

DETECTION OF 1-AMINO -2-NAPHTHOL-BASED AZO DYES (ILLEGAL SUDAN DYES) IN SOME EGYPTIAN FOOD PRODUCTS

Atwa, Manal. A.; M. F. Emar; Akila S. Hamza; M. A. Atwa and Amel M. Ahmed

Regional Center for Food and Feed, Agric. Research Center, Ministry of Agricultural, Giza, Egypt.

ABSTRACT

One hundred random samples of chili products and other related foods either local or imported (spices, mixtures of spices, tomato sauces, dressing, crisps, oils ,pickles and sausages) were collected from retail Egyptian market and analyzed for the presence of Sudan dyes by HPLC procedure . Sudan dyes have been found in a large number of food products samples containing contaminated chilli powder. The prevalence of illegal dyes were detected in a very high percentage of food products out of one hundred samples tested, more than forty five samples were found to contain detectable levels of illegal dyes. Sudan I (Sudan Red) was found as a highest ratio in red hot chili powder (220 mg/kg), also Sudan IV (Scarlet Red) was achieved the highest ratio (1120 mg/kg) in the another chilli powder sample and 326 mg /kg for Sudan II in paprika . Sudan III was detected as the highest ratio in Indian Curry paste sample(165 mg/kg) .

Keywords: Sudan dyes- Spices- Chilli powder- HPLC.

INTRODUCTION

Sudan I, II, III and IV are industrial dyes normally used for coloring plastics and other synthetic materials. Next to Sudan I-IV, Para Red, Butter Yellow, Sudan Red B and Sudan Orange G belongs to the group of azo dyes. Sudan dyes are fraudulently used to enhance the color of the food. Chilli powder is a common spice ingredient used by the food manufacturing industry in the formulation of a wide range of products, especially for oriental dishes, curries, sauces and pickles. The quality of individual batches of chilli powder, which in many cases dictate their economic value, is often arbitrarily judged by the brightness, purity and intensity of the spices color. So, certain irresponsible traders, add synthetic colorants to their spices to improve the appearance, apparent quality and freshness of their products. Several incidents have been reported within Europe, notably in early summer 2003 and 2005 (*Food Standards Agency information,2005*) where imported chilli powders and derivative foods were found to be adulterated with the Sudan dyes or related colorants. In the case of chilli and chilli products, the contamination was traced back to adulterated chilli powder. The price of chilli powder is largely linked to the intensity of the color and its maintenance so, it was suspected that Sudan I was fraudulently used to enhance and maintain the color of the product.. The European Commission extended controls for the dyes to include imports of curcuma and virgin palm oil as well as chilli and chilli products.

The Sudan dyes I-IV have molecular structures that are characterized by the nitrogen: nitrogen azo bridge between aromatic groups (Chen, 2006) as illustrated in the in figure I. Their relatively high molecular weights and low polarity provides these dyes with oil soluble properties (Golka et al. 2004). Sudan dyes are categorized as Class 3 carcinogens by the International Agency for Research on Cancer (IARC) and, these dyes has been reported to cause tumors in the liver or urinary bladder (Stiborova et al. 2005).

Many of azo dyes are suspected to be genotoxic and carcinogenic (An et al.2007). According to the (Federal Institute for Risk Assessment, 2003),sudan dyes are illegal additives in foods. The presence of any dyes not permitted for use in food would be unacceptable at any level according to the European Commission. The limit of detection for common HPLC methods at that time was between 0.5 and 1 mg/kg (Wang et al 2007). Where an illegal dye was found above this level, using this technique, either in the spice itself or a food in which it had been used, the product would need to be withdrawn from the market. Sudan dyes are synthetically produced azo dyes that do not occur naturally in food and are therefore considered to be additives and their degradation products have been shown to cause cancer in laboratory animals and these findings could also be significant for human health (Stiborova et al.2006). Because Sudan dyes may contribute to the development of cancer in people they are not considered safe to eat at any level.

