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Effects of Heavy Metals and Monosodium L-Glutamate in Food Flavors on Albino Rats

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Abstract

Previous studies reported many effects for additives overdoses or some metal contaminants. Flavors of highly reputed commercial product of known as Egyptian pasta were tested for their effects on liver, kidney, brain and other organs histograms. Effects have measured as well in serum analytical functions. Natural standard antioxidants were compared in the biological experiment as vitamin C VC, selinum Se and also the same tasted flavors in natural formula *nf* from spices market. Flavors have been added daily in 10% ratio on male albino rat normal diet comparing to control con group. At the end of experiment time (28 days), rats were sacrificed and analyzed the serum and pathological histograms. On the other side, the existence was followed for Na, Al, Cu, Pb and Cd determinations, *Enterobacteriaceae* and *Escherichia coli* bacterial growth levels. Increase values were noticed in ALT, AST, urea, the lipid risk factor and the fucosidase activity for cf group. As well, liver, kidney, heart, spleen and brain histological sections proved to show congestion and edema. But improved supplement VC, Se, and *nf* groups showed a remarkable enhancement in histopathological patterns. In a trial to find out the main reason of these effects, we measured the presence of monosodium glutamate MSG the food enhancer in the tested flavor.

Keywords: Fast food; Additives; Flavors; Pasta; MDA; Fucosidase; Heavy metals; Oxidative stress; Sodium glutamate.

Introduction

Fast food additives were known since the old man from the ancient civilizations to introduce special color or taste in his food. Nowadays, there are over-use of synthetic chemicals as food additives to preserve or enhance food taste, to improve characters and attract consumers especially children. Junk foods have proved to be a big problem for human health in general and especially for children, for their synthetic flavors preservatives and fabric antioxidants. Food industry has improved through the last century depending on the consumer's appeal and in order to approve that, not safe way to apply. The economic issue and the conscious are the first factors in this healthy equation.

The presence of some metals in the environment is mainly caused by human activity, and their ubiquity, persistence and accumulation in organisms implies that living beings are continuously exposed to them (Garcia-Fernandez *et al.,* 2005) [1]. Uptake of heavy metals and contamination of food during storage, marketing, and processing stages are the major sources of heavy metals in foods (Ward, 1995) [2]. Most heavy metals are non-degradable and form the major non-occupational source of exposure to heavy metals (WHO, 1982) [3]. Excessive intake of Cd, Cu, Pb and Zn have devastating health effects (WHO, 2006) [4] while deficiency of Cu, Zn leads to deficiency syndromes.

Monosodium glutamate MSG (E621) is used as a flavor enhancing agent in our studied product as mention on the label. MSG is one of the most popular flavoring agents of modern time and is widely using in many commercially packed food and household cooking. As glutamate is a major component of protein, it is found naturally in virtually all protein-containing foods. Glutamate is an excitatory neurotransmitter in the central nervous system of mammals (Hinoi *et al.,* 2004) [5]. The endogenous L-glutamate, as the derived L-glutamate of exogenous precursors, is formed in a Ca^{+2} dependent way after a depolarizing stimulus in the CNS (Cotman and Kahle, 2000) [6].

Dietary nutrient scientists help to discover the biological roles of nutrients and their function in diseases. Dietary sources of essential elements are important for correcting the physiological functions of the human body. Reference daily intakes for significant elements were established; Cu (2mg), and Na (2400mg) (Mindel, 2000). Our research applied the common high reputed food additives to biological experiment and tried to find the reason for the high risky results on rat organs and serum analytical tests.

Material and Methods

Vitamin C used from Chemical Industries Development Company, and Selenium tablets manufactured by Sigma for Interpharma UK under license of Wassen International LTD.

