Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2017, 9(1):40-47. DOI: 10.15835/nsb919880
Notulae Scientia Biologicae
Original Article

Biochemical Changes in Fatty Acids, Hydrocarbons and Sterols as well as Total Lipids of Albino Rats Ingested some Synthetic Colourants and Flavourants as Food Additives

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Abstract

The present study was carried out to investigate the effect of ingested synthetic food colourants or flavourants on total lipids content feces. The feces of rats ingested synthetic food colourants or flavourants has more lipids than that of control feces. In treated rat feces some fatty acids were disappeared (C_{19}) or appeared (C_{14}) while the other were increased (C_{15}, C_{20}, $C_{18:2}$) or decreased (C_6) when compared with the control feces. Most of unsaturated fatty acids (un SFA) might bind with the synthetic food colourants or flavourants and secreted in feces, but less consumed than saturated fatty acids (SFA) which, observed on hydrocarbon components, but sterols including cholesterol were more secreted by synthetic food additives treatments. Generally, the increase of total lipids and lipid fractions in feces such as unsaturated fatty acids (un SFA), total sterols (TS) especially cholesterol maybe due to their abilities to bind with food additives and form complex which secreted in feces.

Keywords: fatty acids, hydrocarbon, sterols, colourants, flavourants, food additives and rat feces.

Introduction

Indeed literature has been revealed few reports, which dealt with metabolic changes and other side effects of synthetic food additive. However, several synthetic organic materials are natural constituents of edible food. Acceptance of these substances has been hindered by problems involving assessment of the net benefit of their use, some individuals stressing the absence of any attendant preservative or nutrient function and others often overlooking any possible hazards to health (Lerner and Lerner 2011; Becerril *et al*., 2013). Artificial additives have been pilloried as possible toxic hazards, branded as potential sensitizer (Mister and Hathcock 2012). Progress has been in the testing program of many synthetic food additives, to overcome the "lack of sufficiently comprehensive biological evidence of safety" so common (FAO/WHO 1974; Branen *et al*., 2001; Msagati 2013). Synthetic food flavourants and colourants are considered the most important class of food additives. They have been used in candy, soft drinks, foods, pharmaceuticals and cosmotics for many years, comparatively little work on their metabolic action was published (Gultekin *et al*.,2013; Martyn *et al*.,2013). With increasing awareness of possible health hazards associated with their use, however, more attention has been focused the biological activity (Brown *et al*., 1980; Borzelleaca *et al*., 1983; Abdel-Rahim *et al*., 1992; Lodi *et al*., 2011; Alger *et al*., 2013). Further investigations were carried out on both synthetic food flavourants and colourants to evaluate the acceptability of these compounds as food additives for human and animal feeding (Tsuji *et al*., 1978; Ramadan and El-Damhogy 1994).

It must be born in mind that, with the exception of a few special cases of hypersensitivity to a variety of chemicals including certain additives, no direct evidence has emerged to incriminate any synthetic food additives among the factors responsible for serious illness in man (Lucova *et al*., 2013), about 20% of synthetic food colourants and more of synthetic food flavourants are absorbed from gut (Gaur *et al*., 2003; Sarikaya *et al*., 2012), moreover, they possess a wide range of biological and physiological effects on the metabolism of animals (FAO/WHO 1974; Abdel-Rahim *et al*., 1992; Tanaka 2006; Tanaka *et al*., 2008; Gao *et al*., 2011).

There is not available data in literature about the effect of synthetic food additives on lipid and their fractions in feces (This work is the first of its kind in this area in Egypt and perhaps in all countries of the world). For that, the aim of the present work was to carry out some biochemical studies on rats orally administrated some synthetic food colourants and flavourants in a trial to evaluate their effects on the patterns of fatty acids (saturated and unsaturated), sterols, hydrocarbons composition as well as total lipids content in rat feces.

Received: 11 Aug 2016. Received in revised form: 23 Aug 2016. Accepted: 23 Mar 2017. Published online: 30 Mar 2017.

Materials and Methods

Synthetic food additives

Synthetic food colourants Yellow: 2G, (1-(2,5-dichloro-4-sulphophenyl)-5-hydroxy-3-methyl-4-(P-sulfophenylazo) pyrazole; Ponceau 4R, trisoduim salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-6,8 disulfonic acid and brown, Fb (mixture of `2,`3,`4,5,7-Pentahydroxy flavone and Pentahydroxy-benzophenone) and flavourants: banana (isoamyl-acetate); strawberry (mixture of isoamyl butyric acid and phenyl-ethyl-phytal acetate) and chocolate (amyl-phytal acetate) were obtained from Aromisr Co. Egypt.

