

BIOCHEMICAL EVALUATION ON SOME SYNTHETIC AND NATURAL FOOD COLOURANTS

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ABSTRACT

Acceptability and stability of some synthetic colourants (carmoisine and sunset yellow) and natural pigments (annatto and rouge fraise) were studied. The spectral data indicated that the synthetic colourants were more stable than the natural colourants and the best storage condition was in refrigerator at - 5°C for both types of pigments.

The biological results indicated that the rate of gain in body weight of all studied fed rats was lower than that of the control. Also, the liver wt./body wt. ratio decreased in groups of rats fed on diets containing natural or synthetic colourants relative to control. This might be attributed to that colourants might behave as a hypocholesterolic and hypolipimic effects.

The results pointed out that the amount of food intake decreased for colourants fed rats relative to control. Synthetic colourant (sunset yellow) showed insignificant elevation on food efficiency compared with natural pigments (annatto and rouge fraise). Annatto showed the lowest values of food efficiency relative to control.

The effect of synthetic colourants (carmoisine and sunset yellow) on the activities of GOT, GPT of serum and liver and plasma alkaline phosphatase were studied. The difference between control and colourant treatments was small for alkaline phosphatase activity, while a remarkable increase was recorded for GOP and GPT activities of plasma and liver.

INTRODUCTION

An increase number of synthetic dyes which are used as food colourants over a long periods, is being shown to exhibit a genotoxic effect. Levels of human exposure to such agents vary widely. Such food additives may be

continually ingested over long periods and therefore, the available literature as regards to their toxicity or metabolism revealed that little work was published before the last decade with the increasing awareness of health hazards associated with their use, extensive studies have focused attention in this respect. Recently, more detailed information became available about the biological activity of the synthetic colourings. These studies suggested that certain types exhibit carcinogenic and teratogenic effect such as tartrazine (Arcos and Argus, 1974; Chung *et al.*, 1981; Menoret, 1982 and Augustine and Leviton *1983*). Contrary to abovementioned observation, Drake *et al.* (1978); Carpanine *et al.* (1978) and Borzelleca *et al.* (1983) demonstrated no carcinogenic potential with some synthetic colourings such as erythrosine.

GOT, GPT and alkaline phosphatase activities were insignificantly increased in rat blood by annatto pigment (Hamama *et al.*, 1988) and rouge fraise dye (Hamama *et al.*, 1987). Ahmed *et al.* (1987) found that synthetic dyes (tartrazine, tartrazine/green S mix., and erythrosine) caused a significantly increasing for GOT in both plasma and liver homogenates of rats.

The present study is dealt with some properties of natural pigments (annatto and rouge fraise) and synthetic dyes (sunset yellow and carmoisine) and their acceptability for use as food additives. Some biological effects of these colourants on rats were studied. Also, some specific blood enzymes (GOT, GPT and alkaline phosphatase activities) were achieved in rat feds on both synthetic colourants.

MATERIALS AND METHODS

Colourants :

Two synthetic (sunset yellow and carmoisine) colourants, were provided from Imperial Chemical Industries (ICI, England), and two natural (annatto and rouge fraise) pigments were provided from P. Roberted and Co. France.

Acceptability :

The acceptability of the recovered concentrates as food additives was evaluated by the method reported by Abdel-Rahim *et al.* (1988).

The stability of colourants :

Natural and synthetic colourant solutions (0.4% g/L) were stored under the following conditions :

- (a) darkness at 5°C to -5°C.
- (b) darkness at room temperature (25 - 30°C).
- (d) light at room temperature (25 - 30°C).

Colour intensity was measured after 3,6,9 and 12 months at 520 nm for red colourants (rouge fraise and carmoisine) and at 420 nm for yellow colourants (annatto and sunset yellow) using Beckman Spectrophotometer.

Animals :

A total number of 50 adult male albino rats, 8 weeks old; each weighing about 100 g; raised individually in a well aerated cages, under hygienic conditions for 2 weeks. Rats were fed on a diet consisting of purified casein 15%; cotton seed oil 10%; cellulose 5%; salts mixture 4% (Hegsted *et al.*, 1941); vitamins mixture 1% (Campbell, 1961) and corn starch 65%. Diet and water were supplied *Ad libitum*. They were then divided into 3 groups. The first group (10 rats) kept as control. The second group (20 rats) was divided into two subgroups (each of 10 rats) and used for the two synthetic colourants. The third group (20 rats) was divided into two subgroups and used for the two natural pigments. The rats of each subgroup were orally administrated intragastric tube feeding, a constant dose (0.4 g/kg diet) of only one of the synthetic or natural-food colourants once a day for a period 28 days.

Each rat was weighed every day and its daily food intake was assessed. The animals were killed by decapitation at the end of the 4 weeks treatment period, then liver was dissected out and weighed.

