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BIOCHEMICAL STUDIES ON THE EFFECT OF SODIUM METABISULFITE AND SODIUM BENZOATE ON SOME VITAMINS (ASCORBIC ACID, THIAMIN).

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ABSTRACT: *The effect of sodium metabisulfite and sodium benzoate a food preservative, on the ascorbic acid and thiamin was investigated. Sodium metabisulfite and sodium benzoate administered orally, to rats. Daily doses of sodium metabisulfite (0.35, 0.7, and 1.4 mg kg^{-1}), and sodium benzoate (2.5, 5.0, and 10.0 mg kg^{-1}) were given to rats for 8 weeks. The feeding of sodium metabisulfite (0.7 and 1.4 mg kg^{-1}) considerably decrease thiamin levels in serum and organs (liver, heart, brain, and kidney). Rats treatment of sodium benzoate (5.0, and 10.0 mg kg^{-1}) and sodium metabisulfite (0.7, and 1.4 mg kg^{-1}) produced decrease in concentration of ascorbic acid in serum and all organs. All these results indicated that sodium metabisulfite destroys the thiamin. But sodium metabisulfite and sodium benzoate decrease ascorbic acid.*

Key Words: *Sodium metabisulfite, Sodium benzoate, Ascorbic acid, Thiamin.*

INTRODUCTION

The levels of chemical components in food are an important aspect of their safety. The chemical components include the contaminants of the food chain and the substances intentionally added for technological purposes (food additives). All these substances are submitted to a scientific risk assessment consisting in comparing the deity exposure with the toxicological reference value which is the acceptable daily intake (ADI) in the case of food additives (FAO/WHO, 1974).

The acceptable daily intake (ADI) for a considered chemical is by definition the amount of that substance which can be ingested every day during the life time without appreciable health risk. The theoretical risk of exceeding the ADI for benzoates and sulphites. Among all food additive-containing foods, the highest contributors were: soft drinks to benzoates intake, nuts and canned juices to sulphites intake (Soubra *et al.*, 2006).

Upper concentration limits for food additives are therefore established in the regulation(s) on the basis of their safety assessment. For about 10 year, an obligation to establish monitoring intake systems of these substances was set by law for many countries, e.g., the European Union (European Communities, 1995) and Japan (Ishiwata *et al.*, 1997). Recommendations for

similar procedure were also made by the Codex Committee on Food Additives and Contaminants (Codex Alimentarus Comission, 1997). Many countries therefore, conducted national or regional exposure assessment. Most of these programs found that the potential to exceed the ADI for benzoates and sulphites does exist for a fraction of the population. When food consumption data were related to the maximum permitted levels (MPLs) for these additives established by the Codex Alimentarius and European Union Directive, the ADIs were exceeded (WHO, 1997). In addition, the ADI being established per Kg of body weight, the exposure of children is more likely to exceed this toxicology reference value because their body weights are lower (Leclerq *et al.*, 2000).

The possible adverse effect of those additives include initiation of allergic reactions for benzoates (WHO, 2000) and sulphites in a subset of subjects suffering from asthma or urticaria, local gastric irritations (observed in rats with sulphites), and toxicity in subjects with reduced sulphite oxidase activity (WalKer, 1985). The ADI for benzoates and sulphites were established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at respectively 0.5 (WHO, 1997) and 0-0.7 mg kg^{-1} body weight (WHO, 1987).

Humans are exposed to both endogenous and exogenous sulfites. Considerable amount of sulfites is generated in vivo by the catabolism of sulfur-containing amino acids, systeine and methionine (Cooper, 1983). Exogenous sources of sulfites include food and beverages, ambient air, and pharmaceutical products. Five salts including sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3), and sodium sulfite (Na_2SO_3) are commonly used as antioxidants in food preparation (Gunnison and Jacobsen, 1987). Sulfites are added as a preservative to a variety of pharmaceutical products. There are several amino acid preparations, utilized for potential nutrition, that contain a high level of sulfite (Lakamp and Dobesh, 2000). Similarly, the generic form of protocol, a drug used by anesthesiologists, is reported to contain 25 mg mL^{-1} $Na_2S_2O_5$ (Langevin, 1999).