Animals, diets and treatments

A total of 70 adult male albino rats 80 days old, average weight 90-100g were kept under normal healthy laboratory conditions and fed normal diet which consisted of casein 15%, cotton seed oil 10%, cellulose 5%, salt mixture 4% (Hegested *et al*., 1941), vitamin mixture 1% (Compbell, 1961) and corn starch 65%. Diet and water were supplied ad libitum for a period of month.

The rats were divided into 7 groups; the first group (10 rats) served as control and fed on synthetic additives samples free diets. The other six groups (each of 10 rats) were ingested by stomach tube a dose of 0.4 g/kg diet every 48 hours of each of synthetic food colourants (Yellow: 2G, Ponceau 4R and chocolate) for a period of one month (FAO/Who, 1974). Faces of each group of rats were collected and subjected for analysis. Also, cotton seed oil of diet was analyzed as comparative study with investigated samples.

Lipid extraction

The lipid materials of rat feces were extracted using choloform-methanol mixture (2:1, v/v) according to method of Bligh and Dyer (1959). Lipid content was expressed as g/100 g feces.

Separation of fatty acids and unsaponifiables from lipid samples

Lipid material was saponified with methanolic KOH (40 %, w/v) for 24 h at room temperature according to Ahmed *et al.* (1986). The unsaponifiables were extracted three times with ether. The aqueous layer was acidified with HCl (1:1, v/v) and the liberated fatty acids were extracted three times with ether. The combined extracts of unsaponifiables and fatty acids were washed several times with distilled water and then dried over anhydrous sodium sulfate.

Methylation of lipid materials

The standard and the sample fatty acids were converted to methyl esters using ethereal solution of diazomethane according to Vogel (1975).

Determination of fatty acid composition by (GC–MS)

The fatty acid methyl esters were determined by GC–MS using Trace GC Model 2000 series produced by Thermo equipped with Selective Detector Mass Spectroscopy Model SSQ 7000 produced by Finnigan. This equipment was interfaced via HP chemstation version A 02.12 software (Hewlett-Packard. Avondale, PA). The gas chromatography was equipped with DB-23 (J & W 122-2362) 25 μ capillary column, 60 m × 0.25 mm ID, 0.15 μ m. The operating conditions for gas chromatography were as follows: injector temperature 250 °C, carrier gas: helium at 30 cm/sec, measured at 150 °C, oven temperature 50 °C for 4 min, 150 °C for 4 min and held at 250 °C until the chromatogram was completed. The detector temperature was 280°C. Mass spectroscopy operating parameters were electron ionization at 70 ev, accelerating voltage 10 kV and scan M/Z range from 50 to 500. National Institute of Standards and Technology (NIST) library according to Jiang *et al.* (2006).

Determination of unsaponifiable profile by GC–MS

The unsaponifiable fractions were finally collected in ether and taken to dryness under vacuum. The residue was analyzed using the gas chromatograph HP 5890 (Hewlett Packard) equipped with the MS detector (MSD 5970), EI, 70 ev and fitted with a capillarycolumn DB-1701 (12 m x 0.18 mm × 0.4 mm; J&W Scientific). The column temperature was programmed from 260 to 300 °C while injection temperature was set at 280 °C. Helium was the carrier gas at a flow rate of 0.7 cm^3/min. Identification of peaks was based on the retention time of standard substances and MS spectra. Analyses were run in triplicate. Calculations of percent composition of demethylhydrocarbons and demethylsterol fractions were based on the peak area.

Statistical analysis

All experimental results were expressed as means ± S.D. Analysis of variance was performed by ANOVA procedures. The results with $P < 0.05$ were regarded to be statistically significant. Data were statistically analyzed using Costate Statistical Package (Anonymous, 1989).

Results and Discussion

Effect of synthetic food colourants and flavourants on the patterns of fatty acids (saturated and unsaturated), sterols, hydrocarbons composition as well as total lipids, total saturated acids (SFA), total unsaturated fatty acids (USFA), total sterols (TS) and total hydrocarbons (TH) contents were investigated and the results are tabulated in Table (1-6).

