The relationships between some additives of snacks and biological parameters of rats

Hamdia, H.; Nehad, R. EL-Tahan and Sara, L. Youns
Department of Nutrition and Food Science, Faculty of Home Economics, Menoufia University, Egypt.

Abstract:
Flavor designates all the organoleptic properties that are indirectly perceptible by the olfactory organ when tasting. The term flavor denotes a complex set of olfactory and gustatory properties that are perceived when tasting and that can be influenced by tactile, thermal, painful, and even kinesthetic effects. Therefore, in the present work, study the effect some artificial (tomato and cheese) flavors and chips with the above flavors on the some biological parameters of rats. Thirty adult female albino rats, weighting (115±5g), rats were divided into five groups, first group fed on basal diet as control group. Group (2), rats fed on basal diet with 10% chips with tomato flavor. Group (3) which fed on basal diet with 10% of chips with cheese flavor. Group (4): Rats fed on basal diet with 1% chips with tomato flavor. Group (5): Rats fed on basal diet with 1% cheese flavor. Results showed that increase blood bio markers for liver functions and kidney functions that were increased by feeding on cheese chips and tomato flavors. Also feed with cheese chips and tomato flavor increased in the final body weight, daily feed intake which were diminish by cheese chips, tomato chips and tomato flavor increased WBCS and decrease RBCS.

Keywords: Body weight- cheese chips- tomato flavor- liver functions-kidney functions
Introduction

Due to the changing lifestyles (e.g. running lifestyle, spreading of ready-to-eat and conventional foods) domestic food production and preservation is continuously surpassed, and at the same time the importance of foodstuffs produced by the industry is marked up. Food industry has to fulfill multiple consumer demands. Food industry has to put various, convenient, attractive and affordable foodstuffs on the shelves, and at the same time the consumers’ needs for healthy, chemical free and safe products have to be satisfied. The food additives are one of the solutions to meet consumers’ complex and often conflicting requirements. These additives influence the attributes of the foodstuffs favorably, facilitate the processing of the raw materials, improve the quality of food products and prolong their shelf life (Sohárné, 2005).

According to the Food and Drug Administration (FDA), there are more than 3000 food additives allowed in the United States, which are distributed into 6 groups: Preservatives, nutritional additives, coloring agents, flavoring agents, texturizing agents, and miscellaneous agents. In order to approve new additives or extend the usage of an approved one within the EU, a series of procedures has to be carried out, divided into 4 parts (Carocho et al., 2014).

Food additives are natural or synthetic chemicals added to food to preserve flavor, enhance its texture or appearance, or for other technological functions. Food additives are among the safest chemicals in food due to their low toxicity, rigorous safety testing, and control of use by the law. The permission to use specific food additives is recommended by the Codex Alimentarius Commission and approved by national legislation. The use of food additives is subject to strict controls, underpinned by scientific studies to demonstrate their safety to human health. Their use brings many benefits including increased safety, and greater choice of food products. However, although consumers were aware of the benefits additives could deliver, the automatic assumption that additives were ‘bad’ remained and consumers felt that additives should be reduced in foods (Binnur and Serap 2015).

According to the UNE 87-001-86, standard flavor designates all the organoleptic properties that are indirectly perceptible by the olfactory organ when tasting. The term flavor denotes a complex set of olfactory and gustatory properties that are perceived when tasting and that can be influenced by tactile, thermal, painful, and even kinesthetic effects. The
British Standards Institution defines flavor as the combination of taste and odor that may be influenced by painful, heat-cold and tactile sensations. The aroma and all the sensory characteristics of food represent only a fraction of the phenomena recognized by the individual when the food is consumed (Briz and Garcia 2004).

Cheese flavor is a very complex phenomenon. Every cheese has a unique flavor and a number of specific compounds of varying concentrations and of different chemical classes constitute the flavor of each cheese type. The pursuit of a single unique compound that contributes to a specific cheese flavor in isolation has proven futile. The Component Balance Theory that posits the role of multiple flavor compounds in specific concentrations so as to achieve a delicate but acceptable balance of flavor perception remains the dominant theory that guides current research. This approach has initiated broad surveys that have resulted in development of extensive lists of potential compounds and analytical techniques to define the components of various physicochemical fractions in cheeses. Chemical surveys using chromatographic separation and mass spectrometry have yielded large libraries of compounds without causal connections to their production by bacteria (Manning, 1979 and Urbach, 1995).