Benzoic acid and sodium benzoate inhibit the growth of bacteria. In Japan, these chemical are officially a approved as food preservatives or foods such as caviar, margarine, soy sauce, soft drink and syrups. Maximum allowable levels are 2.5 g kg^{-1} (0.25%) for cavlar, 1 g kg^{-1} (0.1%) for margarine or 0.6 g kg^{-1} (0.06%) for the others, as benzoic acid (0.295, 0.118 or 0.071% as sodium salt, respectively). In Sherman rats, oral LD_{50} of sodium benzoate was 4.07 g kg^{-1} body weight (Smyth and Carpenter, 1984) and male Sherman rats fed 2% of sodium benzoate in the diet for 4 weeks showed growth depression (Fanelli and Halliday, 1963).

Sodemoto and Enomoto (1980) reported that being feed a diet containing 4 or 8% of sodium benzoate for 4 weeks was lethal to F344 rats. Also, 4% of sodium benzoate in the diet resulted death or growth depression in Albino Swiss mice within 5 weeks (Toth, 1984). And, 2.5% of sodium benzoate in the

diet sub acutely produced severe convulsion and mortality in F344 rats of both sexes (Fujitani *et al.*, 1991). While the lethal dietary level (2.5%) of sodium benzoate in F344 rats is only 8-9fold of the maximum allowable level in foods (0.25% as benzoic acid, 0.295% as sodium benzoate), there is no report on more detailed effect of sodium benzoate or benzoic acid. In addition, soy sauce and/or soft drink, in which sodium benzoate is approved for use, are consumed by most of Japanese every day. Thus, toxicity of sodium benzoate in the diet for 10 days was studied in F344 and B6C3F1 mice (Fujitani, 1993).

Sulphur dioxide and sodium metabisulfite inhibit ascorbic acid oxidation and are therefore frequently used in combination with ascorbate, particularly in fruit drinks and sausages (Davies and Wedzicha, 1992). Evidence has been presented that ascorbic acid can interact with benzoic acid and sodium benzoate in the presence of a transition-metal catalyst to yield benzene, a known carcinogen (Gardner and Lawrence, 1993 and Adams, 1997).

The aim of this study was to determine the effect of sodium metabisulfite and sodium benzoate on vitamins (thiamin and ascorbic acid) in the serum and organs (brain, heart, kidney, and liver) of male rats.

2. Materials and methods

2.1. Chemical

All chemicals used were of analytical reagent grade or higher quality and purchased from Sigma, Aldrich Chemicals. Ascorbic acid, thiamin. metaphosphoric acid, potassium phosphate buffer, methanol, trichloro acetic acid, NaOH, and percholoric acid.

Sodium benzoate, Sodium metabisulfite (Merck, Darmstadt, Germany). All solutions were prepared in deionized water.

2.2. Animal treatment

Male rats, 100g were obtained from Ramad hospital, in Cairo. The total 35 rats were divided into 7 equal groups, and one group of them was control. Male rats weighting 100g were housed in stainless steel cages in groups of one rat per cage and given food and water. After 1 week of acclimination, all animal were assigned to a treatment group by randomization of body weight. Rats were divided into 7 groups of 5 animals each: group1, control; group 2, 3, 4, rats treated with a single doses of sodium benzoate (2.5, 5, 10 mg kg^{-1} b.w.) sodium benzoate solved in warm water; group 5, 6, and 7 rats treated with single doses of sodium metabisulfite (0.35, 0.7, and 1.4 mg kg^{-1} b.w.) sodium metabisulfite solved in water. All rats were given by stomach tube for 2 month.

2. 3. Determination of vitamin C

2.3.1. Instrumentation

The HPLC system consisted of an LC.6A pump with a 20 µL injection value, an SPD-6A ultraviolet detector, an SCL-4A chromatopac integrator, and an SCL-6A control system (Shimadzu, Japan). Aspherisob ODS C_{18} column (5µm), 25 ox4mm I.D. (Teknochroma, Barcelona, Spain) was used, the filters of 0.22 µm, 13 mm and 47 mm diameter were from Magna Nylon, MSI (Micron Separations, Westborough, USA).

2. 3. 2. Chromatograpgic conditions

The method used is based on work published by Benlloch *et al.*, 1993 as mentioned above. The method uses an Octadecyl-silica reversed-phase column, amobile phase containing 5 mM cetyltrimethylammonium bromide as the ion-pairing agent and 50 nM potassium dihydrogen phosphate as a buffer, at pH 4.5 (the solution was filtered through a 0.22 µm filter for use in the chromatograph), and 4-hydroxyacetainilide as the internal standard. All measurements were done at room temperature with a flow-rate of 1 mL/min.