Synthetic food colourants

As shown in Table (1), the ratios USFA/SFA and TS/TH were 0.11 and 0.14 for control rat feces, respectively, while total lipids were 8.12%. On the other hand, the ratio un SFA/SFA of rat feces ingested synthetic food colourants was higher than that control, whereas Ponceau 4R gave the highest effect, followed by brown chocolate, while Yellow 2G gave the lowest one, but TS/TH ratio was lower than that control. In case of total lipids, their levels in rat feces ingested the synthetic food colourants were almost two times more than control rat feces. From the above results in Table (1), it was suggested that, unSFA and TS of control feces were consumed and absorbed into intestinal tract, while SFA and TH were markedly increased and secreted in rat feces. From results of treated rat feces with synthetic colourants, it was concluded that, no great difference was observed in SFA between treated rat feces with colourants and control ones, while the increment of unSFA of treated rat feces, relative to control, may be due to their abilities

to bind with synthetic colourants in a complex from secreted in feces. Also, the results are indicated that, TS of treated rat samples are consumed and absorbed of Ponceau 4R and brown chocolate, relative to control rat feces, while the increase of TH in the treated feces may be due to form a new complex with synthetic colourants which secreted in feces. Generally, total lipids are higher in treated rat feces than in control, it means that, synthetic colourants may be linked with different lipid fractions which secreted in feces.

The obtained results in Table (2) indicated that, some fatty acids of control feces were clearly appeared when compared with cotton seed oil, i.e.; C_6 (23.98%), C_{17} (50.50%), and C_{19} (6.48%), also a sharp increase was observed in C_{18} (5.50%) but a significant decrease was noticed in C_{16} (1.65%), $C_{18:1}$ (6.97%) and $C_{18:2}$ (0.18%). In treated rat feces with synthetic Yellow 2G, fatty acids ($C_6$and C_{19}) were disappeared when compared with control, but C_{14} was appeared. On the other hand, C_{15}, C_{16}, C_{18}, C_{20} and $C_{18:2}$ were markedly increased than control to 3.46, 64.89, 11.77, 4.35 and 4.95%, while C_{17} was sharply decreased to 0.99%, relative to control. In Ponceau 4R treatment, C_{19} was disappeared, but C_8, C_{13} and C_{14} were appeared. Also, it was observed that, fatty acids of C_{15}, C_{16}, C_{18}, C_{20}, C_{20}, $C_{14:1}$, $C_{18:1}$ and $C_{18:2}$ were significantly increased to 6.04, 22.76, 14.52, 9.63, 2.70, 3.58, 7.84 and 23.72%, respectively. In contrast, C_6 and C_{17} were largely decreased to 1.62 and 2.43%. In treated rats with brown chocolate, the feces were riched with C_{15} (4.11%), C_{20} (8.22%), C_{22} (10.07%), $C_{14:1}$ (6.36%) and $C_{18:2}$ (14.22%), these were more than of control feces, relative percentage of C_{17} (53.55%) was almost closed to that of control feces (50.50%), while C_{16} and C_{19} were disappeared. From the obtained data in table (2), it was suggested that C_{14}, C_{16}, $C_{18:1}$ and $C_{18:2}$ of control rat feces were absorbed and interred in different processes of metabolism (Gurr and Harwood, 1991; Moutinho *et al*., 2007), since some of them may be consumed as energy source, but the other stored and the residual part was secreted in feces. Therefore, it was concluded that, animals consumed unSFA more than SFA. In connection, unsaturated fatty acids of treated rat feces by synthetic colourants which appeared or clearly increased may be bound with synthetic colourants in a complex from secreted in feces more than saturated fatty acids (Amin *et al*., 2010; Mpountoukas *et al*., 2010).

As presented in Table (3), unsaponifiable mattars of control rat faces, i.e.; C_{14}, C_{16}, C_{17}, C_{20}, C_{21}, C_{26} and C_{32} were appeared when compared with cotton seed oil, while C_{27}, C_{31} and campesterol were disappeared. On the other hand, C_{28} of control feces was appeared in great amount (45.07%), but β-sitosterol was found in low level (1.89%) when compared with

Table 1. Effect of synthetic food colourants on total lipids, saturated, unsaturated fatty acids, sterols and hydrocarbons of rat feces