Bacteriological analysis

Using dangling modifier, 50 g of different flavors weighed into sterile blender jar. Then, 450 mL of sterile Butterfield's phosphate-buffered were added and mixed well with vigorous shaking and blended for 2 min at high speed 10,000-12,000 rpm (Tallent *et al.,* 2012). The plating medium nutrient agar (Himedia) used for aerobic plate count (APC) for different flavors homogenates and incubates for 24 hours at 37 ± 0.5°C. After incubation time, total number of bacterial colonies counted, and APC/gm calculated. Different flavor homogenates were plated on MacConky agar (Himedia) with 0.2% sterile sodium azide medium (Oxford Laboratory company, India) and incubated for 24 hours at 37 ± 0.5°C. *Enterobacteriaceae* and *Escherichia coli* bacteria were identified counted for each gm of different flavors

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homogenate.**Mineral and heavy metal contents**

One gram of chicken and beef flavors was dissolved in one litter of squinting modifier to make homogenates. The estimation of heavy metals Cu, Pb, Cd and Na, Al were carried out using GBC Atomic Adsorption Spectrophotometer (AAS) (Santa AA). Sodium (as an example of macroelement), Al, and Cu (as trace and ultra-trace elements respectively) and Pb, Cd (as contaminant heavy metals) examined.

Biological experiment

Thirty-five male Sprague-Dawley rats, weighing 150 ± 5 g purchased from Research Institute of Ophthalmology, Giza, Egypt. Animals gave two weeks acclimation period, during which they fed *ad libitum* a standard rat chow diet (Compbell, 1961) [7], with alternated 12-h dark/light cycle, and the ambient temperature held at 21-25°C. All studies were performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by research Institute of Ophthalmology, Giza on 2013.

Flavors have chosen as the highly appealed to the food Egyptian consumers especially for children. Effects of tested flavored have enhanced with certified antioxidants. Certain weight (3.0 g) from marked pasta flavors has well mixed with 30 g daily chow diet amount for rat. Animals were divided into five groups (7 rats each), as followed;

Group (1) normal control group con were fed with standard rat chow diet. Group (2) rats were fed the chow diet with 10 % highly reputed commercial pasta flavors cf. Group (3) rats fed with the diet of group 2 and daily injected by esophageal tube with vitamin C VC (1 g/kg rat weight). Group (4) rats fed with the diet of group 2 and daily injected by esophageal tube with selenium Se (100 µg/kg rat weight). Group (5) rats were fed the chow diet with 10 % mixed similar natural flavors *nf* (composed of plant spices market in Cairo).

Assessment of activity

Food consumption monitored daily, and body weight determined once a week. After the experimental period, food was forbidden for 12 hours. The fasting rats were sacrificed, and blood samples collected into clean centrifuge tubes. Blood samples were allowed to coagulate and centrifuge at 3000 rpm for 20 minutes to separate the blood serum. Separated serum was stored at -20°C for subsequent biochemical analysis.

Biochemical analysis

Triglycerides TG, total cholesterol TChl and high density lipoprotein cholesterol HDLc were colorimetrically determined in rat serum using enzymatic colorimetric methods. Low-density lipoprotein cholesterol LDL-c and VLDL-c were calculated (Friedewald *et al*., 1972) [8] by (mg/dl) as follows;

LDLc = *TC – HDLc – (TG/5) VLDLc = TG/5*

Risk factor also has measured by dividing LDL-c by HDL-c values. Liver transaminase enzymes as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) measured with colorimetric method (Reitman and Frankel, 1957) [9]. Blood urea was determined as well (Fawectt and Scott, 1960) [10]. The determination of serum creatinine performed (Larsen, 1972) [11].

Histopathological study

Autopsy samples were taken from the rats in different experimental groups. Then, samples were fixed in 10% formal saline solution for twenty-four hours. Washing was done in tap water then serial dilutions of absolute ethyl alcohol were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56°C in a hot air oven for twenty-four hours. Paraffin beeswax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stain for histopathological examination under the light microscope (Banchroft *et al*., 1996) [12].

