

**QUANTITATIVE DETERMINATION OF COCHINEAL EXTRACT  
(*Adctylopius coccus*), CARMINE, CARMINIC ACID AND 4-  
AMINOCARMINIC ACID (E120) IN SOME FOOD-STUFFS.**

**Manal A. Atwa**

**Regi. Center for Food & Feed Agric. Res. Center**



**ABSTRACT**

Carmines and carminic acid are red food colors (E 120) can be classified as natural colors; obtained from alcoholic extracts of cochineal, which consists since they are of dried bodies of the female insect *D. coccus costa*.

In the present investigation a detection of carmine, carminic acid and 4-aminocarminic acid in the studied samples of red colored beverages containing E120 as ingredient or in unlabeled samples has been used.

The study has been applied for the analysis of around 140 food and beverages samples in red colored beverages classified in more than 25 categories; containing or May containing E120 as ingredient.

There were around 29.5% unlabeled of the total samples.

The ratio of detected samples were 55.4% from the total examined samples given 77 samples contained carmine, carminic acid and 4-aminocarminic acid. 13% of the detected samples contained 4-aminocarminic acid.

Results of this study proved and revealed alarming illegal presence of 4-aminocarminic acid in E120-labelled colorants. A mislabeling are documented from many commercial products, and varied widely in dye content.

Carmine, carminic acid and 4-aminocarminic acid were not detected in cocoa, coffee, processed cheese, chocolate, canned fruits and vegetables products; but presented in 54.5% and 32.5% of the studied samples respectively. Also 60% of the labeled investigated samples no mention of the adding of these dyes. They use only sentence "Natural colors added" or "No artificial colors" and on the rest of labeled samples 40% it was mentioned on label either E120, cochineal extract or carmines without any mention for levels of adding.

Half samples of the fresh juices were contained the different types of dyes. The highest value of carminic acid detected was found in one fresh juice sample giving 3670 ppm. In one sample of flavored tea the carmine was detected in level 35 ppm. Dye concentration in 4 samples of seasoning salad dressing was 43 ppm and 182 ppm for carmine and carminic acid, respectively and no 4-aminocarminic acid was detected. The fish products category include red colored the level of detected carmines was ranged from 16 -290 ppm in two caviar samples, however, no dye detected in salmon.

In seven meat products samples include minced meat, burger, and sausages four samples contained carminic acid level ranged from 98 to 1020 ppm while one sample contained 67 ppm carmine. In flavored dairy products and soya milk, it was found that one sample of soya milk contained 18 ppm as carmine. While the carminic acid was ranged from 26 to 1020 ppm on the other samples include yoghurt and processed milk.

In one sample of three soup powder samples carmine dye was detected in level of 21 mg/kg. The value of carminic acid in one concentrated syrup sample was 1440 ppm. However, in four of seven samples confectionery products collected from bakery and dessert stores was ranged from 39 to 900 ppm for carminic acid and from 1400 to 1087 ppm for carmine. The highest level of carmine detected in cake sample was 2070 ppm; followed by the value of 1810 ppm in sweet category in chilled sweet

drink sample .The cakes samples also contained ratio of carminic acid from 100 up to 980 ppm. Moreover, some samples of candy, gums, jelly sweet, marshmallow, toffee, liqueur and filling cream of biscuits contained carmine in values ranged from 43 to 1750 ppm as a carminic acid and ranged from 40 to 1810 ppm as carmine ;Aminocarminic acid was detected in one sample in this category. Instant flavored drink powdered was also found to be containing carminic acid level of value ranged from 120-477 ppm and one sample contained amino carminic acid. In the frozen ice cream level of carminic acid was ranged from 400-1340 ppm and from 360 to 1680 ppm in powder. Also, carmine values of 80 ppm and 160 ppm were determined for frozen and powder samples respectively. However, aminocarminic acid was detected in one of frozen ice cream samples. Presence of aminocarminic acid content was confirmed in one sample of jam, fruits jelly powder and tomato sauce.

Carmine dye detected in one sample of pickles salty syrup given value of 68 ppm. In some carmines free samples, other colors such as Carmoisine , Allure red, Ponceau 4R, Indigotine and Red 2G were detected .However, instant flavored drink powder, red pepper paste ,fruits milk powder ,milk check powder, ketchup, tomato sauce and fruit jam were proved to be free of carmine dye.

This study proved that the identification of 4-aminocarminic acid in food colors labelled was either E120 or not labelled at all. Analysis results of beverages and food samples showed the widespread presence of this semi-synthetic carmine acid derivative, which is not permitted by law in food industry, with unknown effects on human health.

These studies have to be continued in the future to investigate more colors and additives.