Components	Cotton seed oil	Control (untreated feces)	Treated feces with synthetic food colourants			LSD 5%
			Yellow 2G	Ponceau 4R	Brown chocolate	
Saturated fatty acids (SFA)	28.38	90.35±1.78^a	87.64±1.22^b	64.63±1.43^d	78.53±1.56^c	1.33
Unsaturated fatty acids (un SFA)	71.18	9.65±0.54^d	12.36±0.43^c	35.37±0.57^a	21.47±0.49^b	1.59
un SFA/SFA	2.52	0.11±0.003^d	0.14±0.005^c	0.55±0.01^a	0.27±0.008^b	0.005
Total sterols (TS)	74.42	12.41±0.63^a	10.17±0.44^b	0.69±0.06^d	1.21±0.09^c	0.25
Total hdyrocarbons (TH)	25.58	87.59±0.34^c	89.83±0.39^b	99.31±0.27^a	98.79±0.21^a	0.68
TS/TH	2.91	0.14	0.11	0.007	0.012	-
Total lipids (g/100g feces)	-	8.12±0.82^c	17.20±0.67^a	15.81±0.57^b	15.45±0.55^b	0.43
Relative %	-	100.00	211.82	194.70	190.27	-

-Each values represents as the mean of 10 rats (Means ± SE).
- The same letters in each column represent insignificant difference at P < 0.05.

Table 2. Relative percentage of fatty acids in rat feces affected by synthetic food colourants

Fatty acids	Cotton seed oil	Control (untreated feces)	Treated feces with synthetic food colourants		
			Yellow 2G	Ponceau 4R	Brown chocolate
Saturated fatty acids:					
$C_{6:0}$ (Caproic)	-	23.98±2.40	-	1.62±0.15	0.12±0.01
$C_{8:0}$ (Caprilic)	0.28	-	0.20±0.01	1.69±0.16	0.16±0.02
$C_{10:0}$ (Capric)	0.41	0.12±0.10	0.20±0.02	0.23±0.02	-
$C_{11:0}$ (Undecanoic)	0.24	-	-	-	-
$C_{12:0}$ (Lauric)	0.30	0.12±0.10	0.59±0.06	0.23±0.02	1.01±0.10
$C_{13:0}$ (Tridecanoic)	0.34	-	-	0.82±0.07	0.16±0.01
$C_{14:0}$ (Myristic)	1.50	-	1.19±0.12	1.96±0.20	0.71±0.06
$C_{15:0}$ (Pentadecanoic)	-	0.96±0.01	3.46±0.35	6.04±0.60	4.11±0.41
$C_{16:0}$ (Palmitic)	23.16	1.65±0.16	64.89±0.6.43	22.76±2.23	-
$C_{17:0}$ (heptadecanoic)	-	50.50±5.06	0.99±0.10	2.43±0.23	53.55±5.36
$C_{18:0}$ (Stearic acid)	2.15	5.50±0.54	11.77±1.11	14.52±1.44	0.42±0.04
$C_{19:0}$ (Nonadecanoic)	-	6.48±0.65	-	-	-
$C_{20:0}$ (Aracidic)	-	0.62±0.05	4.35±0.42	9.63±1.00	8.22±0.81
$C_{22:0}$ (Behenic)	-	0.42±0.04	-	2.70±0.26	10.07±0.01
Unsaturated fatty acids:					
$C_{14:1}$ (Myristolic)	1.30	1.35±0.14	1.48±0.15	3.58±0.40	6.36±0.62
$C_{16:1}$ (Palmitoleic)	0.40	0.73±0.06	-	0.23±0.02	0.20±0.0.2
$C_{18:1}$ (Oleic)	16.32	6.97±0.07	5.93±0.60	7.84±0.80	0.69±0.07
$C_{18:2}$ (Linoleic)	53.60	0.18±0.02	4.95±0.50		14.22±1.43
$C_{18:3}$ (Linolenic)	-	0.42±0.03	-	-	-

-Each values represents as the mean of 10 rats (Means± SE).

Table 3. Relative percentage of unsaponifiable matters in rat feces affected by synthetic food colourants