Serum and liver glutamate-pyruvate (GPT) and glutamate-oxalacetate (GOP) activities were determined according to Ritman and Frankel (1957). Serum alkaline phosphatase activity was evaluated adopting the method of King and King (1954).

Statistical analysis :

Statistical analysis was carried out using the analysis of variance (t - test); Snedocor and Cochran (1967).

RESULTS AND DISCUSSION

The available literature as regards to stability, acceptability and biological evaluation of the food-stuff colourants is characterized by consistency results. But the magnitude of change is partially dependent on the type of colourants, chemical structure, administration dose, duration of treatment, time of sampling after treatment, degree of absorption from the gastrointestinal tract after oral dosing ----- among other factors.

Acceptability studies :

A number of prepared solutions and artificial colourants with different dosages were judged by a number of four panelists as a consumers.

All doses (0.2%, 0.4%, 0.6%, 0.08% W/V) of annatto, rouge fraise, carmoisine and sunset yellow were in the normal acceptability. It seems that over the permissible maximum dose (0.4%), (FAO/WHO, 1974) was normal for acceptability.

Stability studies :

The effect of temperature and light on colourant solutions were studied. Changes in absorbancy within 12 months storage were illustrated in Table (1).

The results in Table (1) showed that in daylight at room temperature about complete fading was observed in rouge fraise. Also a great damage was noted during the storage period.

The changes in absorbancy of synthetic colourants were less than of natural colourants. The maximum effect was observed in the daylight at room temperature for all colourants, but in refrigerator at -5°C there was no effect on both the natural and synthetic colourants.

The spectral data suggests that the synthetic colourants were more stable than the natural colourants and the best storage condition was in refrigerator at -5°C . Such results were in agreement with that obtained by Abdel-Rahim *et al.* (1988).

Biological evaluation :

The present investigation was carried out in order to study the relationship between synthetic or natural food colourants and body weight gain, food intake and food efficiency. The results are summarized in Tables (2, 3 and 4).

Results of body weight gain of rats fed the different natural and synthetic food-stuff colourants are illustrated in Table (2). The body weight increased with the increase of age for all rats with control diet or with the different natural or synthetic food colourants. But the rate of increasing in body weight was different among groups. It was observed that control showed the higher values at the end of the experimental period. Values of daily increase in body weight relative to control can be arranged in the following increasing order:

Carmoisine < Sunset yellow < Annatto < Rouge fraise

Low and significant growth rate in body weight was observed (Table, 2). These effects of the colourants on growth rate might be due to the disturbances effect of colourants in different metabolic system.

Table (1): Effect of storage temperature and day light on absorbance of natural and synthetic food colourants.

Storage period	Absorbance											
	Carmoisine			Sunset yellow			Annatto			Rouge fraise		
	1	2	3	1	2	3	1	2	3	1	2	3
Control	0.85+ 0.08-	0.85+ 0.08-	0.85+ 0.08-	0.61+ 0.05-	0.61+ 0.05-	0.61+ 0.05-	0.73+ 0.07-	0.73+ 0.07-	0.73+ 0.07-	0.55+ 0.06-	0.55+ 0.06-	0.55+ 0.06-
3 months	0.78+ 0.08-	0.62+ 0.05-	0.85+ 0.08-	0.60+ 0.05-	0.57+ 0.05-	0.61+ 0.06-	0.66+ 0.06-	0.48+ 0.05-	0.73+ 0.07-	0.42+ 0.04-	0.10+ 0.01-	0.55+ 0.05-
6 months	0.70+ 0.06-	0.57+ 0.05-	0.84+ 0.07-	0.55+ 0.06-	0.50+ 0.04-	0.61+ 0.06-	0.51+ 0.05-	0.26+ 0.02-	0.73+ 0.06-	0.30+ 0.03-	0.05+ 0.005-	0.54+ 0.05-
9 months	0.69+ 0.07-	0.56+ 0.04-	0.84+ 0.07-	0.54+ 0.05-	0.48+ 0.04-	0.60+ 0.06-	0.44+ 0.05-	0.25+ 0.02-	0.72+ 0.07-	0.25+ 0.02-	0.01+ 0.001-	0.55+ 0.05-
12 months	0.66+ 0.06-	0.50+ 0.05-	0.84+ 0.08-	0.52+ 0.05-	0.45+ 0.05-	0.54+ 0.06-	0.42+ 0.04-	0.22+ 0.02-	0.71+ 0.07-	0.23+ 0.02-	0.00+ 0.00-	0.54+ 0.04-

1: In darkness at room temperature

2: In day light at room temperature

3: In refrigerator at -5°C.

* P values were calculated by the t-test were < 0.5 for all samples.

Table (2): Gain in body weight of rats fed on different natural or synthetic food-colourants.