## INTRODUCTION

Food colorants can be divided into four categories: natural, nature-identical, synthetic and inorganic colors (Scotter, 2011). Natural colors are pigments made by living organisms. Among them, carminic acid (E120) is a red glucosidal hydroxyanthrapurin that occurs naturally in some insect scales, such as *Dactylopius coccus* and *Polish cochineal*. The chemical structure of carminic acid consists of an anthraquinone core, linked to a glucose sugar unit (Dapson, 2007and Scotter, 2011). Carmines and carminic acid are obtained from aqueous, aqueous alcoholic or alcoholic extracts from cochineal, which consists of dried bodies of the female insect *D. coccus Costa* (2008/128/EC). *Dactylopius coccus* which lives on various cactus plants also known as *Coccus cacti*( Feller, 1986).Throughout the world, the use of natural-type food colors continues to increase. Many consumers believe, even without valid proof, that natural colors are less harmful and therefore more acceptable than synthetic dyes.

In recent years, a new dye, acid-stable carmine, has appeared on the market .This has been identified as 4-aminocarminic acid, it is considered a semi -synthetic color. Carmine in foods may initiate or provoke allergic reactions in some individuals The use of E120 has increased in recent years since (EC Regulation 1333/ 2008)b, requiring the mandatory statement “may have an adverse effect on activity and attention in children” on products that contain E102, E104, E110, E122, E124 and E129 colorants, came into force (European Commission, 2008)a. Commercial preparations of carmine may contain not less than 2.0% carminic acid in extracts containing carminic acid

and not less than 50% carminic acid in chelates( European Commission ,2012). Carmine is normally containing 50% or more carminic acid. Both colorants produce pink shades in candy, confections, chewing gum, concentrated fruit juice (except orange), , smoked fish, liqueurs and soft drinks, yogurt, ice cream. In recent years, a new dye, acid-stable carmine, has appeared on the market in response to needs in the food industry for a brighter red color in acidic foods. This has been identified as 4-aminocarminic acid (Sugimoto et al., 2002). Carminic acid was transformed into a different type of pigment, named acid-stable carmine, through amination when heated in ammonia solution. The features of the structure were clarified using a model compound, purpurin, in which the orientation of hydroxyl groups on the ring of the anthraquinone skeleton is the same as that of carminic acid. By spectroscopic means and the synthesis of acid-stable carmine and purpurin derivatives, the structure of the major pigment in acid-stable carmine was established as 4-aminocarminic acid, a novel compound.

Among additives, colorants are added to food for one or more of the following reasons: to replace color loss during processing, to impart or enhance color already present, to minimize batch-to-batch variations and to color otherwise uncolored food (Ash and Ash ,1995). The use of food colors is strictly controlled by laws and regulations. Carmine, one of the food dyes, is authorized for use in (European Commission, 1994) except for Japan. The acceptable daily intake (ADI) value of carmine, which is formulated by Joint FAO/WHO Expert Committee on Food Additives, is 5 mg /kg based on weight (JECFA, 2001). As the Committee's conclusion, carmine in foods may initiate or provoke allergic reactions in some individuals.

Recovery and purification of carminic acid from raw cochineal is regarded as a difficult and complicated process. Cochineal consists principally of carminic acid, but also contains varying amounts of other biological molecules and body parts. Current industrial technology suffers from low and irreproducible yields, while generating a low-quality final product (Borges et al., 2012). The International Numbering System for approved food colors in the countries of the European Union and elsewhere lists E120 for carminic acid, specifying the molecular formula ( $C_{22}H_{20}O_{13}$ ) and molecular weight (492.39) of the molecule responsible for the colour (Table 1). (Dapson, 2005 and 2007) noticed that many E120 additives contained substantial amounts of aminocarminic acid, a synthetic derivative obtained after heating carminic acid with ammonia. Different from carminic acid, aminocarminic acid maintains its deep red colorant power at very low pH and it is sold as "acid-stable carmine" suitable for acidic food, even if it is not approved for use in food. The aminocarminic method of preparation was illustrated in a United States Patent( Schul, 1992) and molecular formula ( $C_{22}H_{21}NO_{12}$ , M.W.491.10) was firstly elucidated by (Sugimoto et al., 2002).

Most research has been related to the analysis of carminic acid, a major component of cochineal extract, using high-performance liquid chromatography (Ishikawa, et al., 2003 ; Lancaster and Lawrence, 1996; Merino,et al., 1997).Also, capillary electrophoresis was used according to (Liu et al., 1995). Spectrophotometric analysis was determined by (Tripathi et al., 2004).

The prevalence of sensitization and asthma caused by carmine in facing a new example of an allergen that can act through digestion, giving rise to an allergical syndrome. Many insects are known to appropriate defensive substances from plants. The concentration of carminic acid was found to vary based on the size and life cycle stage of the cochineal, *Dactylopius coccus Costa*. That with others industrial processes, shall allow an improvement of the current classification criteria of the commercial quality of dry cochineal.

The studies have showed negative results of non-mutagenic; non-carcinogenic and genotoxicity data activity of carminic compound. Recently, the number of literatures described about increased incidence of type 1 allergy to cochineal dye. As the usage of cochineal dye is increasing in the Japanese market, we should keep in mind that cochineal dye can be a cause of urticarial in daily practice. Carmine should be added to the list of agents capable of producing asthma, whose mechanism, according to (Tabar et al., 2003) would be immunological mediated by IgE antibodies in the face of diverse allergens of high molecular weight, which can vary from one to one. Nonetheless, given the existence of different components in carmine, it cannot be ruled out that substances of low molecular weight, such as carminic acid, might act as haptens. Besides, since we are dealing with a coloring that is widely used as a food additive, it is not surprising that allergic reactions can appear both through ingestion and through direct cutaneous contact, giving rise to an Allergenic syndrome.