Components	Cotton seed oil	Control (untreated feces)	Treated feces with synthetic food colourants		
			Yellow 2G	Ponceau 4R	Brown chocolate
Hydrocarbons:					
C_{14} (tetradecane)	-	1.67±0.17	-	1.11±0.10	1.51±0.14
C_{16} (hexadecane)	-	3.34±0.32	-	0.71±0.06	-
C_{17} (heptadecane)	-	4.75±0.44	-	3.46±0.35	0.77±0.69
C_{18} (octadecane)	-	0.50±0.04	3.81±0.32	85.30±7.49	2.42±0.23
C_{20} (eicosane)	-	2.97±0.30	2.33±0.21	-	33.89±3.40
C_{21} (hencosane)	-	1.34±0.12	23.73±2.40	1.94±0.20	-
C_{22} (docosane)	-	0.66±0.07	1.59±0.16	2.91±0.30	1.51±0.15
C_{24} (tetracosane)	1.55	0.75±0.07	0.42±0.04	-	18.15±1.84
C_{25} (pentacosane)	-	0.75±0.06	-	-	-
C_{26} (hexacosane)	-	1.00±0.10	1.59±0.12	-	-
C_{27} (heptacosane)	2.39	-	-	-	-
C_{28} (octacosane)	12.98	45.07±4.41	0.64±0.05	-	0.61±0.06
C_{30} (triacontane)	0.53	-	44.07±4.44	-	35.39±3.54
C_{31}(Squalene)	1.68	-	-	-	-
C_{32} (dotriacontane)	6.45	24.79±4.41	11.65±1.11	3.88±0.40	4.54±0.44
Sterols:					
Cholesterol	7.60	7.51±0.74	9.53±1.00	0.69±0.07	1.21±0.11
Campesterol	2.43	-	0.64±0.06	-	-
Stigmasterol	6.25	3.01±0.30	-	-	-
β-sitosterol	58.14	1.89±0.20	-	-	-

-Each values represents as the mean of 10 rats (Means ± SE).

cotton seed oil. Relative percentage of cholesterol in control feces was almost closed to that of cotton seed oil (7.51-7.60%). In treated rat samples with Yellow 2G, hydrocarbons of C_{14}, C_{16}, C_{17}, stigmasterol and β-sitosterol were disappeared when compared with control rat feces, while C_{30} was appeared with high level (44.07%). On the other hand, hydrocarbons of C_{18} (3.81%), C_{21} (23.73%), C_{22} (1.59%), C_{26} (1.59%) and cholesterol (9.53%) were markedly increased more than control, but C_{28} and C_{32} were sharply decreased from 45.07% and 24.79% in control rat feces to 0.64% and 11.65% respectively in Yellow 2G treated rat feces. In case of Ponceau 4R, C_{20}, C_{26}, C_{28}, stigmasterol and β-sitosterol were disappeared, relative to control rat feces. While C_{14}, C_{16}, C_{17}, C_{32} and cholesterol were clearly decreased to 1.11, 0.71, 3.46, 3.88 and 0.69%, respectively, but C_{18}, C_{21} and C_{22} were significantly increased to 85.30, 1.94 and 2.91%, respectively. By using Brown chocolate, it was observed a great increase in C_{18} (2.42%), C_{20} (33.89%), C_{22} (1.51%) and C_{24} (18.15%) than that of control feces. But a sharp decrease was noticed in C_{17} (0.77%), C28 (0.61%), C32 (4.54%) and cholesterol (1.21%). Moreover, C_{16}, C_{21}, C_{26}, stigmasterol and β-sitosterol were disappeared, but C_{30} was appeared in a high level (35.39%). As presented in Table (3), it was suggested that, hydrocarbons and sterols of control rat feces such as C_{24}, C_{27}, C_{30}, C_{31}, campesterol, stigmasterol and β-sitosterol were almost consumed and absorbed in different processes of metabolism (Gurr and Harwood, 1991; Moutinho *et al*., 2007). In treated rat samples with synthetic colourants, C_{18}, C_{22} and C_{30} were (for all colourants); C_{20} and C_{24} (for brown chocolate), C_{26} and cholesterol (for yellow 2G) may be bound with one or more of synthetic colourants secreted in feces. As reported in Tables 1, 2 and 3, cotton seeds oil composition was similar to that obtained by Farag *et al*. (1990), also the effect of the present synthetic colourants on incriminate of lipid fractions in treated rat feces are confirmed with others (Gaunt *et al*., 1972; Phillips *et al*., 1980; Maha 1990; Abou-Donia *et al*., 2008; Brusick *et al*., 2009). Also, these results are paralleled with the results of Abdel-Rahim *et al*. (1989) who found that, synthetic colourants influenced as hypocholesterolemic and hypolipimic agents. The same observation was reported by Abdel-Rahim *et al*. (1995), since they concluded that, total lipids and total cholesterol of blood were decreased by ingestion of synthetic colourants as food additives (Shi *et al*., 2010; Rezaei *et al*., 2013).