Mono sodium glutamate (MSG) determination

Sodium L-glutamate was extracted and determined by high-performance liquid chromatography HPLC (Lateef *et al.,* 2012) [13]. HPLC Knauer, Germany with Agilent Forbes reversed phase C18 column at 25°C and 40% humidity equipped with two pumps. UV detector used at 254 nm with clarity-chromatogram software.

Statistical analysis

Using SPSS program, (SPSS, 1998) [14] means were calculated from ten replicates, with their Standard Deviations (± SD) for each group. Analysis of variance was applied to make statistical comparisons (ANOVA) with Dennett's post hoc test in between 5% probability.

Results and Discussions

Bacterial experiment

The bacteriological analysis of different flavors (50 g / 450 ml buffer) showed results (Table 1) that were acceptable for different bacteriological indicators. Aerobic plate count (APC) for different flavors homogenate at 37±0.5°C which indicates the level of microorganisms in a product was ranged from 8–83 CFU/g, while standard range is less than 10^4 colony forming unit CFU/g. Also, for *Enterobacteriaceae* and *E. coli* results were not detected for both bacterial species as the standard reference (Gilbert, 2000 [15]; Food Standards Australia New Zealand, 2001 [16] and Hocking, 2003 [17]). Results proved safe bacteriological pattern for all the different flavors for pasta additive.

Metal determination

For the most flavors accepted for Egyptian consumers through the last 10 years as shown in sensory evaluation study (not mention), chicken and beef flavors were examined. Sodium was ranged from 230-267 ppm, however, Costa *et al*. (2013) [18] found that the most abundant elements were K, S, Cl and Na (above 500 mg kg^{-1}) in raw fish. They proved that the dietary reference intake DRI values for adults as follows; Na (1500 mg/d) in macroelements and Cu (0.9 mg/d) for trace and ultra-trace elements. Sodium sources determination either came from the salt content or mono-sodium glutamate MSG level.

Values of estimated weekly intake of contaminants (Pb, Cd) were very limited in Table 2, and were below the limits established by the European community (EC, 2008) [19]. Lead (Pb) recorded high level in chicken by 32.5 ppb that is very dangerous concentration, while cadmium (Cd) and aluminum (Al) concentrations were below the limits established. On the other hand, copper (Cu) was 10 and 7.6 ppb for two flavors chicken and beef, respectively. Subramanian *et al*. (2012) [20] determined some elements in medicinal plants regularly used in cooking in Indian curries. They found average concentrations detected in ranged from 150.22 to 521.98 (Na); 6.94 to 49.76 (Cu); 0.478 to 9.890 (Pb); 11.51 to 94.05 (Cd) mg/kg, respectively. The different data in these experiments are a kind similar to our result showed in Table 2.

Flavor cf	APC (CFU/g)	*Enterobacteriaceae*	*Escherichia coli*
chicken	8	ND	ND
Cari chicken	12	ND	ND
beef	23	ND	ND
shrimp	15	ND	ND
vegetarian	11	ND	ND
Spicy chicken	9	ND	ND
Onion chicken	83	ND	ND

Table 1: Bacteriological results of different flavors.

Flavor cf	Sodium (Na) ppm	Aluminum (Al) ppm	Copper (Cu) ppb	Lead (Pb) ppb	Cadmium (Cd) ppb
chicken	230	6.39	10.032	32.512	0.09
beef	267	2.10	7.684	0.178	ND

ND = Not detectable; ppm = mg/L; and ppb = µg/L

Table 2: Metal contents in chicken and beef flavor additives.

Biological experiment

Different values have compared to infected meal with fast food flavors according to the normal chow rat meal. Liver function aminotransferase enzyme activities have dramatically increased by flavor mixed diet compared with the normal diet (Table 3). Values increased at the end of the biological test in the infected group rather than the increase in the negative control.