Terminology related to these dyes is not followed accuracy in the food, which leads to potential errors in labeling. Accordingly, monitoring of carmines in foods is necessary and investigating aminocarminic acid occurrence in E120-labelled red-colored-beverages to ensure food safety and consumer confidence. The aim of the present study was designed to elucidate the carmines derivatives in red colored food containing E120 as ingredient or illegal additives in some unlabeled samples

**Keywords:** 4-aminocarminic acid; Carminic acid; Carmine; Food colorants, Cochineal, Beverages, Dairy, Meat, and Confectionery.

## **MATERIALS AND METHODS**

### **Sampling:**

Around 140 samples of E120 additives and some samples of commercial red-colored beverages (such as strawberry, pomegranates, roses, melons, grapes, cherries, cocktail, tomatoes and hibiscus flavored) may containing E120 as additive commercially available imported or local production; were purchased from retail stores in Cairo and Giza in Egypt in the framework of investigation study performed

The samples were categorized into more than 25 food types such as; as canned fruits drinks (12), confections (7), fruit flavored soft drinks (5), canned fruit vegetable product (3), meat product (7), cakes (5), fish product (4), processed cheese (3), fresh juices (12), Dairy products (7), fruit jam (3) seasoned food (4), soup (3), concentrated flavored sweet syrup

sherbets(3) chocolate (3) , coffee, cocoa and tea (4) ,ketchup, tomato sauce, red pepper paste (7),sweets ,candy, gum(15) ,fruits jelly powder (6),instant flavored drink powder(3),frozen ice cream(7)and (4) ice cream powder.

Checking the labels and colors written on some products. In addition, some samples of unlabeled or (fresh); all samples were stored at 4 °C.

**Chemicals and reagents:**

Carmine ,4-aminocarminic acid and carminic acid used as standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals used in extraction and preparation of mobile phase, such as sodium hydroxide, sodium phosphate dibasic, and sodium phosphate monobasic were of analytical reagent grade and also supplied by Sigma–Aldrich. Water for all applications was obtained from an Milli-Q ultra-pure water system (Millipore, Bedford, MA, USA).

**Preparation of standard solutions:**

The stock solution (1000 ppm) of dyes was prepared by adding 0.05 M NaOH solution to give a final volume of 100 ml. Calibration standard solutions at serial concentrations of dyes were obtained by mixing subsequent dilution (1–100 ppm) with water.

For spectrophotometer method, test solutions were prepared by suspending 0.1 g of dye in approximately 900 ml deionized water in a large beaker. Enough 1.0 N sodium hydroxide was added to bring the pH to 12.50 - 12.60. A Labomed, INC UV-VIS double Beam UVD-3500 spectrophotometer was used to scan from 700 to 400 nm at slow speed with an absorbance range of 0.0 to 2.0. This constituted the high pH assay, which is quantitative, a aliquot of the solution (20 ml ) used in the high pH scan was adjusted to a pH of 2.09 .

UPLC waters H -class coupled with PDA was used to quantify detection of carmine and carminic acid after filtration on 0.2µ filters. An Nova. Pak. C18 column (150 - 3.9 mm, 5 µm) purchased from Waters Corporation (Milford, MA, USA) was used for chromatographic separation. All separations were carried out isocratically at room temperature with a mobile phase consisting of methanol-phosphate buffer (pH 6.0) at ratios of 15:85 (v/v). The flow-rate was maintained at 0.8 ml /min and a 20 µL sample volume was injected into HPLC. Carmine, eluted from the column, was monitored by photodiode array detector set at 281 nm. (the absorption spectra of carmine).

**Preparation of samples:**

Ground food samples (5.0 g) was transferred into a 100 ml beaker and 0.05 M NaOH solution was added to give a final volume of 50 ml. then mixed by a homogenizer for 2 min, the sample was shaken mechanically for 10 min with a shaking rate of 300 rpm. The extract was centrifuged for 5 min at 5000 rpm. 10 ml supernatant was then filtered with 0.2 µm membrane; whereas yoghurt, meat and fish samples required pre digestion with proteases and extraction with dilute sodium hydroxide to release color during the extraction step .

**METHOD:**

Method followed was applied to samples; retention time and spectrum of carmine were different from carminic acid, which was detected in samples

for distinguishing carmine from carminic acid, which is a major component of cochineal extract. In addition, it is a quantitative method that can be applied to various food products such as beverages, confectionery, and candies. HPLC method to differentiate carmine and carminic acid in foods. This method is applicable for the effective surveillance of carmine in food products (Lim, et al., 2014).

The analytical methods of carmine as carminic acid by hydrolysis with hydrochloric acid have a disadvantage that carmine or carminic acid in foods could not be distinguished (Carvalho and Collins, 1997 and Merino et al., 1997).