Synthetic food flavourants

As shown in Table (4), it was observed that, SFA are the major fatty acids either in control or in treated rat feces with synthetic food flavourants, since their levels were ranged from 76.85 to 90.35%, while USFA levels of treated rat samples (except banana flavourant treatment) are two times more than that of control rat feces. Also, the ratio of unSFA/SFA under the effect of synthetic flavourants gave the same trend as of colourants treatment. Total sterols (TS) in treated rat feces with banana flavourant is higher than that of control rat feces, while strawberry flavourant represents the highest value, but chocolate flavourant gave the lowest one, relative to control. No significant difference was observed in TH between control and treated rat feces except of strawberry flavourant treatment. In treated rat feces with banana and strawberry flavourans, ratio of TS/TH was higher than that of control feces, while the chocolate flavourant gave the lowest one. Levels of total lipids in treated rat feces with synthetic food flavourants are about two times or more higher than that of control. From the above results in Table (4), it was concluded that, large amounts of synthetic food flavourants may be bound with unSFA and TS to give a new complex secreted in feces when compared with control feces. Generally, this trend was observed in total lipids which secreted in and lipid fractions which secreted in feces via bound with synthetic flavourants (Chen and Ni 2009; Ohtsuki *et al*., 2012; Pundir and Rawal 2013).

From the obtained data in Table (5), it was found that, some fatty acids of rat feces under synthetic banana flavourant treatment were appeared, such as C_8, C_{11} and C_{14}, but C_{16}, C_{19} and $C_{14:1}$ were disappeared; when compared with control rat feces. On the other hand, some fatty acids were greatly

Table 4. Effect of synthetic food flavourants on total lipids, saturated, unsaturated fatty acids, sterols and hydrocarbons of rat feces

Components	Cotton seed oil	Control (untreated feces)	Treated feces with synthetic food flavourants			LSD 5%
			Banana	Strawberry	Brown chocolate	
Saturated fatty acids (SFA)	28.38	90.35±1.78[a]	89.45±1.69[a]	80.93±1.50[b]	76.85±1.42[c]	1.18
Unsaturated fatty acids (un SFA)	71.18	9.65±0.54[c]	10.36±0.39[c]	19.07±0.48[b]	23.15±0.70[a]	0.49
un SFA/SFA	2.52	0.11±0.003[c]	0.12±0.002[c]	0.24±0.003[b]	0.30±0.005[a]	0.006
Total sterols (TS)	74.42	12.41±0.63[b]	15.66±0.61[b]	49.22±1.13[a]	8.33±0.45[c]	1.57
Total hdyrocarbons (TH)	25.58	87.59±0.44[b]	84.43±0.59[c]	50.78±0.82[d]	91.67±0.74[a]	0.60
TS/TH	2.91	0.14	0.19	0.97	0.09	-
Total lipids (g/100g feces)	-	8.12±0.82[c]	16.18±0.58[a]	16.64±0.64[a]	13.06±0.71[b]	0.58
Relative %	-	100.00	199.26	204.93	160.93	-

-Each values represents as the mean of 10 rats (Means ± SE).
- The same letters in each column represent insignificant difference at P < 0.05.

Table 5. Relative percentage of fatty acids in rat feces affected by synthetic food flavourants

Fatty acids	Cotton seed oil	Control (untreated feces)	Treated feces with synthetic food flavourants		
			Banana	Strawberry	Brown chocolate
Saturated fatty acids:					
$C_{6:0}$ (Caproic)	-	23.98±2.40	2.02±0.20	0.23±0.02	-
$C_{8:0}$ (Caprilic)	0.28	-	2.83±0.23	-	3.89±0.40
$C_{10:0}$ (Capric)	0.41	0.12±0.10	5.38±0.53	-	-
$C_{11:0}$ (Undecanoic)	0.24	-	3.23±0.33	-	-
$C_{12:0}$ (Lauric)	0.30	0.12±0.10	0.81±0.07	2.64±0.24	-
$C_{13:0}$ (Tridecanoic)	0.34	-	0.31±0.03	0.66±0.07	0.16±0.10
$C_{14:0}$ (Myristic)	1.50	-	13.30±1.40	3.61±0.32	1.30±0.12
$C_{15:0}$ (Pentadecanoic)	-	0.96±0.01	44.64±4.45	2.34±0.24	4.32±0.42
$C_{16:0}$ (Palmitic)	23.16	1.65±0.16	-	-	47.92±4.80
$C_{17:0}$ (heptadecanoic)	-	50.50±5.06	15.12±1.45	55.43±5.60	0.43±0.04
$C_{18:0}$ (Stearic acid)	2.15	5.50±0.54	1.81±0.17	0.15±0.01	9.74±1.00
$C_{19:0}$ (Nonadecanoic)	-	6.48±0.65	-	-	-
$C_{20:0}$ (Aracidic)	-	0.62±0.05	-	7.39±0.74	9.09±0.91
$C_{22:0}$ (Behenic)	-	0.42±0.04	-	8.66±0.80	-
Unsaturated fatty acids:					
$C_{14:1}$ (Myristolic)	1.30	1.35±0.14	-	-	3.03±0.30
$C_{16:1}$ (Palmitoleic)	0.40	0.73±0.06	0.94±0.14	-	-
$C_{18:1}$ (Oleic)	16.32	6.97±0.07	6.65±0.07	0.69±0.07	6.06±0.61
$C_{18:2}$ (Linoleic)	53.60	0.18±0.02	2.96±0.30	18.38±1.49	14.06±1.41
$C_{18:3}$ (Linolenic)	-	0.42±0.03	-	-	-