2.3.3. Samples

Venous blood samples were drawn from a forearm vein of volunteers using a syringe, and were transferred to tubes without heparin to obtain serum. Determinations were carried out immediately after the separation of serum by centrifugation at 3000 rpm for 15 min (Esteven *et al.*, 1997).

2.3.4. Sample treatment

An aliquot of serum was diluted (1:1) with 10% metaphosphoric acid. Internal standard (4 hydroxy acetanilide) in the amount needed to obtain a final content of 4.5 µg/mL was added, and the mixture was then centrifuged at 3300 rpm for 10 min at room temperature. The supernatant was filtered through a 0.22 µm filter, and a 20 µL aliquot was injected directly into the HPLC system (Esteven *et al.*, 1997).

One gram of tissue liver, brain, kidney, and heart was mixed with 1 mL of cold 5% (w/v) metaphosphoric acid, and centrifuged to remove the precipitated protein. An aliquot of the supernatant was subjected to HPLC (Sanchez-Moreno *et al.*, 2003).

2.4. Determination of thiamin

2.4.1. Chromatographic conditions

The HPLC system consisted of an LC.6A pump with a 20 µL injection value, with a multi λ fluorescence detector (Waters 2475). The column (15 cm×4 mm) and the precolumn (2 cm×4 mm) were packed with a RP-amide C_{16} stationary phase with a particular size of 5 µm (Supelco, USA). The column

and the guard column were placed in an oven at 30°C. The mobile phase was potassium phosphate buffer (50 mM, pH 6) methanol (80/20, v/v) delivered at flow rate of 1 mL/min. the injection volume was 20µL and the duration of the analytical run was 10 min. Fluorescence detection was operated at 366 nm excitation and 435 nm emission.

2.4.2. Sample treatment

To 0.2 mL of serum in 1.5 mL polyethylene centrifuge tube, add 0.2 mL of trichloro acetic acid, 100 g/L, and vortex mix vigorously. Then centrifuge at 3500 rpm for 5 min. inject 20 µL of supernatant into the HPLC (Kimura *et al.*, 1982 and Kimura and Itokawa, 1983). 1 g tissue liver, brain, kidney, and heart was homogenized in 7 mL of $HClO_4$ 0.4 M. the suspension was then centrifuged at 8000 rpm for 10 min and aliquot of the supernatant were immediately used for derivatization. 30.4 mM NaOH was added to 200 µL of percholic extract, mixed by vortex during 10s and left to stand exactly during 60 s and then 5 µL of NaOH (15%) was added. An aliquot was subjected to HPLC (Batifoulier *et al.*, 2005).

2.5. Statistical analysis

The data are expressed as mean ± standard error. Significant differences between the experimental groups were determined for each measurement using a one-way ANOVA. A corrected *p*-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

We designed this study to investigate the effect of sodium benzoate and sodium metbisulfite on vitamin C and thiamin. Information on biochemical aspect of sodium benzoate and sodium metbisulfite is very limited. It was found that sodium metbisulfite caused a decrease in vitamin C and thiamin in serum of rats. The results obtained in Table 1, showed generally that the highest value of vitamin C was located for G1 of rats which fed base diet which recorded 7.36 µg mL^{-1}. The group (G4, and G6) of rats fed with 5 mg/kg sodium benzoate, and 0.7 mg/kg sodium metabisulfite had lower value (1.78, and 2.16 µg mL^{-1}) than G2, G3, and G5.

The sodium metabisulfite and sulfur dioxide damage can be reduced by different antioxidants such as vitamins E and C (Stocks and Dormandy, 1971 and Etlik *et al.*, 1995). The decreased vitamin C might be due to formation of SO_2 is produced in aqueous SO_2 solutions by the action of light, chemical-reducing agents or biochemical-reducing agents. Vitamin C acts as a potent reducing agent. It reduces oxygen-, nitrogen-, and sulphur-centered radicals (Niki, 1991 and Gűműslű *et al.*, 2000).

Table (1). Effect of different concentrations of sodium benzoate and sodium metabisulfite on ascorbic acid and thiamin (µg mL^{-1}) concentration in serum of rats after 8 weeks.