Aminocarminic acid dye is relatively new to the food trade as an acid-stable red colorant and may eventually enter the biological stains market. The assay proposed here was a two-step procedure using quantitative spectrophotometric analysis at high pH (12.5-12.6) followed by a qualitative scan of a low pH (1.90-2.10) solution. Carmine is distinct at high pH, and the remaining dyes were easily distinguished at low pH. While the color of carminic acid changed from yellow to red in the pH range of McIlvaine buffer (3.0-7.0), the color of 4-aminocarminic acid was always red, and also the ultraviolet/visible (UV/Vis) spectra did not change. These characteristics are useful to distinguish 4-aminocarminic acid from carminic acid as reported by (Kawasaki et al., 2002 and Lim et al., 2014).

Spectroscopic characteristics of both analysis were in agreement with literature data (Schul, 1992; Sugimoto et al., 2002 and Dapson, 2007). Under acidic conditions, carminic acid had a broad absorbance peak at 490 nm, while the aminocarminic acid had a main absorbance peak at 525nm with a secondary peak about 35nm further in the spectrum. These UV absorbance are in agreement with (Samari et al., 2010), who published the influence of pH on UV absorbance of carminic acid.

The determination of colors in sweet, confectionery and soft drinks by HPLC was applied to check the presence of many artificial colors in some samples (Narumol et al., 2008). All samples had triplicates analyses in separate runs.

## **RESULTS AND DISCUSSION**

The present study has been applied for the analysis of around 140 food and beverages samples to investigate the levels of carmines, carminic acids and the occurrence of 4-aminocarminic acid in red colored beverages containing or may be containing E120 as ingredient.

There were around 41 samples out of 138 samples unlabeled such fresh juices, ice cream, cakes, confectionery, pickles syrup. The meat products represent 29.5% of the total samples.

The ratio of detected samples were 55.4% from the total examined samples given 77 samples contained carmine , carminic acid and 4-aminocarminic acid.

Samples were processed as previously described and the results are reported in Table (1).As showed ,the results demonstrated that (10 out of 77 samples) represent 13% of the detected samples contained 4-aminocarminic acid, and in 100% of positive samples there weren't any sign for the presence of this additive in the products. Occurrence of aminocarminic acid in E120-labelled food additives and beverages, which is almost always associated with minimum quantities of carminic acid, probably as unreacted residue. Although aminocarminic acid is a non-permitted color additive in the food industry, evidencing the alarming illicit use of this semi-synthetic carmine acid derivative.

These results proved and revealed alarming illegal presence of aminocarminic acid in E120-labelled colorants.

Carmine is a "semi-synthetic" dye; also, confusion in terminology has led to mislabeling. Pressure from the food industry for a more satisfactory colorant for acidic foods led to the introduction of a new dye, aminocarminic acid (Dapson, 2007).A mislabeling are documented from many commercial products, and varied widely in dye content.

Dye of carmine, carminic acid and aminocarminic acid were not detected in cocoa, coffee, processed cheese, chocolate, canned fruits and vegetables products.

The present study is useful for distinguishing carmine from carminic acid, which is a major component of cochineal extract. In addition, to differentiate carmine and carminic acid in foods. Carmine and carminic acid didn't occur together in all examined foods, carmine and carminic acid are not used at the same time due to the similar color (from red to dark red colors);but they may found as a impurities in small quantities.

Data in Table (1) indicated that the number of samples contain carminic acid dye was higher than the number of samples contain carmine given ratio 54.5% and 32.5% respectively from the detected samples. Carminic acid is the common dye used by the food industry may be because it is an alkaline solution of carmine, which is stable at all pH values above ca. 3.5 and is stable to heat, light and oxygen.

It was noted that on the label of 60% investigated samples no mention of the adding of these dyes; they use only sentence "Natural colors added" or "No artificial colors ".On the rest of labeled samples 40%. It was mentioned on label ether E120, cochineal extract or carmines without any adding for level of concentration.

As shown in Table (1), half samples of the fresh juices were contained the different types of dyes. The highest value of carminic acid was achieved in fresh fruit juice giving 3670 ppm while the carmine levels ranged from 490-1100 mg/L on one sample. The presence of aminocarminic acid had confirmed in the other two of samples. In one sample of flavored tea the carmine was detected in level 35 ppm.