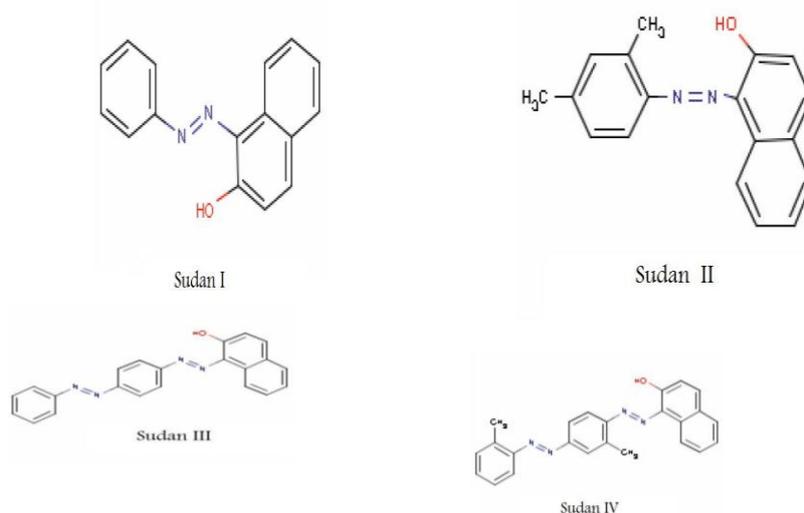


Figure I: The molecular structures of Sudan dyes I-IV (Chen ,2006).

MATERIALS AND METHODS

Materials

One hundred random different kind of spices and related food products (Chili products and spices, mixtures of spices, curry, curcuma, cayenne pepper, capsicum and sumac) were collected from Egyptian market in three replicates and were checked for the presence of Sudan dyes .

Due to the widespread use of chili powder, curry, curcuma, paprika as common ingredients in different kind of food processed products so, many kind of samples like ketchup, salad dressing, tomato sauces, pastas sauce , sausages, Worcester sauce, pizzas, pot noodles, smoked salmon, seafood sauces, palm oil, butter, crisps, mustard and pickles were chosen to carried out random checks for presence of Sudan dyes in investigated samples.

Sudan Dyes standards:

Sudan I (solvent yellow 14) 97% pure Sigma-Aldrich ,Sudan II (solvent orange 7) 90% pure Merck ,Sudan III (solvent red 23) 90% pure Merck, and Sudan IV (scarlet red, Solvent red 24) 80% pure Merck were chosen as recommended by (*Budavari and Neil. 2001*).

Methods:

HPLC Model (Dionex Instruments, USA) with version 6.8 chromeleon software was using to record the absorption spectra of the Sudan dyes as the method described by (*Uematsu et al 2007*) .The sudans I, II, III, and IV in chili- and curry-containing foodstuffs are extracted from the samples with dichloromethane. Sample extracts are filtered and analyzed by reverse phase HPLC using a 3.6 pH Acetonitril or methanol (*Puoci et al.2005*). The chromatographic separation could carry out on a reverse phase using (HCOL48) HPLC column separates the four Sudan dyes. Sudan I can be detected at 476nm (Tateo and Bononil 2004). To achieve the best detection limits for the dyes a high-performance liquid chromatography coupled to a UV/Visible detector was selected and operated at wavelength of 510 nm. The 15 cm octadecylsilica column was achieved the best separation of the four dyes. A Flow rate (0.8ml/min) was carried out and the injection volume was (20 ul) . Gradient conditions from two mobile phase were used, mobile phase A was ammonium acetate (pH adjusted with acetic acid),while mobile phase B was acetonitrile .Limits of detection were ranged from 0.2 to 0.5 mg/kg for sauces and from 1.5 to 2 mg/kg for spices samples.

RESULTS AND DISCUSSION

The Average Contents of illegal dyes (Sudan I, II, III, and IV)(ppm)in the investigated Food Products samples were given in table (1).

Results showed that the highest ratios of Sudan I,III and IV were recorded in Indian Curry paste sample(122,165 and 628 mg/kg ,respectively), While levels of sudan II were 20 ppm for pizza sauce and oriental sausage samples.

Table 1: The Average Contents of the Food Products samples from illegal dyes (Sudan I, II, III, and IV ppm).