Group	Vitamin C	Thiamin
G1 (control)	7.36 ± 0.31^{a}	$\pm0.003^{a}$ 0. 2
G2 (2.5 mg kg^{-1} b.w. C_6H_5COONa)	6.06 ± 0.39^{b}	$\pm0.004^{b}$ 0.15
G3 (5.0 mg kg^{-1} b.w. C_6H_5COONa)	3.32 ± 0.16^{d}	0.10 ± 0.002^{c}
G4 (10.0 mg kg-1 b.w.C_6H_5COONa)	1.78 ± 0.15^{ef}	$\pm0.005^{d}$ 0.08
G5 (0.35 mg kg-1 b.w. $Na_2S_2O_5$)	4.86 ± 0.05^{c}	0.15 ± 0.005^{b}
G6 (0.7 mg kg-1 b.w. $Na_2S_2O_5$)	2.16 ± 0.06^{e}	0.07 ± 0.002^{d}
G7 (1.4 mg kg-1 b.w. $Na_2S_2O_5$)	1.16 ± 0.10^{f}	0.037 ± 0.006^{e}
L.S.D	0.89	0.017

Neal *et al.*, (1993) examined the potential for benzoate and ascorbic acid to react to produce benzene. The decarboxylation of benzoate is a well-known probe for hydroxyl radicals (Winston and Cedarbaum, 1982) and it seems likely that this is the mechanism for benzene formation in the beverages (Gardner and Lawrence, 1993). The role of ascorbic acid is probably as asource of reducing equivalents in the udenfriiendtype generation of hydroxyl radicals from molecular oxygen and a transition metal-chelate catalyst (Castle, 1980 and Scotter and Castle, 2004).

Even traces of metals in potable water, or in other ingredients used to make beverages, are sufficient when combined with ascorbic acid, to produce the levels of hydroxyl radicals responsible for formation of such low levels of benzene, benzene known carcinogen. The metal-catalysed reduction of oxygen and hydrogen peroxide by ascorbic acid forming hydroxyl radical which can then decarboxylate benzoic acid . The condition for this reaction in a model system suggests that benzene could be produced in low yield (Adams, 1997).

Thiamin level in serum is shown in Table (1) serum thiamin levels detected in control rats (0.20 µg mL^{-1}) was significantly higher ($p<0.01$) than those detected in rats treated with $Na_2S_2O_5$ (0.15, 0.07, and 0.037 µg mL^{-1}) and in rats treated with C_6H_5COONa (0.15, 0.1, 0.08 µg mL^{-1}). Similarly, serum thiamin levels detected in G2 (2.5 mg kg^{-1}) C_6H_5COONa treated rats (0.15 µg mL^{-1}) and in G5 (0.35 mg kg^{-1}) rats treated with $Na_2S_2O_5$ (0.15 µg mL^{-1}) was significantly higher ($p< 0.01$) than this detected in G7 (1.4 mg kg^{-1}) rats treated with $Na_2S_2O_5$. Treatment of rats with sulfites ($Na_2S_2O_5$) reduced their thiamin levels in serum. In G7, about 30% of thiamin was destroyed in two month.

Feeding 0.6% $Na_2S_2O_5$ to rats was associated with reduced body weight gain caused by thiamin deficiency induced by the in vitro destruction of the vitamin in diet by the sulfite. Such effect occur neither in vivo nor when sulfites are administered in drinking water (ACGIH, 1991). The information concerning oral exposure of experimental animals to benzoic acid is very limited.

Sulfites cause a nucleophilic displacement on the methylene bride of thiamine, which is this cleaved to form the free thiaziole and pyrimidyl methanesulfuric acid. The reaction occurs faster at neutral pH (Scotter and Castle, 2004). Fujiwara *et al.*, (1964) and Kimura and Itokawa (1983) reported that finding a total thiamin concentration of 4 µg/mL in rabbit of erythrocytes 30 min after oral administration of 5 mg of thiamin propyl disulfide (TPD) and total thiamin content of 0.2 µg/mL in serum of the rabbit.

Sulfite compounds react with end products or intermediate products and inhibit enzyme chain reactions. Sulfur dioxide and $Na_2S_2O_5$ cleave essential disulfide linkage in proteins and induces changes in the molecular confirmation of enzymes. This modifies the enzyme active site or destroys the coenzymes. It destroys the activity of thiamin and thiamin-dependent enzymes by cleave and produces cytotoxic effects by cross-linking individual nucleic acid residues or nucleic acid residues and proteins (Hammods and Carr, 1976 and Stevenson and Simon, 1981).