**Table 1: Determination of carmine, carminic acid and 4-aminocarminic acid for the different types of food samples**

SN.	Food type	Number of sample	Detected samples	Carmine level (mg/kg)	Carminic acid level (mg/kg)	Aminocarminic acid (Qualitative)
1	Fish product ,caviar ,salmon	4	2	16-290	ND	ND
2	Meat product, minced, sausages, burger	7	5	67	98-116-201-1020	ND
3	Canned fruit&vegetable product	3	-	ND	ND	ND
4	Processed flavored milk, flavored yoghurt, soya milk	7	5	18	26-132-980-1020	ND
5	Confectionery products	7	4	1400-1087	39-900	ND
6	Beverage, flavored canned fruits drinks	12	9	30-550-980	226-300-764-1020	confirmed presence on two sample (++)
7	Red pepper paste	3	1	ND	81	ND
8	Coffee, cocoa ,flavored tea, herbal tea	4	1	35	ND	ND
9	Processed cheese	3	-	ND	ND	ND
10	Seasoned salad dressing	4	2	43	182	ND
11	Fresh fruits juices	12	6	490-1100	190-1665-3670	confirmed presence on one sample (+)
12	Chocolate	3	-	ND	ND	ND
13	Soup powder	3	1	21	ND	ND
14	Ice cream powder,	4	3	160	360-1680	ND
15	Ice cream frozen	7	5	80	400-730-1340	confirmed presence on one sample (+)
16	Milk shake powder, fruits milk dessert powder	5	3	ND	113-990-1030	ND
17	Cakes	5	3	2070	100-980	ND
18	Ketchup and tomato sauce	4	2	ND	58	confirmed presence on one sample (+)
19	Pickles salty syrup	3	1	68	ND	ND
20	Flavored filling cream of biscuits	3	1	ND	51	ND
21	Fruits flavored Jelly powders	6	4	48-490	45	confirmed presence on one sample (+)
22	Fruits flavored soft drinks	5	4	69	49-70	confirmed presence on one sample (+)
23	Fruits jam	3	2	ND	104	confirmed presence on one sample (+)
24	Instant flavored drink powder	3	3	ND	120 - 477	confirmed presence on one sample (+)
25	Sweets, toffee, lolita, wafers cream, sour straw sweet, liqueur, jelly Candy, gums ,marshmallow	15	9	40-82-129-1810	43-70-1030-1750	confirmed presence on one sample (+)
26	Concentrated flavored syrup	3	1	ND	1440	ND

a ND: not detected means below limits of quantification (1.0 ug /g).

Note: in all positive presence of 4-aminocarminic acids there are also a small amount of carminic acids.



In the fish products category include red colored caviar and salmon indicated that the level of detected carmines were ranged from 16 to 290 ppm in two caviar samples but no dye detected in salmon.

In four samples of seasoning salad dressing carmine and carminic acid levels were 43 and 182 ppm for the two dyes, respectively. The absence of aminocarminic acid was observed in the four samples.

In seven meat products samples include minced meat, burger and sausages, four samples contained carminic with acid level ranged from 98 to 1020 ppm while one sample contained 67 ppm carmine.

In flavored dairy products and soya milk one sample of soya milk contained 18 ppm of carmine; while the carminic acid was ranged from 26 to 1020 ppm on the rest of samples include yoghurt and processed milk.

The results demonstrated that 9 samples out of analyzed 12 beverages canned fruits drinks had carmine value ranged from 30 to 980 ppm. While the carminic acid was in higher level ranged from 226 to 1020 ppm and existing of aminocarminic acid was conformed other two samples.

In one sample of three soup powder samples carmine dye was detected in level of 21 mg/kg.

The value of carminic acid in one concentrated syrup sample was 1440 ppm. While in four of seven confections samples carminic acid and carmine levels ranged from 39 to 900 ppm and 1400 to 1087 ppm as these compounds, respectively.

The highest level of carmine was detected in cake sample (2070 ppm) followed by the value of 1810 ppm found for sweet category in chilled sweet drink. The cakes samples also contained ratio of carminic acid from 100 up to 980 ppm.

Moreover, some samples of candy, gums, jelly sweet, marshmallow toffee, liqueur and filling cream of biscuits contained carmine in values ranged from 43 to 1750 ppm of carminic acid and ranged from 40 to 1810 ppm as carmine; but aminocarminic acid was detected in one sample.

Instant flavored drink powdered was also found to be containing carminic acid level of value ranged from 120 to 477 ppm and one sample contained aminocarminic acid.

In the frozen ice cream, level of carminic acid was ranged from 400 to 1340 ppm and from 360 to 1680 ppm in powder. Carmine levels were 80 and 160 ppm in frozen and powder samples, respectively. Also aminocarminic acid was detected in one of frozen ice cream samples;

The level of carminic acid in fruits flavored milk and milk shake powder given values ranged from 113 to 1030 ppm and from 49 to 70 ppm in fruits flavored soft drinks. Presence of aminocarminic acid was confirmed in one sample of jam, fruits jelly powder and tomato sauce. Carmine dye detected in one sample of pickles syrup given value average of 68 ppm.

In some carmine free samples, other colors such as Carmoisine, Allure red, Ponceau 4R, Indigotine and Red 2G were detected. However, red pepper paste, fruits flavored milk powder, milk shake powder, ketchup, tomato sauce and fruit jam were proved to be free of carmine dye.

Many samples not conforming to the local and international regulations on additives were sold as "no artificial or synthetic colors" although the label

not reported the content of carminic acid level as the molecule responsible for the color and some samples contained more than one color non-complying with the permissible limits of colors combined together or individual.

## **CONCLUSIONS**

This study proved that the identification of aminocarminic acid in food colorants labelled was either E120 or not labelled at all.

Analysis results of beverages and food samples showed the widespread presence of this semi-synthetic carmine acid derivative, which is not permitted by law in food industry, with unknown effects on human health.