-Each values represents as the mean of 10 rats (Means ± SE).

Table 6. Relative percentage of unsaponifiable matters in rat feces affected by synthetic food flavourants

Components	Cotton seed oil	Control (untreated feces)	Treated feces with synthetic food flavourants		
			Banana	Strawberry	Brown chocolate
Hydrocarbons:					
C_{14} (tetradecane)	-	1.67±0.17	4.54±0.44	0.40±0.04	0.03±0.00
C_{16} (hexadecane)	-	3.34±0.32	2.59±0.26	9.26±1.00	0.12±0.01
C_{17} (heptadecane)	-	4.75±0.44	-	0.44±0.42	-
C_{18} (octadecane)	-	0.50±0.04	0.24±0.02	1.81±0.12	7.20±0.66
C_{20} (eicosane)	-	2.97±0.30	10.71±1.10	14.83±1.42	8.57±0.84
C_{21} (hencosane)	-	1.34±0.12	0.32±0.30	0.93±0.08	0.33±0.03
C_{22} (docosane)	-	0.66±0.07	0.24±0.02	1.24±0.12	0.41±0.04
C_{24} (tetracosane)	1.55	0.75±0.07	0.30±0.03	0.03±0.00	0.59±0.06
C_{25} (pentacosane)	-	0.75±0.06	0.04±0.01	-	-
C_{26} (hexacosane)	-	1.00±0.10	12.98±1.30	12.29±1.23	0.38±0.04
C_{27} (heptacosane)	2.39	-	0.61±0.06	0.20±0.02	0.06±0.01
C_{28} (octacosane)	12.98	45.07±4.41	-	0.29±0.03	-
C_{30} (triacontane)	0.53	-	41.38±4.09	0.40±0.03	56.75±5.58
C_{31}(Squalene)	1.68	-	-	-	-
C_{32} (dotriacontane)	6.45	24.79±4.41	10.39±1.30	9.11±0.90	17.23±1.67
Sterols:					
Cholesterol	7.60	7.51±0.74	11.68±1.11	35.62±3.54	7.82±0.77
Campesterol	2.43	-	3.25±0.32	-	0.51±0.05
Stigmasterol	6.25	3.01±0.30	0.73±0.06	7.98±0.80	-
β-sitosterol	58.14	1.89±0.20	-	5.62±0.53	-

-Each values represents as the mean of 10 rats (Means ± SE).

increased than of control, for examples: C_{10} (5.38%), C_{15} (44.64%) and $C_{18:2}$ (2.96%) while C_6, C_{17} and C_{18} were sharply decreased to 2.02%, 15.12% and 1.81% when compared with control rat feces which are 23.98, 50.50 and 5.50%, respectively. In case of synthetic strawberry flavourant, fatty acids; C_{16}, C_{19}, $C_{14:1}$ and $C_{16:1}$were disappeared, but C_{14} was appeared, when compared with control rat feces. On the other hand, C_{12}, C_{15}, C_{17}, C_{20}, C_{22} and $C_{18:2}$ were greatly increased (more than control) to 2.46, 2.34, 55.43, 7.93, 8.66 and 18.38%, respectively, but C_6, C_{18} and $C_{18:1}$ were sharply decreased from 23.98%, 5.50% and 6.97% in control rat feces to 0.23%, 0.15% and 0.69% respectively, in treated rat feces. Under the effect of synthetic chocolate flavourant, C_6, C_{19} and $C_{16:1}$ were not detected when compared with control rat feces, while C_8 and C_{14} were appeared. On the other hand, C_{15}, C_{16}, C_{18}, C_{20}, $C_{14:1}$ and $C_{18:2}$ were sharply increased more than control to 4.32, 47.92, 9.74, 9.09, 3.03 and 14.06%, respectively, but $C_{17}$was greatly decreased from 50.50% in the control rat feces to 0.43% in treated rat feces. Data in Table (5) indicated that, the appearance or incriminate of some fatty acids in treated rat feces, may be due to form a new complex between them and synthetic flavourants more than 80% of food additives were secreted in rat feces (Gaunt *et al*., 1972, FAO/WHO, 1974, Phillips *et al*., 1980; Maha, 1990; Gaur *et al*., 2003; Saad *et al*., 2005; Yoshikawa *et al*., 2011; Sarikaya *et al*., 2012).