Table (2) shows the levels of total vitamin C in tissues of rats treatment C_6H_5COONa and $Na_2S_2O_5$. No significant difference was observed in ascorbic acid concentration in liver of G3, G4, G6, and G7 (245.82, 164.83, 221.70, and 165.77 µg g^{-1}). Significant increases occurred in G1 G2. Brain vitamin C of different groups of rats were statistically significant difference as shown in Table 2, obviously, brain of G1 rats fed on normal diet containing the highest value (349.09 µg g^{-1}). Mean wile, the lowest concentrate of vitamin C was found to be with liver of G7 (98.64 µg g^{-1}). Vitamin C was found to be significantly higher ($p< 0.01$) in the kidney of G1 (108.56 µg g^{-1}) and in G7 (47.38 µg g^{-1}). Rats treatment of C_6H_5COONa and $Na_2S_2O_5$ produced significant decrease in concentration of vitamin C in G6 (62.53 µg g^{-1}). However, no significant difference could be shown among the G2, G3 and G5 (85.09, 76.64, and 85.84 µg g^{-1}). Concerning the vitamin C in heart, our data show that, G1 "rats fed on based diet" recorded the highest value (95.32 µg g^{-1}). Which was statistically significant difference comparing with other groups of rats. Currently, all the groups were in significant difference. The G7 had the lowest value of vitamin C (41.90 µg g^{-1}). Heart of G2, G3, and G5 rats had higher values of vitamin C than the heart of G4, and G6. So, the results indicated that, rats given sodium benzoate at concentration of 10 mg kg^{-1} or those given sodium metabisulfite at 1.4 mg kg^{-1} had the capacity to lower the concentration of vitamin C comparing with sodium benzoate at 2.5 mg kg^{-1} or sodium metabisulfite at 0.35 mg kg^{-1}.

Table (2). Ascorbic acid (µg g^{-1}) concentration in tissues of rats after treatment with sodium benzoate and sodium metabisulfite.

Group	Liver	Brain	Kidney	Heart
G1 (control)	429.85±40.79^{a}	349.10±9.84^{a}	108.36±4.64^{a}	95.32±2.44^{a}
G2 (2.5 mg kg^{-1} b.w. C_6H_5COONa)	491.21±34.93^{a}	307.29±0.23^{b}	85.09±1.00b	82.40±1.093^{bc}
G3 (5.0 mg kg^{-1} b.w. C_6H_5COONa)	245.82±20.46^{c}	236.29±11.53^{c}	76.64±1.91^{b}	75.30±1.06^{c}
G4 (10.0 mg kg-1 b.w.C_6H_5COONa)	164.83±10.24^{c}	179.39±6.61^{d}	54.92±2.1^{cd}	56.06±0.79^{d}
G5 (0.35 mg kg-1 b.w. $Na_2S_2O_5$)	343.43±10.81^{b}	245.47±14.71^{c}	85.84±2.64^{b}	84.97±2.78^{b}
G6 (0.7 mg kg-1 b.w. $Na_2S_2O_5$)	221.70±12.27^{c}	160.35±2.96^{d}	62.53±1.74^{c}	61.06±1.46^{d}
G7 (1.4 mg kg-1 b.w. $Na_2S_2O_5$)	165.77±7.33^{c}	98.64±1.94^{e}	47.38±0.50^{d}	41.90±1.89^{e}
L.S.D	80.03	35.66	10.17	7.52

Sulfite salts react with water leading to the generation of bisulfite (HSO^{-}_3), sulfite (SO^{-2}_3), and sulfur dioxide (SO_2). Sulfur dioxide caused a marked decrease in vitamin C. thus, the observed decrease in vitamin C in response to SO_2, might constitute protection against superoxide anion elevation. Because the oxidation of SO^{-}_2 free radical can from superoxide. Vitamin C reacts with superoxide (Gunnison, 1981; Halliwell and Gutteride, 1990; and Gűműslű *et al.*, 2000). The levels of vitamin C was decreased in young, middle-aged, and old groups in response to SO_2 (Adams, 1997 and Scotter and Castle, 2004).

Results of thiamin in liver, brain, kidney, and heart could be seen in Table (3). Obviously, significant difference was noticed for thiamin for each organ separately. "G7" group of rats fed with 1.4 mg kg-1 b.w. $Na_2S_2O_5$ through the experimental recorded the lowest value of thiamin. On contrary, control group (G1) recorded the highest value of thiamin. Thiamin levels decreased with the increasing of food additives. Thus, G4 and G7 of sodium benzoate or sodium metabisulfite were superior to the other tested groups of rats. In spite of ADI of sodium benzoate and sodium metabisulfite affect on thiamin levels in G2 and G5 of different organs of rats were high concentration than the other groups.