Colourants	Initial body weight (g)	Final body weight (g)	% Gain in body weight	Mean of daily increase in body weight (g)	% Daily increase in body weight relative to control
Annatto	102.0 \pm 9.60	149.04 \pm 15.01	46.12	1.68 \pm 0.20	84
Rouge fraise	101.0 \pm 10.00	150.28 \pm 14.97	48.79	1.76 \pm 0.18	88
Carmoisine	105.0 \pm 11.01	134.40 \pm 12.99	28.00	1.46 \pm 0.15	73
Sunset yellow	104.0 \pm 10.00	146.56 \pm 15.00	40.92	1.52 \pm 0.14	76
Control	103.0 \pm 9.00	159.00 \pm 16.00	54.37	2.00 \pm 0.23	100

* Each value is the average analysis of 10 rats.

* P values were calculated by the t-test were ≤ 0.05 for all experiments.

Table (3): Liver weight/body weight ratio of rats fed on different natural or synthetic food-colourants.

Colourants	Liver weight (g)	Body weight (g)	Liver weight/body ratio	% Liver weight/body weight relative to control
Annatto	6.26 \pm 0.70	149.04 \pm 15.01	0.042 \pm 0.005	82.35
Rouge fraise	6.16 \pm 0.62	150.28 \pm 14.97	0.041 \pm 0.004	80.39
Carmoisine	5.38 \pm 0.50	134.40 \pm 12.99	0.040 \pm 0.004	78.43
Sunset yellow	6.01 \pm 0.59	146.56 \pm 15.00	0.041 \pm 0.004	80.39
Control	8.11 \pm 0.78	159.00 \pm 16.00	0.051 \pm 0.006	100

* Each value is the average analysis of 10 rats.

* P values were calculated by t-test were ≤ 0.05 for all experiments.

The results obtained are in agreement with those obtained by Hansen *et al.* (1973), Sekigawa *et al.* (1979) who found that rats fed synthetic colourants (erythrosine or tartrazine) were characterised by two growth rates compared control. Abdel-Rahim *et al.* (1988) showed that natural pigments (curcumin, chlorophylls or anthocyanins) slight decreased the growth rate of rats.

Rats fed on control diet showed the highest values of liver weight/body weight as shown in Table (3). Other groups of the rats fed on the different natural or synthetic colourants showed lesser ratio as compared with the control groups (between 78.43% to 82.35% at control). The decrease in the average of fresh liver weight by the colourants induction may be attributed to that colourants might have a hypolipimic and hypocholesterolemic effects (Abdel-Rahim *et al.*, (1987a). The present results agree with those reported by Abdel-Rahim *et al.* (1988) who found that erythrosine, tartrazine, curcumin, chlorophylls and anthocyanine slightly decreased the liver weight/body weight ratio.

Data summarizing the average food intake and food efficiency for the different tested diets with or without food-colourants are reported in Table (4). At the end of the experimental period, the amount of food intake decreased for colourants fed rats relative to control, although changes in food intake were not parallel to the growth rates. Synthetic colourants (carmoisine and sunset yellow) reduced the food intake values than natural ones (annatto and rouge fraise). These results are in partial agreement with the results of Abdel-Rahim *et al.* (1988) who found that no abnormal effects were noted on food intake of rats fed natural pigments (chlorophylls, anthocyanins and curcumin). They observed that synthetic colourants slightly reduced the food intake relative to control.

The results in Table (4) show that in case of addition of food colourants in 0.4% level to control diet, annatto, rouge fraise, carmoisine and sunset yellow gave a decrease of 12%, 9%, 10% and 9%, respectively in food efficiency than control. Synthetic colourant (sunset yellow) showed the highest food efficiency than natural colourants when compared with control.

It is worthy to note that although natural colourants (annatto and rouge fraise) showed the highest values for food intake, annatto showed the lowest values of food efficiency. Also, it can be noticed that synthetic colourants (carmoisine and sunset yellow) fed rats showed lowest values for body weight gain but they showed high values for food efficiency. These results supported the hypothesis that the digestion of diet may be inhibited in a certain manner by adding natural or synthetic colourants. Synthetic colourants had more inhibition effect on food digestion than the natural colourants.

The present results are in agreement with the observations of Abdel-Rahim *et al.* (1988) who found that both food colourants (natural and synthetic) reduced food efficiency. Consequently, it could be suggested that, the highest and residual effect of the synthetic colourants might be referred to their uptake and accumulation by the body.

Serum enzymes activity :

From the data presented in Table (5), it is clear that either carmoisine or sunset yellow had a significant increase effect on the activities of GOT, GPT and alkaline phosphatase activities. However, the magnitude of difference between the control and each colourant treatment was small for alkaline phosphatase activity. Remarkable increase was recorded for GPT and GOT. Also, the obtained results illustrate that the effect of carmoisine on the mentioned enzymes activity was more than sunset yellow effect. Similar results for both GPT and GOT activities were demonstrated in rats ingested by synthetic colourants (Erythrosine and tartrazine) by Abdel-Rahim *et al.* (1987 b). Also the obtained results were in agreement with that obtained by Ahmed *et al.* (1987).