Results of the present study give support to the hypothesis of (Dapson, 2007) for possible improper use of aminocarminic acid as carminic acid, which needs consideration by risk assessors and organizations with an interest in protecting consumer's health.

### **Recommendations:**

Regulations have to force the food manufacturers that use cochineal and carmine in their food products must list them by name in ingredient lists. Until now, these colorings, have been hidden under the terms "natural colors" "No artificial colors" or "color added." Naming those ingredients on labels will help people who suffered allergic reactions determine if the colors were the culprits. Individual's sensitive to them is to suffer repeated reactions, including potentially life-threatening anaphylactic reactions.

Carmine and cochineal extract even they are permitted in many countries should not be used for the food items as followings: natural food (meat and seafood), fruits, ice cream, cakes, vegetables and fresh juices.

The found amount of carmine and carminic acid exceeded the legal limits set for permitted colorants in food categories and beverages. As regards the analysis of the colors additives, a ratio of noncompliance more than 60 % was determined.

These studies have to be continued in the future to investigate more colors and additives.

Improper use of aminocarminic acid as carminic acid, which needs consideration by risk assessors and organizations with an interest in protecting consumer's health. A regulation has to force the food manufacturers that use carmine in their food products and cochineal must list them by name in ingredient lists. Until now, these colorings, have been hidden under the terms "natural colors" "no artificial colors" or "color added." Naming those ingredients on labels will help people who suffered allergic reactions determine if the colors were the culprits. Individual's sensitive to them is to suffer repeated reactions, including potentially life-threatening anaphylactic reactions.

The found amount of carmines and carminic acid exceeded the legal limits set for permitted colorants in food categories and beverages. As regards the analysis of the colors additives, a ratio of noncompliance more than 60 % was determined.

Peak No.	Component Name	Retention Time	Area mAU*min	Height mAU	Amount	Relative Amount %
1	carmine	1.70	24.048	196.341	49.3099	100.00

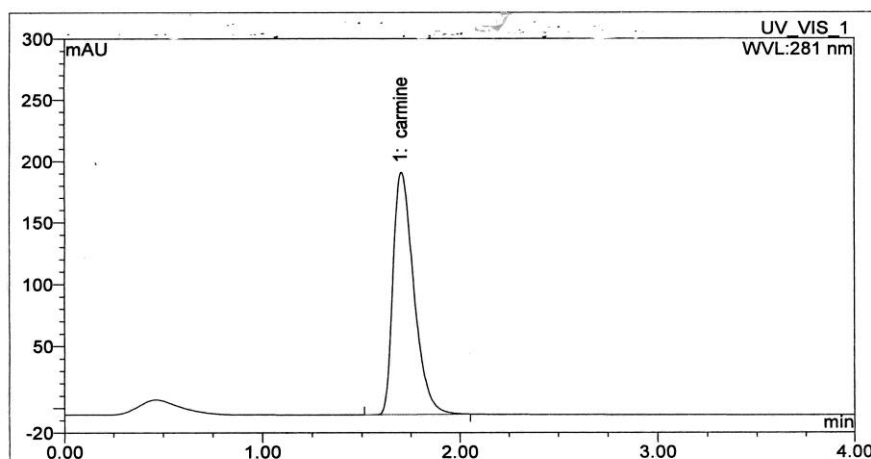


Fig 1. The UPLC chromatogram of carmine content in one analyzed sample.

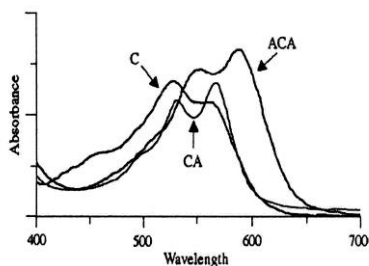


Fig. 2. Spectrophotometric scans of carmine (C), carminic acid (CA) and aminocarminic acid (ACA) using high pH (12.50–12.60) solutions.

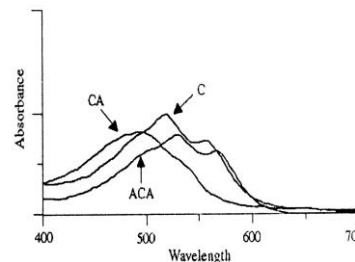


Fig. 3. Spectrophotometric scans of carmine (C), carminic acid (CA) and aminocarminic acid (ACA) using low pH (1.90–2.10) solutions.

## REFERENCES

- Ash, M. and Ash, I.(1995). Handbook of food additives: an international guide to more than 7000 products by trade name, chemical, function, and manufacturer. Farnham (Surrey, UK): Gower Pub Co.
- Borges, ME., Tejera, RL., Di'az, L., Esparzab, P. and Iba'n'ez, E.( 2012). Natural dyes extraction from cochineal (*Dactylopius coccus*). New extraction methods. Food Chem. 132:1855–1860.
- Carvalho, P. R. N., and Collins, C. H. (1997). HPLC determination of carminic acid in food stuffs and beverages using diode array and fluorescence detection. Chromatographia, 45, 63–66.

