and Carmine http://edocket.access.gpo.gov/2009/p df/E8- 31253.pdf. 2 71 Fed. Reg. 4839.

Ghorpade, V.M.; Desphande, S.S. and Salunkhe, D.K. (1995): Food Colors. In: Maga, J.A. and Tu, A.T. eds. Food Additive Toxicology.Chapter4.Marcel Dekker, Inc., New York, pp. 179-233.

Gonzalez, M.; Mendez, J. and Garnero, A. (2002): Optimizing condition for traction of pigments in cochineals using response surface methodology.J.Agric Food Chem. 50: 6968-6974.

Harris, D.C. (2006): Quantitative chemical analysis. Textbook, 1st Ed. (W.H.Freeman), New York, USA.

James, L.; Baldwin, M.D.; Alic, H. C. and William, R.S. (1997): Popsicle- induces anaphylaxis due to carmine dye allergy. Ann. Allergy Asthma Immunol 79: 415 - 419.

Ke, E.; Nazzal, S. and Tseng, Y. (2012): Erythrosine-mediated photodynamic inactivation of bacteria and yeat using green light-emitting diode light .J. Food Drug Anal 20: 951- 956.

Kotobuki, Y. (2007): Case of urticaria due to cochineal dye in red-colored diet Arerugi; 56 (12): 1510 - 4. Department of Dermatology, Osaka University.

Lloyd, A. (1990): Extraction and Chemistry of Cochineal. Food Chemistry, 5: 91-107.

Mccann, D.; Barrett, A.; Cooper, A.; Crumpler, D.; Dalen, L.; Kitchin, E.; Lok, K.; Porteous, L.; Prince, E.; Warner, J. and Stevenson, J.(2007): Food additives and hyperactive behaviour in 3-8-9 year-old children in the community: a randomised, double-blinded, placebo-controlled trial, The Lancet, 370 [9598] 1560-1567.

Méndez-Gallegos, S. De J; Tarango-Arámbula, L.A. and González-González, M. (2010): Preliminary analysis of cochineal insects with high performance liquid chromatographic (HPLC) Instituto Canario de Investigaciones Agrarias. Apdo. 60. La Laguna 38200, Spain.

Noonan, J. (1995): Color Additives in Food. 2nd ed, ed. H.o.F. Additives., Cleveland, Ohio: Furia. 587-615.

Perez, G. and Kosztarab, M. (1992): Biosystematics of the family Dactylopiidae (Homoptera: coccinea) with emphasis on the life cycle of Dactylopius coccus Costa, in Studies if Morphology and Systematics of Scale Insects. Blacksburg, Virginia.

Perez, J. (1992): Anteproyecto de una planta procesadora de grana de cochinilla para la obtencion de sus principales derivados: extracto de cochinilla, carmin de cochinilla y acido carminico, in Escuela de Quimica., Universidad de La Salle: Mexico. 137.

Sabatino, L.; Scordino,M.; Gargano,M.; Lazzaro, F.; Borzı, M.;Traulo, P. and Gagliano, G. (2013): Aminocarminic acid in E120-labelled food additives and beverages. Food Additives and Contaminants 5 (4): 295-300.

Sáenz,C.; Garrido, J. and Carvallo, M. (2004): Colorantes Naturales Derivados de la Cochinilla (Dactylopius coccus Costa),in La Alimentacion Latinoamericana. 56-62.

Sampson, H. A. (2003): Pediatric, Vol 111, 1601-1608.

Shunck, E.and Marchlewski, L. (1994): Zur kenntniss der carminäure. Berichte Der Deutchen Chem. Gesellschaft.27:2979-2985.

Sloan, A.E., (2002): The natural and organic foods marketplace - Mother Nature moves mainstream as the natural and organic foods market grows worldwide. Food Technology. 56 (1): p. 27-34.

Sugimoto, N. (2002): Structure of acid-stable carmine. Journal of the Food Hygienic Society of Japan, 43 (1): p. 18-23.

Sugimoto, N.; Kawasaki, Y.; Sato, K.; Aoki, H.; Ichi, T.; Koda, T.; Yamazaki, T. and Maitani, T. (2002): Structure of acid-stable carmine. J. Food Hyg. Soc. Jpn. 43 (1):18-23.

Ummihan, T. Y.; Fevzi, E. and Hasim, y. (2014): Determination of the food dye carmine in milk and candy products by fifferential pulse polarography. Journal of food and drug analysis 22: 329-335

Zeinab, M. L. and EL- Fetouh, M.S. (2010): Pathological studies on the adverse effect of food color carmine cochineal on male albino rats. Zag Vet. J. Vol 38, N 1:103-116.