Table (4): Food intake and food efficiency of rats fed on different natural or synthetic food-colourants.

Colourants	Food intake (g)	Daily food intake (g)	% at control	Daily body weight (g)	Daily body food gain/100 g food	% at control
Annatto	267.40±25.67	9.55±1.00	95.50	1.68±0.20	17.60±1.80	88
Rouge fraise	270.76±26.11	9.67±0.97	96.70	1.76±0.18	18.18±1.77	91
Carmoisine	227.08±23.00	8.11±0.83	81.10	1.46±0.15	18.00±1.81	90
Sunset yellow	233.80±22.34	8.35±0.80	83.50	1.52±0.14	18.20±1.97	91
Control	280.00±27.77	10.00±0.99	100	2.00±0.23	20.00±1.97	100

* Each value is the average analysis of 10 rats.

* P values were calculated by the t - test were <0.05 for all experiment.

Table (5): Effect of synthetic colourants on serum alkaline phosphatase and GOT and GPT of liver and serum

Colourants	Plasma Alkaline phosphatase I.U.		GOT I.U.		GPT I.U.		Liver GOT I.U.		Liver GPT I.U.	
	I.U. at control	% at control	I.U. at control	% at control	I.U. at control	% at control	I.U. at control	% at control	I.U. at control	% at control
Control	30.00±2.99	100	29.01±2.44	100	10.11±1.00	100	112.00±12.00	100	40.00±4.00	100
Carmoisine	34.79±3.00	116	39.23±3.12	135	14.40±1.12	142	145.60±15.00	130	54.40±5.34	136
Sunset yellow	33.12±2.99	110	36.12±3.33	125	13.53±1.11	134	134.40±12.91	120	51.20±5.11	128

* I.U: International units.

* Each value is the average analysis of 10 rats.

* P values were calculated by t-test were < 0.5 for all experiments.

Elevation in serum activity of GOP, GPT and alkaline phosphatase demonstrated liver dysfunction and necrosis, since serum transaminases are considered to be very sensitive indicator on liver cell damage and their activities increase in the condition that produce hepatic dysfunction (DeRitis, 1958). However, SGPT is more specific for liver damage than SGOT (Sherlock, 1975).

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“التقييم الكيميائي الحيوي لبعض الملونات الطبيعية والصناعية المستخدمة

غذاثيا“

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فرع بنها

يهدف هذا البحث الى دراسة مدى ثبات بعض الصبغات الطبيعية والملونات للأغذية تحت ظروف مختلفة . وكذلك إجراء بعض التقييم البيولوجى لهذه المواد .

وقد أوضحت النتائج أن الملونات الصناعية أكثر ثباتا من الصبغات الطبيعية وأن أفضل الظروف لتخزينها هي على درجة حرارة التلاجة (- 5 ° م) وفى الظلام .

أوضحت النتائج البيولوجية أن معدل زيادة وزن حيوانات التجارب (الفئران البيضاء) كان منخفضا عن معاملة المقارنة . أما نسبة وزن الكبد / وزن الجسم قد أنخفضت فى المجموعات الغذاء على العلائق المحتوية سواء على الصبغات الطبيعية أو الملونات الصناعية وقد يعزى ذلك الى تأثير هذه الملونات على تمثيل الدهون وكذلك مركب الكوليسترول .

وقد أظهرت النتائج أيضا أن معدل امتصاص الغذاء لحيوانات التجارب قد أنخفض بالنسبة للحيوانات الغذاء على الملونات مقارنة بمعاملة المقارنة .

وقد بينت النتائج أن اللون الصناعى (الصبغة الصفراء الذهبية) قد سبب ارتفاع غير معنوى فى كفاءة الغذاء وأن صبغة الأناثو الطبيعية قد سببت أقل نسبة انخفاض فى كفاءة الغذاء مقارنة بمعاملة المقارنة .

وقد درس تأثير الملونات الصناعية (كارموزين - الصبغة الصفراء الذهبية) على نشاط أنزيم جلوتامات / أوكسال خلات ترانز أمينيز (GOT) وأنزيم جلوتامات / بيروفات ترانز أمينيز (GPT) فى كل من السيرم والكبد . وكذلك أنزيم الفوسفاتيز القلوى فى البلازما وقد أوضحت الدراسة أن الأختلاف فى نشاط الفوسفاتيز القلوى لكل من معاملة المقارنة والعلائق المعاملة بالملونات الصناعية أختلافا بسيطا بينما كانت هناك زيادة واضحة فى نشاط كل من الانزيميين الأخرين (GOT , GPT) .