- Dapson, RW. (2005). A method for determining identity and relative purity of carmine, carminic acid and aminocarminic acid. *Biotech Histochem.* 80(5–6):201–205.
- Dapson, RW. (2007). The history, chemistry and modes of action of carmine and related dyes. *Biotech Histochem.* 82(4–5):173–187.
- EC (European Commission)( 2012). European Commission Regulation (EC) No. 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation No. 1333/2008 of the European Parliament and of the Council. *Off. J. Eur. Union.* L354:16–33.
- EC (European Commission)(2008)a .the Commission of the European Communities, Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colors for use in food stuffs.
- EC (European Commission) (2008)b. European Commission Regulation (EC) No. 1333/2008 of the European Parliament and of the council of 16 December 2008 on food additives. *Off. J. Eur. Union.* L83:1–295.
- EC (European Commission)(1994). European parliament and council Directive 94/36/EC. colours for use in foodstuffs. *Off. J. Eur.Communities,* L237, 13–29.
- Feller, L.(1986). *Artists' pigments, a handbook of their history and characteristics (Vol.1).* London, UK: Cambridge University Press.
- Lim,H.S.,Choi,J.C., Song,S.B and Kim, M. (2014) Quantitative determination of carmine in foods by high-performance liquid chromatography. *Food Chem.,* 158 :521–526.
- Ishikawa, F., Shigeoka, S., Nagashima, M., Takahashi, M., and Kamimura, H. (2003). Simultaneous determination of eleven dyes and their aluminum lakes in drugs. *J.Ass. Anal. Chem.Inter.,* 86, 215–221.
- JECFA (Joint FAO/WHO Expert Committee on Food Additives) (2001). Evaluation of certain food additives and contaminants, WHO Technical Report Series, No. 901. Geneva: World Health, Organization pp. 10–12.
- Kawasaki, Y., Sugimoto, N., Stao, K., Yamazaki, T., Ishiwata, H., and Maitani, T. (2002) Identification of acid-stable carmine in imported apple syrup product. *Sho. Eis. Zas.* 43: 191-195.
- Lancaster, F. E., and Lawrence, J. F. (1996). High-performance liquid chromatographic separation of carminic acid, alpha- and beta-bixin, and alpha- and betanorbixin, and the determination of carminic acid in foods. *J. of Chromatog. A,* 732: 394–398.
- Liu, H., Zhu, T., Zhang, Y., Qi, S., Huang, A., and Sun, Y. (1995). Determination of synthetic colourant food additives by capillary zone electrophoresis. *J. Chromatog. A,* 718: 448–453.
- Merino, L., Edberg, U., and Tidriks, H. (1997). Development and validation of a quantitative method for determination of carmine (E120) in foodstuffs by liquid chromatography: NMKL collaborative study. *J. Ass. Anal. Chem. Intern.,* 80: 1044–1051.

- Narumol, V., Juthamas, M. and Wonnop, V.(2008). Identification and determination of seven synthetic dyes in foodstuffs and soft drinks on monolithic C18 column by high performance liquid chromatography. *J.Food Drug Ana.*, 16(5): 77-82
- Samari,F.,Hemmateenejad,B., Shamsipur, M. (2010). Spectrophotometric determination of carminic acid in human plasma and fruit juices by second order calibration of the absorbance spectra-pH data matrices coupled with standard addition method. *Anal. Chim Acta.* 667:49-56.
- Schul, J. (1992). Colorant based on carminic acid, method of preparation, and method of coloring a foodstuff. United States Patent Number. 5, 147, 673.
- Scotter. MJ. (2011). Methods for the determination of European Union-permitted added natural colours in foods: a review. *Food Addit. Contam. A.* 28(2) 527-596.
- 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.
- Tabar, Al., Acero, S., Arregui, C., Urdános, M. and Quirce, S. (2003). Asthma and allergy due to carmine dye. *pubmed ; US National Library of Medicine National Institutes of Health; 26 Suppl 2:65-73.*
- Tripathi, M., Khanna, S. K., and Das, M. (2004). A novel method for the determination of synthetic colors in ice cream samples. *J. Ass. Anal. Chem. Intern.*,87:657-663.

**التقدير الكمي لمستخلص الكوشينيل (الكارمين) حمض الكارمينك,  
و حمض 4-الامينوكارمينك (اي 120) في بعض الاغذية  
منال عبد المظلع عطوه  
المركز الاقليمي للاغذية والاعلاف - مركز البحوث الزراعية**

الوان الكارمين, حمض الكارمينك , (اي 120) القرمزية و التي تعطي لونا احمر مرغوبا للأغذية و منتجاتها و يتم استخلاصها من الاناث المجففة لحشرة الكوشينيل والتي تعيش على نبات الصبار.