Nishino and Itokawa (1977) observed that the role of thiamin in nervous tissues is that thiamin is locatized in the membranous structure in the nerve and plays a role in the sodium transport system.

Bhagat and Lockett (1980) and Davidson *et al.*, (2002) observed that feeds containing 0.6% sodium metabisulfite produced two types toxic effects in rats. Feeds stored for 7 weeks resulted in thiamin deficiency symptoms. However, feeds stored 3-4 months produced diarrhea and growth retardation that were not reversed by thiamin administration (Stevenson and Simon, 1981).

Wedzichar (1984) showed that disadvantage of using sulfur dioxide or sulfite (sodium sulfate, sodium bisulfite, and sodium metabisulfite) in foodstuff is their adverse destructive effect on thiamin.

Table (3). Thiamin (μg g^{-1}) concentration in tissues of rats after treatment with sodium benzoate and sodium metabisulfite

Group	Liver	Brain	Kidney	Heart
G1 (control)	7.83±0.13^{a}	2.69±0.15^{a}	2.84±0.06^{a}	4.97±0.40^{a}
G2 (2.5 mg kg-1 b.w. C_6H_5COONa)	6.99±0.19^{ab}	2.54±0.25^{a}	2.53±0.04^{a}	4.12±0.34^{a}
G3 (5.0 mg kg-1 b.w. C_6H_5COONa)	5.62±0.24^{cd}	1.320±0.03^{b}	1.33±0.14^{b}	1.76±0.15^{b}
G4 (10.0 mg kg-1 b.w. C_6H_5COONa)	2.42±0.20^{e}	0.81±0.09^{bc}	0.89±0.01^{c}	0.82±0.04^{bc}
G5 (0.35 mg kg-1 b.w. $Na_2S_2O_5$)	6.05±0.28^{bc}	2.36±0.11^{a}	2.44±0.05^{a}	4.31±0.39^{a}
G6 (0.7 mg kg-1 b.w. $Na_2S_2O_5$)	4.95±0.43^{d}	0.87±0.08^{bc}	0.81±0.09^{c}	0.57±0.03^{c}
G7 (1.4 mg kg-1 b.w. $Na_2S_2O_5$)	0.69±0.05^{f}	0.43±0.06^{c}	0.34±0.01^{d}	0.18±0.05^{c}
L.S.D	1.03	0.54	0.42	1.07

REFERENCES

ACGIH (1991). Documentation of the threshold limit values and biological exposure indices. 6th Edition. Volume III. American Conference of Governmental Industrial, Inc. Cincinnati, Ohio, USA.

Adams, J. B. (1997). Food additive-additive interactions involving sulphur dioxide and ascorbic and nitrous acids: areview. Food Chemistry 59 (3):401-409.

Batifoulier, F., M. A. Verny, C. Besson, C. Demigé and C. Rémésy (2005). Determination of thiamin and its phosphate easter in rat tissues analyzed as thiocromes on a RP-amide C_{16} column. Journal of Chromatography B 816:67-72.

Benlloch, R., R. Farré and A. Frigola (1993). J.Liq. Chromatogr. 16:3113.

Bhagat, B. and M. F. Locketi (1980). The effect of sulphite in solid diets on the growth of rats. Food Cosmet Toxicol 2:1.

Castle, L. (1980). Astudy of oxy-radicals as simple models for sytochrome P450-dependent monooxygenation. Thesis, Department of Chemistry, University of York.

Codex Alimentarus Commission, (1997). Report of the 29th session of the CODEX Committee on food additives and contaiminants. The hague, The Netherlands, 17-21 March (ALINORM 97/12).

Cooper, A. j. (1983). Biochemistry of sulfur-containing amino acids. Annu. Rev. Biochem.52:187-222.

Davidson, P. M., V. K. Juneja and J. K. Branen (2002). Antimicrobia agents. In "food additives". Branen, A. L.; Davidson, P.M.; Salminen, S., and Thorngate, J. H (EDs). Marcel Dokker, Inc. pp.563-616.

Davies, C. G. and B. L. Wedzicha (1992). Kinetics of the inhibition of ascorbic acid browning by sulphur. Food Addi. Contam. 9:417-477.

Esteven, M. J., R. Farré, A. Frigola and J. M. Garcia-Cantabella (1997). Dtermination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography. J. Chromatography B 688:345-349.