Our result is correlated with that of Ortiz et al. (2006) [21], who mentioned high levels of ALT and AST serum concentration with the hepatic damage. MSG cytotoxic effect induced tissue damage and enzyme release increasing serum levels. The reason might be due to the dissociation in sodium Na^+ and L-glutamate, which crosses the mesothelial peritoneal cells and arrives at the bloodstream. Results showed as well enhancing in liver enzymes especially with natural flavor.

Kidney function values for urea and creatinine in serum were observed after 4 weeks. Values were increased for the group fed on the diet mixed with commercial flavors (Table 4). As previously mentioned, the increase by the end of the biological experiment was much higher for infected group rather than that for negative control. As shown in hepatic enzymes, natural flavor decreased blood urea and creatinine values, and vitamin C and Se did the same.

Ortiz *et al.* (2006) explained that arriving L-glutamate in high concentrations through the renal artery, increase their excretion from the kidney. Absorption, filtration make MSG crossing the membrane and damaging the cell. The convoluted proximal tubules were more susceptible to damage in comparison to the distal convoluted tubules (Figure 2). That might show edema, hydropic degeneration, and necrosis patterns. Risk factor was shown increase the ratio of low-density lipoprotein to high-density lipoprotein for group fed on the diet mixed with synthetic flavors (Table 5). Clear results have shown a decrease in cholesterol and triglyceride for the infected diet group with fast food flavors.

By the end of the biological experiment, lipid peroxidation end product as marker of oxidative stress and the fucosidase activity as a biomarker of hepatic tumors are doubled approximately (data not shown). One of the logical reason information of reactive oxygen species ROS is the metal related oxidative stress that can lead to oxidative damage to lipids, DNA, and proteins. In addition, Ortiz et al. (2006) declared that MSG (4 mg/g BW) increased products of lipid peroxidation (MDA and 4-HAD).

Positive relationship has been found in our results correlated with other results (Espin *et al.*, 2014) [22]. TBARS or MDA concentrations related to the levels of Pb and Cd significantly (Tables 2, 6). Antioxidant defense responds differently depending on pollution levels, or contamination occurred in food flavors. On the other hand, analysis of supplement of beef and vegetable soup acquired in supermarkets (Krejcova et al., 2007) [23] found Na 1.50; K 12; Ca 0.32; Mg 0.13; P 1.1; Cd 0.16; Cr 0.21; Cu 0.32; Fe 0.30; Mn 0.17; Ni 0.42; Pb 2.1 and Zn 0.21 (mg kg^{-1}).

Histopathological examination

Histological patterns consider a big prove to pass or refuse the products after scientific experiments. Comparing to control rats, liver of rats fed on pasts flavor meal cf were showing sever dilatation and congestion (Figure 1) in the portal veins (pv) and hepatic sinusoids (s) associated with edema (o). Inflammatory cells infiltration (m) noticed in the portal area with diffuse Kupffer cells proliferation in between the hepatocytes (h). Ortiz *et al.* (2006) mention that MSG is toxic to liver and kidney, and attributed the high level of serum ALT and AST release for the hepatic damage. On the other hand, no histopathological changes have been proven with Se additives or natural flavors. While vitamin C showed focal hepatic necrosis with inflammatory cell infiltration.

Comparing to control rats, kidney of rats fed on flavor meal cf were showing focal haemorrhage (h) as well as sever congestion in the blood vessels (v) with perivascular oedema in between the tubules (Figure 2). Glomerular tuft (g) was detected at the cortex with perivascular oedema (o) (H&E × 40). Good protection appeared since normal histopathological pattern was showed with vitamin C and nf

	ALT	AST
control	5.6 ± 3.54	3.5 ± 0.77
cf	15.4 ± 1.77	8.2 ± 1.7
vC	10.2 ± 2.2	6.75 ± 2.07
Se	11.3 ± 1.4	7.3 ± 2.0
nf	7.5 ± 2.5	6.0 ± 1.8

The values are mean ± SD of 7 rats in each group.

Table 3: Effects of feeding fast food flavors on transfer enzymes activity (IU/dl) after four weeks.