sample NO.	sample type	SI	SII	SIII	S IV
1	B B Q sauce	T	0	0	8
2	mix of Olean ,soya and sunflower seed oil	0	0	0	0
3	mix. of Olean, soya oil	0	0	0	0
4	butter of palm oil	0	0	0	0
5	canned cooked red bean	0	0	0	0
6	chicken wings sauce	0	0	0	0
7	chips with tomato	T	0	0	0
8	chips with lemon and flavored hot pepper	0	0	0	0
9	crisps with paprika	0	5	0	0
10	French salad dressing	0	0	0	0
11	harisa tomato sauce	T	0	T	0
12	hot sauce	0	0	0	0
13	hot sauce	5	1	0	0
14	ketchup	0	0	0	0
15	mixed spices of kebab	0	0	0	0
16	mustard	0	0	54	0
17	noodle spices	20	0	0	0
18	sunflower oil	0	0	0	0
19	corn oil	0	0	0	0
20	cotton seed oil	0	0	0	0
21	paprika cheese	6	0	0	0
22	pickles cocktail	0	0	0	0
23	red bean	0	0	0	0
24	chips flavored with lemon and hot pepper	4	T	0	0
25	chips with tomato	0	0	0	0
26	smoked salmon	0	0	0	0
27	snack with paprika	3	8	0	0
28	snack with tomato	3	0	0	0
29	sausage	0	0	0	0
30	tomato ketchup	0	0	0	0
31	tomato pasta	0	0	0	0
32	tomato sauce	0	0	0	0
33	tomato sauce	0	0	0	0
34	tomato sauce	0	0	0	0
35	tomato sauce	0	0	0	0
36	tuna with chilli	5	0	0	0
37	Turkish sausage	0	0	0	0
38	Worcester sauce	0	0	0	0
39	noodles sauce	0	0	1	4
40	pizza sauce	0	20	18.2	5
41	koshari hot sauce	3	0	0	5
42	mesh with paprika	13	0	44	6
43	tomato sauce 22%	6	0	24	10
44	tomato sauce	9.6	0	0	16
45	tomato sauce	0	3	10	30
46	tomato sauce	0	T	35	55
47	mustard	0	0	45	63
48	chilli ketchup	4.9	0	0	88
49	mustard	0	0	0	103
50	hot ketchup	1.4	0	0	109
51	oriental sausage	48	20	2	250

Con. Table 1:

52	harisa ready to eat	14	2	30	370
53	hot sauce	0	0	0	520
54	Indian curry paste	122	0	165	628
55	pickles cocktail	T	0	0	T
56	koshari tomato sauce	0	0	0	T
57	butter	T	0	0	T
58	chips with curry and spices	T	0	0	T
59	crisps with hot ketchup	0	T	0	T
60	crisps with lemon and chilli	T	0	0	T
61	ketchup	0	0	0	T
62	ketchup	0	0	T	T
63	noodle spices with curry and fish flavor	T	0	0	T
64	snack hot chilli	T	0	0	T
65	tomato sauce	T	T	T	T

**Sudan I (SI); Sudan II (SII); Sudan III (SIII) and Sudan IV(S IV).
T:Traced amount (<1ppm).**

Many samples were contaminated by sudan dyes as a range from 4 to 624 ppm as given in the same table. The most samples contaminated by high levels of sudan dyes were oriental sausage, hot sauce ,harisa ready to eat,hot ketchup, mustard ,chilli ketchup, tomato sauce ,mech with paprika ,koshari hot sauce and Indian curry paste.

On the other hand many samples were contaminated by trace limits (<1ppm) such as chips, pickles, butter, ketchup ,tomato sauce crisps and snacks.

It could be also noticed that all samples of vegetables oils (sunflower seed ,soy and palm) were free from sudan dyes.

Cheese with paprika achieved (6 ppm) for Sudan I while in mesh with chili it was (13ppm) and (44 ppm) for Sudan III and 10 ppm for Sudan IV. 5ppm of Sudan I was recorded in hot chili sauce and canned tuna with chili and (20 ppm) in mustard.

In kosharie tomato sauce which is very popular meal in Egypt the Sudan I was detected in level (3ppm) for Sudan I and (5 ppm) for Sudan IV; and traced ratio of Sudan IV in tomato sauces.

The average contents of illegal sudan dyes of the spices samples were determined as shown in table (2).

Sudan I was found as a highest ratio with level of 220 ppm in red hot chilli powderd ,also sudan IV was achived 1120ppm in the same sample, while sudan II was 326 ppm in paprika. Our results showed lower levels than those detected by Mazzetti et al,2004 who suggested that, from a small survey of 30 retail samples, 11 samples of crushed chilli, Italian pasta, chilli-snack and vegetable sauce contained levels of Sudan I ranging from 24 to 5591 ppm. One paprika powder sample achieved 180 ppm Sudan I. In curcuma powder (200ppm) of Sudan I was detected and 2ppm Sudan I was found in mixed spiced for taamia.

In one kind of spices called sumac ,high ratio of Sudan IV (166ppm) was detected and 10ppm of Sudan I in another sumac sample.