وقد تم تطبيق هذه الدراسة للكشف عن الكارمين و حمض الكارمينك و حمض 4-الامينوكارمينك وتم تحليل حوالي 140 عينة غذاء و مشروبات ذات لون احمر مقسمة لأكثر من 25 فئة قد تحتوي او لا تحتوي على ( اي 120 ) كميون منصوص عليه. منهم 29.5% عينة غير مغلقة من اجمالي العينات. و اوضحت النتائج ان نسبة العينات التي وجد فيها مجموعة الكارمينات كانت 55.4% من اجمالي العينات التي تم تحليلها, بينما احتوت 13% من العينات على حمض الامينوكارمينك الغير مسموح باستخدامه. مجموعة الكارمينات لم تحدد في اي من الكاكو, القهوة, الجبنة المطبوخة, الشوكولاتة, و الخضروات و الفاكهة المعلبة. و لكن ارتفعت نسب العينات التي تحتوي على حمض الكارمينك عن تلك التي تحتوي على الكارمين فكانت النسب 54.5% و 32.5% على التوالي. ايضا 60% من العينات المغلقة لم تشير لاستخدام هذه الصبغات واستبدالتها بعبارات مثل " بدون الوان صناعية" و " الوان طبيعية مضافة ". و في 40% من العينات المغلقة تم ذكر ( اي 120 ) او كارمين او مستخرج الكوشينيل فقط و لكن بدون تحديد اي نسبة للاضافة. احتوت نصف عينات المشروبات على انواع مختلفة من الكارمينات. في احدى عينات الشاي المنكهه وجد الكارمين بنسبة 35 جزء في المليون. وفي اربعة عينات من صوص السلطات كان

مستوى الكارمين 43 جزء في المليون, 182 جزء في المليون من حمض الكارمينك مع غياب حمض الامينوكارمينك في الاربع عينات. و في فئة المنتجات السميكية و التي تحتوى على الكافيار الاحمر , السلمون تراوحت نسب الكارمين بين 16, 290 جزء في المليون في عينتان من الكافيار بينما السلمون لم يحتوى على اى نسب. وفي عينات منتجات اللحوم التي تشتمل على انواع مختلفة كاللحم المفروم, البرجر, السجق. احتوت اربعة عينات على حمض الكارمينك بنسبة تتراوح بين 98, 1020 جزء في المليون بينما احتوت عينة واحدة على نسبة 67 جزء في المليون. بينما في منتجات الالبان ذات النكهة, لبن الصويا, احتوت عينة واحدة من لبن الصويا على الكارمين بنسبة 18 جزء في المليون بينما حمض الكارمينك تراوح بين 26, 1020 في بقية العينات و من ضمنها الزبادى و اللبن المنكهه.و في احدى عينات مسحوق الحساء المجفف و جدت صبغة الكارمين بنسبة 21 جزء في المليون. اما نسبة حمض الكارمينك في عينة الشراب المركز كانت 1440 جز في المليون, في اربعة من سبع عينات من كريمات تزيين الحلوى تم الكشف عن حمض الكارمينك في مدى من 39 الى 900 جزء في المليون , الكارمين من 1087 الى 1400 جزء في المليون. اعلى نسبة من الكارمين و جدت في عينات الكيك وهي 2070 جزء في المليون تليها نسبة 1810 جزء في المليون في نوع من حلوى الاطفال. و اشارت النتائج لاحتواء عينات الكيك ايضا على نسب من حمض الكارمينك تتراوح من 100 الى 980 جزء في المليون. ايضا, في بعض عينات الحلوى, اللبان, الجيلي, حلوى المارشملو, التوفى, كريمة الحشو للبسكويت وجد نسب الكارمين من 43 الى 1750 جزء في المليون , حمض الكارمينك بنسب من 40 الى 1810 جزء في المليون, الامينوكارمينك تاكد تواجده في احدى عينات هذه الفئة بينما في مساحيق المشروبات سريعة التحضير احتوت على حمض الكارمينك من 120 الى 477 جزء في المليون , احتوت عينة واحدة على حمض الامينوكارمينك في عينات الاليس كريم المتلج , تراوح حمض الكارمينك من 400 الى 1340 جزء في المليون بينما حققت من 360 الى 1680 جزء في المليون في مساحيق الاليس كريم , كانت نسب الكارمين 80-160 جزء في المليون للعينات المتلجة و المساحيق بالتوالي. تم التاكيد على وجود الامينوكارمينك ايضا في احدى عينات المربى, وعينة لمسحوق جيلي الفواكه و صوص الطماطم. و وجدت صبغة الكارمين ايضا في احدى عينات الشراب الملحى للمخلل بنسبة 68 جزء في المليون. اما عينات معجون الفلفل الاحمر, مسحوق لبن الفواكه, مسحوق اللبن المخفوق, الكاتشب, صوص الطماطم و مربى الفواكه ثبتت انها خالية من صبغة الكارمين. وفي بعض العينات التي لم يوجد فيها اى نسب للكارمين, وجد فيها ملونات اخرى كالكارموزين, الالورا ريد والانديجوتين, بونسوار, احمر 2 جى. يمكن استكمال هذه الدراسة في المستقبل لتحقيق المزيد من الكشف عن الملونات و الاضافات الغذائية الاخرى و للتحقق من نسب اضافتها و مدى مطابقتها و تاثيراتها والتي قد تكون اكثر خطورة و أقل أمانا.