	Creatinine	Urea
control	0.61 ± 0.01	40.7 ± 12.00
cf	1.14 ± 0.02	46.04±17.06
vC	0.98 ± 0.02	42.1 ± 8.1
Se	0.91 ± 0.01	42.8 ± 6.2
nf	0.64 ± 0.02	41.5 ± 6.8

The values are mean ± SD of 7 rats in each group.

Table 4: Effects of fast food flavors feeding on serum Creatinine and Urea (mg/dl) after four weeks.

	T.Chl	LDL	TG	vLDL	HDL
control	127.64 ± 18.3	39.02 ± 12.9	42.26 ± 7.4	8.45 ± 1.5	80.16 ± 17.5
cf	155	120.6	47	9.4	25
vC	173	120.8	71	14.2	38
Se	167	116.2	59	11.8	39
nf	115.38 ± 2.8	72.47 ± 14.4	36.84 ± 4.3	7.37 ± 0.9	36.83 ± 14.2

The values are mean ± SD of 7 rats in each group.

Table 5: Effects of fast food flavors on serum low density lipoprotein LDL, high density lipoprotein HDL, triglycerides TG, very low density lipoprotein TG/5, Total cholesterol Chl and Risk Factor RF (mg/dl).

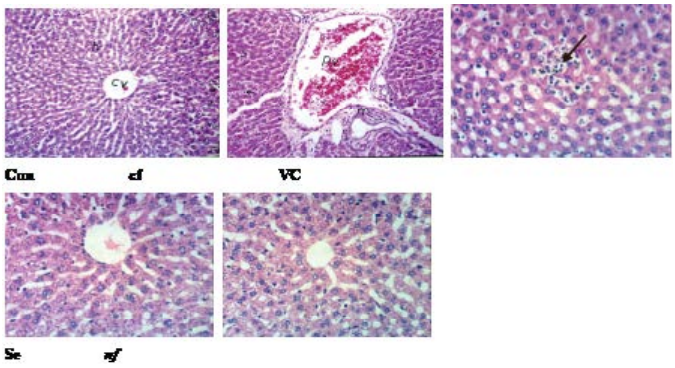

Figure 1: Liver histograms for a) normal rat group, b) rats fed on cf 10% flavor meal, c) vitamin C supplement, d) Se supplement and e) meal of 10% nf (H, E × 400).

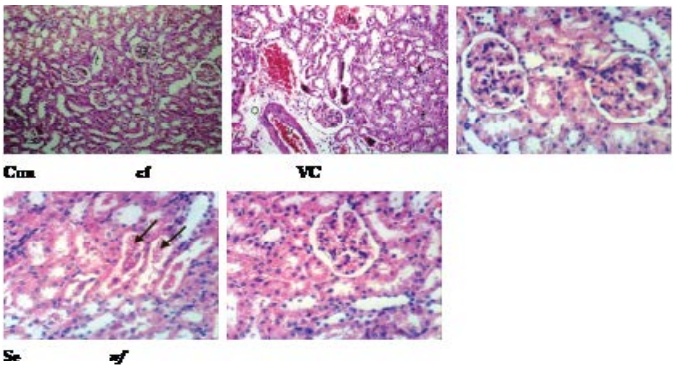

Figure 2: Kidney histograms for a) normal rat group, b) rats fed on 10% flavor meal, c) vitamin C supplement, d) Se supplement and e) meal of 10% nf (H, E × 400).

substitution. While, mixed diet with Se showed protein cast in the lumen of renal tubules.

Comparing to normal male rat group, brain of rat fed on 10% flavor cf in chow diet were showing congestion (Figure 3) in the meningeal blood vessels (v-m) as well as cerebral blood vessels (v-cr) (H&E × 40). Metal related oxidative stress or excess of radicals can cause oxidative damage to the membrane, and their oxidation may ultimately lead to cellular dysfunction and tissue injury (Valvanidis et al., 2006) [24]. Since SMG is an excitatory neurotransmitter in the central nervous system of mammals, our protective standard antioxidants; VC and Se showed no histopathological changes. While, *nf* showed neuronophagia of necrotic neurons in slight effects in comparison with cf.