Etlik, Ö., A. Tomur, M. Kutman, S. YÖrűkan and O. Duman (1995). The effects of sulfur dioxide in halation and antioxidant vitamins on red blood cell lipoperoxidation. Environmental Research 71: 25-28.

European Communities (1995). European parliament and council Directive No.95/2/EC of February 1995 on additives other than colors and sweeters for use in foodstuffs. Official Journal, No.L61, 18.3.95, pp.1-40.

Fanelli, G. M. and S. L. Halliday (1963). Relative toxicity of chlortetracycline and sodium benzoate after oral administration to rats. Arch. Int.Pharmacodyn 144:120.

FAO/WHO, (1974). WHO food additives series No5. Feinberg, M.; Favier, J.C., and Ireland-Rippert, J.(1991). Repertoire Géneéral des Aliments. In:Doc, T. (ed). Lavoisier, Paris, p 281.

Fujitani, T. (1993). Short-term effect of sodium benzoate in F344 rats and B6c3F1 mice. Toxicology Letters 69:171-179.

Fujitani, T., H. Ando, K. Fujitani, T. Ikeda, J. Kabashima, N. Kamiya, A. Kojima, Y. Kubo, A. Ogata, S. Oishi, Y. Tada, H. Takahashi, O. Takahashi, M. Yoneyama and H. Ichikawa (1991). Sub-acute toxicity of sodium benzoate and piperonyl but oxide in F344 rats. Ann.Rep.Tokyo. Metr. Res.Lab.P. H 42:285.

Fujiwara, M., S. Sasagawa, Itokawa. and J. Ikeeda (1964). Affinity of thiamin propyl disulfide-S^{35} to blood J. Vitaminal 10: 70-78.

Gardner, L. K. and G. D. Lawrence (1993). Benzene production from decarboxylation of benzoic acid in the presence of ascorbic acid and atransition-metal catalyst. Journal of Agricultural and Food Chemistry 41:693-695.

Gardner, L. K. and G. D. Lawrence (1993). Benzene production from decarboxilation of benzoic acid in the presence of ascorbic acid and a transition-metal catalyst. J. Agric. Food Chem 41: 63-695.

Gűműslű, S., D. K. Korgun, S. Bilmen, P. Yargicoglu and A. Agar (2000). Effect of sulfur dioxide inhalation on plasma vitamin C and ceruloplasmin in Ageing rats. Industrial Health 38: 319-322.

Gunnison, A. F. (1981). Sulphite toxicity: a critical review of in vitro and in vivo data. Food Cosmet.Toxicol.19:667-6820

Gunnison, A. F. and D. W. Jacobsen (1987). Sulfite hypersensitivity. Acritical review. CRC. Crit.Rev. Toxicol.17:185-214.

Hammonds, S. M., and J. C. Carr (1976). The antimicrobial activity of SO_2-with particular reference to fermented and nonfermented fruit juices. Soc.Appl. Bacteriol.Symp.ser.5:89.

Halliwell, B. and J. M. C. Gutteridge (1990). The antioxidants of human extra cellular fluids. Arch. Biochem.Biophys.280:1-8.

Ishiwata, H., M. Nishijima, Y. Fukasawa, Y. Ito and T. Yamada (1997). Evaluation of preservatives content in foods and the daily intake deduced from the results of official inspection in Japan infical year 1994. Journal of the food Hygiene Sciety of japan38:145-154.

Kimura, M. and Y. Itokawa (1983). Determination of thiamin and thiamin phosphate ester in blood by liqid chromatography with post-column derivatization. Cli.Chem. 29(12): 2073-2075.

Kimura, M., B. Panijpan and Y. Itokawa (1982). Separation and determination of thiamin and its phosphates esters by reversed-phase high performance liquid chromatography. J. Chromatogr. 245: 141-143.

Lakamp, J. E. and P. P. Dobesh (2000). Propofol and too much sulfite?.Chest118:277.

Langevin, P. B. (1999). Propofol containing sulfite-potential for injury. Chest 116:1140-1141.

Leclerq, C., M. G. Molinaro, R. Piccinelli, M. Baldini, D. Arcella and P. Stacchini (2000). Dietary intake exposure to sulfites in Italy-Analytical determination of sulphite-contaning foods and their combination into standard meals for adults and children. Food Additives and Contaminants 5:979-989.