Traced amount (< 1ppm) of Sudan I and IV were detected in curcuma, chilli , paprika and curry samples.

Table 2: The average Content of the Spices samples from illegal dyes (Sudan I, II, III, and IV)(ppm).

sample NO	sample type	SI	SII	SIII	SIV
1	curry powder	0	T	0	0
2	paprika Spanish	0	0	0	T
3	red crushed pepper	5	0	0	0
4	sumak Indian	T	0	0	166
5	B B Q spices	19	49	0	168
6	curcuma	0	0	0	0
7	curcuma	T	0	0	0
8	curcuma	T	0	0	0
9	curcuma	T	0	0	0
10	curcuma	0	0	0	0
11	curcuma	0	0	0	T
12	curry indium	200	0	0	0
13	curry powder	0	0	0	0
14	curry powder	0	0	0	T
15	ground red pepper	25	0	0	0
16	hot pepper chilli	20	0	0	0
17	hot pepper chilli	4	70	0	460
18	hot pepper chilli	105	0	0	1120
19	kabsa mixed spices	0	0	70	120
20	kabsa mixed spices	0	0	0	T
21	mixed spices for meat	T	0	0	T
22	paprika	0	0	0	0
23	paprika	10	0	0	0
24	paprika	0	0	0	0
25	paprika	5	T	0	4
26	paprika	15	326	43	19
27	paprika	4	0	0	T
28	paprika powder	12	278	10	0
29	paprika powder	180	0	0	T
30	red ground hot pepper	220	0	0	246
31	red pepper chilli	T	0	0	0
32	safflower	T	T	0	0
33	sumak	10	5	0	T
34	taamia spices	2	0	0	0
35	tikka spices	T	0	0	T

T:Traced amount (<1ppm).

It was noticed that most of samples contain Sudan I dye it is often contain also Sudan IV; the same in the samples contained Sudan II always contain Sudan III. (326 ppm) Sudan II,(43ppm) for Sudan III, (19ppm) for Sudan IV and 15ppm Sudan I was detected in a paprika powder ;also in chilli powder spice sample (1120 ppm) Sudan IV and (105ppm) Sudan I was recorded.

BBQ spices were contaminated by sudan dyes I,II,IV with levels of 19,49 and 168 ppm, respectively.

Three paprika samples from eighteen were contaminated by high levels of sudan dyes I,II,III and IV, which recorded level of 15,326,43 and 19 ppm, respectively as shown in the same table.

As an example, figure(2) indicated the chromatogram of sudan I and IV dyes detected in one hot chilli powder sample .

This results were similar to that obtained by (Daood and Biacs, 2005) ,who reported that more than 350 different food products, including soups, ready meals and curry powders, were withdrawn from sale as part of a frantic hunt for products tainted with the dye Sudan I. The dye had been added to a batch of Worcestershire sauce that was subsequently used to prepare many different types of food, so they all became contaminated.

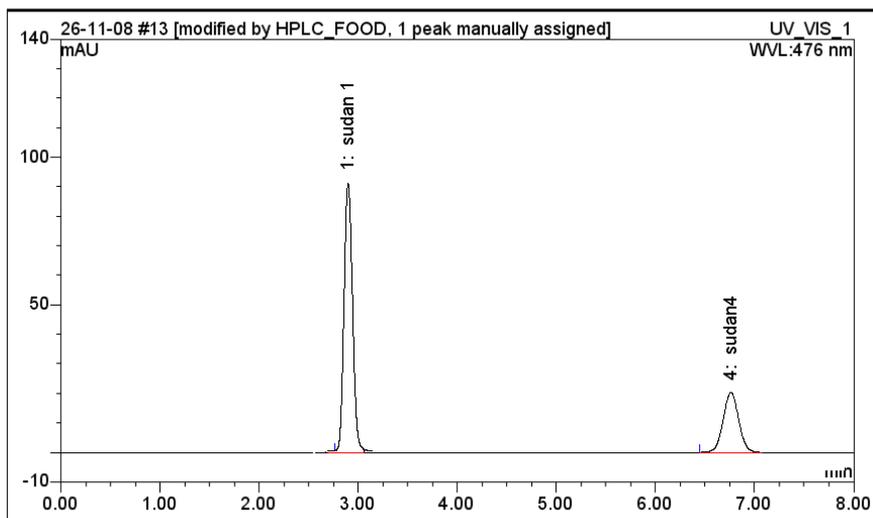


Fig (2): Chromatogram of a SI and S IV dyes detected in one hot chilli powder sample.