Heart sections for rats fed on 10% fast food flavors cf have been examined and proved to have heart congestion (Figure 4) in the myocardial blood vessels (v). In the same time, protective groups of vitamin C, Se, and natural flavors showed normal cardiac myocytes in enhancement pattern.

While, spleen section showed sever congestion in the splenic blood vessels (v) and red pulps (Figure 5) associated with lymphoid hyperplasia in the white pulps (w). Normal lymphoid follicles in the white pulp have appeared in the protective vitamin C, Se supplements and substituted natural flavors.

Group of rat fed on 10% fast food flavor cf showed fibrous osteodystrophy in the peripheral portion of the shaft (f) as well as in the condyle of the head femur bone (Figure 6 a, b), While, the articular joint (ct) collagenous surface was intact (Figure 6c) with osteoporosis in the bone trabeculae (b).

Olvera-Cortes et al. (2005) [25] reported that neonatal exposure to MSG (4 mg/g BW) in rats and mice causes many effects like learning

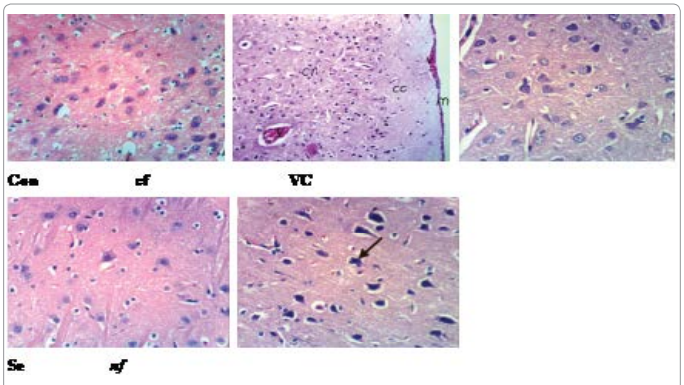

Figure 3: Brain histograms for a) normal rat group, b) rats fed on 10% flavor meal, c) vitamin C supplement, d) Se supplement and e) meal of 10% nf (H, E × 400).

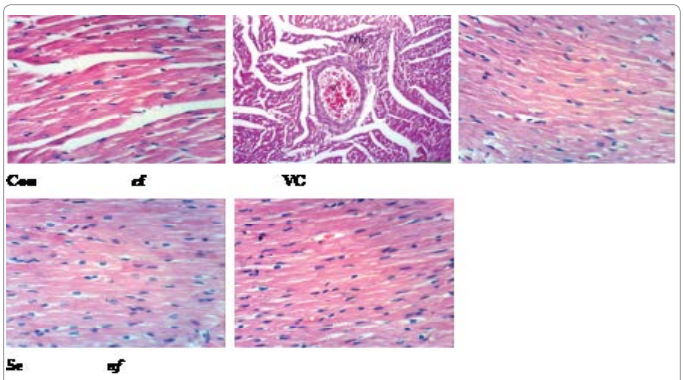

Figure 4: Heart histograms for a) normal rat group, b) rats fed on 10% flavor meal, c) vitamin C supplement, d) Se supplement and e) meal of 10% nf (H, E × 400).