As shown in Table (6), C_{17}, C_{28} and β-sitosterol of treated rat sample with banana flavourant were disappeared, relative to control, while C_{30} was appeared. On the other hand, the following components were sharply increased, relative to control, such as C_{14} (4.54%), C_{20} (10.71%), C_{26} (12.98%) and cholesterol (11.68%), but the hydrocarbons of C_{16}, C_{18}, C_{21}, C_{22}, C_{24}, C_{25}, C_{32} and stigmasterol were significantly decreased more than that of control to 2.59, 0.24, 0.32, 0.24, 0.30, 0.04, 10.39 and 0.73%, respectively. By using the strawberry flavourants, C_{25} was disappeared, while C_{30} was slightly appeared by comparison with control feces. Moreover, C_{16}, C_{18}, C_{20}, C_{22}, C_{26}, cholesterol, stigmasterol and β-sitosterol were sharply increased more than that of control to 9.26, 1.81, 14.38, 1.24, 12.29, 35.62, 7.98 and 5.63%, respectively, but C_{14}, C_{17}, C_{21}, C_{24}, C_{28} and C_{32} significantly decreased to 0.40, 0.44, 0.93, 0.03, 0.29, and 9.11, respectively. On the other hand, some unsaponifiable components of treated rat feces with synthetic chocolate flavourant were disappeared when compared with control feces, such as C_{17}, C_{25}, C_{28}, stigmasterol and β-sitosterol, while, C_{30} and campesterol were appeared with level of 56.75% and 0.51%, respectively. Also, with the same treatment, C_{18} and C_{20} were greatly increased than control to 7.20 and 8.57, respectively; in contrast, C_{14}, C_{16}, C_{21}, C_{26} and C_{32} were markedly decreased to 0.03, 0.12, 0.33, 0.38 and 17.23%. As shown in Table (6), it was suggested that, the increase or appearance of some unsaponifiable matters in treated rat feces may be due to their abilities to bind with synthetic flavourants in a complex from secreted in feces (Patel *et al*., 2010; Gultekin *et al*., 2013).

From obtained results in Tables (1-6) it was observed that, synthetic food additives (colourants and flavourants) have similar effects on lipid fractions, these may be due to that, synthetic food colourants and flavourants have several function groups such as –OH, -COOH, -N=N-, $-NH_2$, HSO_4, ...etc (FAO/WHO, 1974; Ferreira *et al*., 2000; Stolz *et al*., 2001; Chudgar and Oakes 2003; Isaac *et al*., 2006). These synthetic food additives were bound with the different lipid fractions to prevent the absorption of them including cholesterol through intestinal tract and their secretion in feces. The appearance or disappearance and increase or decrease of some lipid fractions which observed in the present work may be due to intestinal microflora (Rencuzogullari *et al*., 2001; Vally *et al*., 2009; Garcia-Gavin *et al*., 2012). These microorganisms have different biological systems for degradation or synthesis of lipids (Dawes and Sutherland, 1976; Jawetz *et al*., 1978; Brul and Coote, 1999; Tajkarimi *et al*., 2010) which may be altered synthetic food additives (Grasso *et al*., 1974; Hveland-Smith and Combos, 1980 and 1982; Maha, 1990; Zhang and Ma 2013). These data are confirmed by those of FAO/WHO (1974); Abdel-Rahim *et al*. (1992) and Beezhold *et al*. (2014). On the other hand, the elevation of total lipids and lipid fractions in treated rat feces were paralleled with decrease of them in blood and liver (Abdel-Rahim *et al*., 1989; Caroch *et al*., 2014).

Conclusions

Finally, other efforts must be supported to investigate the capability and safety of these tested synthetic colourants and flavourants as hypocholestrolimic and hypolipidimic agents for lipid fractions. Also, more investigations are required to assess the significance of the present findings with regard to general undesirable effects of synthetic additives in different organisms.

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