Conclusion

During the study to check the presence of illegal dyes sudan I, II, III and IV in a spices and related food products in Egyptian markets even it is local or imported, around forty five samples out of one hundred samples were positive. Sudan I was detected in thirty two samples ,twelve samples were positive for Sudan II and III and twenty five samples out of one hundred samples were contaminated by Sudan IV. The prevalence of Illegal dyes were detected in a very high percentage of food products out of one hundred samples tested, more than forty five samples were found to contain detectable levels of illegal dyes. Spices is an important ingredient used by the food manufacturing industry in the formulation of a wide range of products , especially for oriental dishes, snacks, sauces, cheese ,meat product and pickles. Some of tested samples were unknown the origin, such this

contaminated food commodities with illegal dyes must withdraw from the market, for the contaminated imported samples it must be destroyed at point of entry. Sudan I-IV is a dye that should not be added to food and is banned because it can contribute to an increased risk of cancer. At the levels present the risk is likely to be very small but it is sensible to avoid eating any food known to be contaminated.

As part of a regular quality assurance, it is highly recommended to screen products such as spices, spice mixtures, tomato based sauces and gravies, etc, for Sudan I-IV.

Cargoes of dried and crushed or ground chilli and curry powders coming from any country to Egypt must be accompanied by a certificate showing that it had been tested and found to be free of Sudan dyes. Random sampling is also being carried out at ports and by local authorities to investigate the presence of Sudan dyes.

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الكشف عن بعض صبغات (١- أمينو ٢- نافثول) ذات قاعدة الأزو الغير مسموح بها ومنها صبغات السودان في بعض المنتجات الغذائية المصرية.
منال عبد المظلع عطوة ، محمد فهمي عمارة ، عقيلة صالح حمزة ، محمد عبد المظلع عطوة و امل مصطفى أحمد
المركز الاقليمي للاغذية والاعلاف- مركز البحوث الزراعية

مجموعة صبغات الأزو هي صبغات لها تركيب جزيئي يتميز بوجود مجموعة الأزو $N = N$ وهي رابطة تعطي المركبات قطبية منخفضة ووزن جزيئي مرتفع وبالتالي قدرة عالية على الذوبان في الوسط الدهني.

ومنها صبغات السودان ١ ، ٢ ، ٣ ، ٤ ، والسودان أحمر G والسودان أحمر ٧ والباراريد وأصفر الميتانيل واصفر الباتر والأورنج ٢ ، والسودان أورنج G وأحمر التوليدين والرودامين B. وقد منعت دول الاتحاد الأوروبي وأمريكا ومنظمة (الاعدية والادوية) استخدامها كمادة مضافة وقد تستخدم هذه الصبغات كأحد وسائل الغش لأعطاء ألوان زاهية (تتراوح بين الأحمر والأصفر) لبعض التوابل مثل الشيلي بودر والكرم والكارى والبابريكا والفلفل الأحمر المطحون (الشطة) وتم عمل تحليل للكشف عن هذه الصبغات في المنتجات الغذائية المطروحة بالأسواق المصرية على مستوى التجزئة سواء محلية أو مستوردة.

- وتم اختيار مائة منتج وتم عمل التحليل لـ ٣ مكررات من كل منتج والحصول على المتوسط. وكانت أنواع العينات من التوابل كما هي (مثل البابريكا - الشطة - الكارى - الكرم - السماق - العصفور) أو من المنتجات التي قد تدخل أحد هذه التوابل في تصنيعها مثل الكاتشب - صوص السلطات - صلصة الطماطم - المستردة - الجبن - المخلل - الزيوت - السناك). وقد تم استخدام جهاز التحليل الكروموتوجرافي السائل عالي الكفاءة في التحليل وتم الكشف عن صبغات السودان ١ ، ٢ ، ٣ ، ٤ وكانت النتائج الإيجابية لـ ٤٥% من العينات المختبرة وتتراوح أعلى نسبة من صبغة سودان ٤ في أحد عينات الشطة الحمراء إلى ١١٢٠ جزء في المليون وكانت أعلى نسبة من السودان (١) في عينة شطة حمراء معبأة (٢٢٠ جزء في المليون). وقد وجدت صبغات السودان في عديد من العينات مختلطة او منفردة.