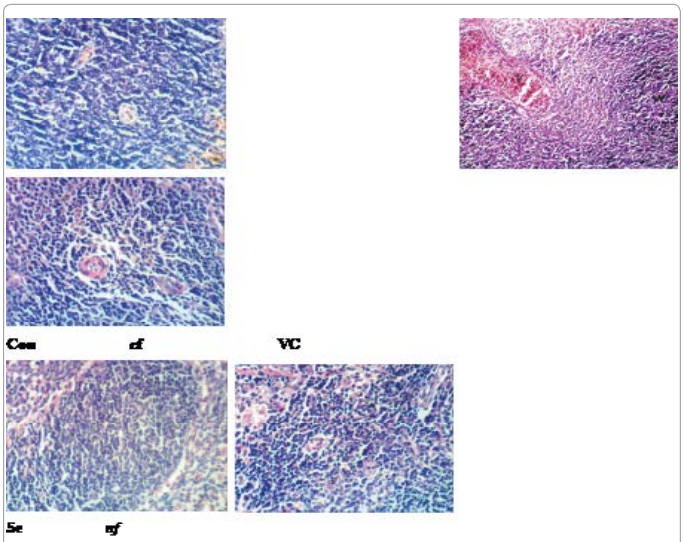

Figure 5: Spleen histograms for a) normal rat group, b) rats fed on 10% flavor meal, c) vitamin C supplement, d) Se supplement and e) meal of 10% nf (H, E × 400).

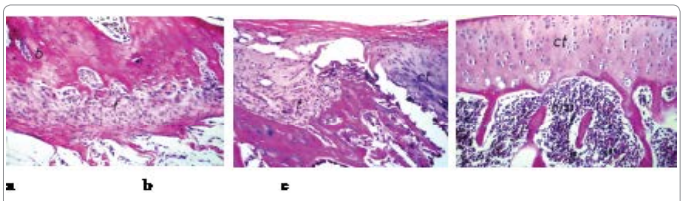

Figure 6: Showed bone femur of rat fed on flavor chow diet cf showing fibrous osteodystrophy (a, b and c) (H and E × 40).

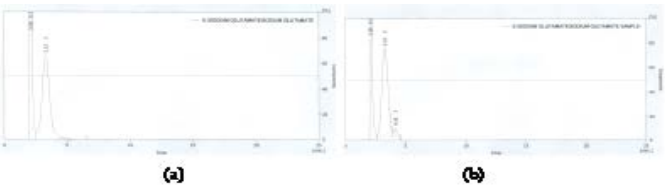

Figure 7: HPLC chromatogram for (a) MSG standard, and (b) flavor enhancer sample.

difficulty, obesity, and gonadal dysfunction. It has also been associated with oxidative stress in the hepatic tissue of young rats (Diniz et al., 2004) [26]. Brain damage induced by the neurotoxicity of MSG (Robinzon et al., 1974) [27], and some of the neurotransmitters like serotonin and dopamine in the hypothalamus region were found to be depleted in MSG treated rats (Nakagawa et al., 2000) [28].

MSG determination by HPLC

MSG is the sodium salt of glutamic amino acid eluted after 3.33 min. L-glutamic acid is one of the most amino acids found in nature. It is made commercially by the fermentation of molasses but exists in many products such as pre-cooked pastas and noodles. These products are heated, boiled, steamed, cooked, pre-gelatinized or frozen. The highest dose for food additives in these products, reached 50 g/kg (FAO/WHO Food Standards, 2013) [30]. Figure 7 showed the level of MSG in pre-cooked sample flavor reach 80 g/100 g. While, Institute of food technologists (1987) [31] found glutamate content around 10g / 100g milk products, 3.5g / 100g poultry products and 5.5g /100g vegetables. They also found that daily intake of MSG is vary between 0.37g/day in Malaysia and 1.57g/day in Korea.

Arriving L-glutamate at the blood stream by transport system using ATP and be divided into the cell conjugates to be eliminated, and part is transformed into glutamine. The cells try to repair some of the damages using endoplasmic enzymes but still not able to completely remove the excess glutamine. For this reason, the liver presented cloudy swelling with vesicular degeneration and necrosis (Walker and Lupien, 2000) [31].

Conclusion

In conclusion words, study mention the risk of miss-use of commercial junk flavors without making good investigations in bacteriology, mineral evaluation, main preservative or an enhancer content and study the biochemical and histopathological effects. Either the food process mineral contaminants or miss-use the right potent concentration from the preservative/ additive material could lead to many hazardous circumstances.

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