Neal, T. P., P.J. Nyman, G. W. Diachenko and H. C. Kollifield (1993). Survey of benzene in foods using headspace concentrayion techniques and capillary gas chromatography. Journal of the AOAC International 76:1213-1219.

Niki, J. (1991). Action of ascorbic acid as ascavenger of active and stable oxygen radicals. Am.J.Clin. Nutr 54: 119S-204S.

Nishino, K. and Y. Itokawa (1977). Thiamin metabolism in vitamin B6 or vitamin B12 deficient rats. J.Nutr.107:775-782.

Sánchez-moreno, C., M. Paniague, A. Madrid and A. Martin (2003). Protective effect of vitamin C against the ethanol mediated toxic effects on human brain glial cells. J. Nutritional Biochemistry 14: 606-613.

Scherrer, R. (1982). Interaction of bisulfite with unsaturated fatty acids. J. Toxicol.Enviro. Health 10:479-491.

Scotter, MiJ. and L. Castle (2004). Vhemical interactions between additives in foodstuffs. Food Additives and Contaminants 21(2):93-124.

Smyth, Jr. H. H. and C. P. Carpenter (1984). Further experience with the range finding test in the industrial toxicology laboratory. J.Industr. Hyg. Toxicol 30:63.

Sodemoto, Y. and M. Enomoto (1980). Report of carcinogenesis bioassay of sodium benzoate in rats. J. Environ. Pathal.Toxicol 4:87.

Soubra, L., D. Sakis, C. Hilan and Ph. Verger (2006). Dietary exposure of children and teenagers to benzoates, sulphites, butyl hydroxyanisol (BHA) and butyl hydroxytoluen (BHT) in Beirut (Lebanon). Regulatory Toxicology and Pharmacology 39:1-10.

Stocks, J. and T. L. Dormandy (1971). The autoxidation of human red cell lipids induced by hydrogen peroxide. Br.J.Haemotol 20:95-111.

Toth, B. (1984). Lock of tumorigenicity of sodium benzoate in mice. Fundam.Appl. Toxicol 4: 494.

Walker, R. (1985). On the dietry intake of butylated hydroxytoluene. Food and Chemical Toxicology 29:74-75.

Wedzichar, B. (1984). Chemistry of sulphur dioxide in foods, Elsevier Applied Sci., Londno.

WHO, (1987). Evaluations of certain food additives and contaminants. Thirtieth report of the joint FAO/WHO Expert Committee on Food Additives. Wold Health Organization, Geneva.

WHO, (1997). Evaluation of certain food additives and contaminants. Forty-sixth report of the joint FAO/WHO Expert Committee on Food Additives. World Health Organization, Geneva, Technical Report Series No. 891, Geneva.

WHO, (2000). Benzoic acid and sodium benzoate. CICAD, Concisr International Chemical Assessment Document 26.Geneva.

Winston, G. W. and A. I. Cedarbaum (1982). Oxidative decarboxylation of benzoate to carbon dioxide by rat liver microsomes: aprobe for oxygen radical production during microsomal electron transfer. Biochemistry 21:4265-4270.

دراسات كيميائية وحيوية عن تأثير صوديوم ميتا باى سلفيت وصوديوم بنزوات على بعض الفيتامينات (الأسكوربيك أسيد، الثيامين)

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الملخص العربي

لقد تم بحث دراسة تأثير صوديوم ميتا باى سلفيت وصوديوم بنزوات على الأسكوربيك أسيد، الثيامين. واعطيت الفئران عن طريق الفم جرعات من صوديوم ميتا باى سلفيت (٠,٣٥، ٠,٧، ١,٤ملجم/كجم)، وصوديوم بنزوات (٢,٥، ٥ ،١٠ ملجم/كجم) لمدة ٨ أسابيع ونلاحظ حدوث نقص فى نسبة الثيامين فى السيرم والأعضاء (الكبد- القلب- المخ- الكلية).

ينتج من معاملة الفئران بصوديوم بنزوات (٥ ، ١٠ ملجم/كجم) و صوديوم ميتا باى سلفيت (٠,٧ , ١,٤ملجم/كجم)نقص فى تركيز الأسكوربيك أسيد فى السيرم والأعضاء.

والنتائج عامة تشير أن صوديوم ميتا باى سلفيت تحدث هدم للثيامين ولكن المعاملة بصوديوم ميتا باى سلفيت وصوديوم بنزوات تحدث نقص فى نسبة الأسكوربيك أسيد.