Tomato is the highest value fruit crop in the world and a major component of healthy diets as it provides ready sources of vitamins A, C, E and K, minerals including K and Fe and lycopene as an antioxidant. However, modern varieties are often described as having little flavor, especially in comparison to traditional or heirloom types. One of the major challenges is to breed fruit that have a long shelf life fit for the modern supply chain, but maintain excellent eating quality. The route taken by many breeders has been to introduce non-ripening mutations, such as ripening inhibitor (rin), into elite backgrounds. Hybrids containing rin produce firmer fruits that ripen slowly, but they often have poor flavor, fail to develop full color and have reduced nutritional value. Another reason for poor flavor is that the modern cultivated tomato has been selected for yield, disease resistance and size. Breeders will select for sugars and acids as these are known to be important, but there is a negative correlation between fruit weight and sugar content. Tomato flavor results from not only a complex mix of taste metabolites, but also the perception of volatile compounds (Kitagawa et al., 2005 and Tieman et al., 2017).
For that, this research aimed to investigate the effect of some flavor and flavor products on some biological parameters of rats.

MATERIALS AND METHODS

Cheese and tomato flavors were obtained from Gomhoria Co., Giza and spiced potato with cheese and tomato flavor obtained from local market from Menoufia Governorate. Thirty adult female albino rats, weighting (115±5g), were obtained from Institute of Ophthalmology, Medical Analysis Dep., Giza, Egypt. Rats were housed in wire cages under the normal laboratory condition and were fed on standard diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid looser conditions of food, water was provided to the rats by glass tubes supported to one side of the cage, food and water provided ad-labium and checked daily.

The basal diet consisted of casein (10%), cellulose (5%) salt mixture (4%), vitamin mixture (1%), corn oil (10%) and corn starch (70%) according to Reeves et al. (1993).

Experimental design

The experimental was done in the Faculty of Home Economics, Menoufia University, Shebin EL-Kom. Rats were housed in wire cages in a room temperature 25 °C and kept under normal healthy conditions for 7 consecutive days. After this adaptation period, rats are divided into 5 groups, each group which consists of 6 rats as follows:

- **Group (1):** Rats fed on basal diet as control group.
- **Group (2):** Rats fed on basal diet with 10% snacks 1 (chips with tomato flavor).
- **Group (3):** Rats fed on basal diet with 10% of snacks 2 (chips with cheese flavor).
- **Group (4):** Rats fed on basal diet with 1% of snacks 3 chips with tomato flavor.
- **Group (5):** Rats fed on basal diet with 1% of snacks 4 chips with cheese flavor.

During the experimental period (28 days), the diet consumed was recorded every day and body weight was recorded every week. The body weight gain (BWG), feed efficiency ratio (FER), and relative organ weight were determined according to Chapman et al., 1959. Using the following equations:
BWG = Final weight – Initial weight

FER = \frac{\text{Gain in body weight (g)}}{\text{Food consumed (g)}}

Blood sampling: After fasting for 12 hours, blood samples in initial times were obtained from retro orbital vein, while it obtained from hepatic portal vein at the end of each experiment. Blood samples were collected into a dry clean centrifuge glass tubes and left to clot in water bath (37°C) for 28 minutes, then centrifuged for 10 minutes at 4000 rpm to separate the serum, which were carefully aspirated and transferred into clean cuvette tube and stored frozen at -20°C till analysis according to the method described by Drury and Wallington (1980).

Serum total cholesterol was determined according to the colorimetric method described by Allen (1974). Serum triglycerides was determined by enzymatic method using kits according to Fossati and Prencipe (1982). HDL-c was determined according to the method described by Lopez (1977) while VLDL-c was calculated in mg/dl according to Lee and Nieman (1996) using the following formula:

VLDL-c (mg/dl) = Triglycerides / 5

LDL-c = total cholesterol - (VLDL-c + HDL-c)

Serum glucose was measured using the modified kinetic method according to Kaplan (1984).

Histological investigation:

Small Specimens from liver were collected from all experimental groups, fixed in 10% neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%) cleared in xylene and embedded in paraffin. Sections of (4-6) µm thickness were prepared and stained with Hematoxylin and Eosin according to Bancroft et al. (1996).

Statistical analysis:

The data were analyzed using a completely randomized factorial design (SAS, 1985) when a significant main effect was detected; the means were separated with the Student-Newman-Keuls Test. Differences between treatments of (P≤0.05) were considered significant using Costat Program. Biological results were analyzed by One Way ANOVA.
RESULTS AND DISSECTION

Table (1) showed the effect of flavor snacks on FI, FER, and BWG.

Data in table (1) indicated that the mean highest value of feed intake in the control group was 14.321 g/day, while the lower mean value of group that fed on basal diet containing cheese chips was 12.213 g/day.

The mean value of FER for control was 0.191 ± 0.011, while it was 0.101 ± 0.01 for group fed on cheese chips. Results in this table showed decreasing in FER of all groups as compared to control group. There is no significant between all groups and control group.

The result of BWG in rats fed on basal diet tested material under the current investigation were shown and summarized in table (1).

BWG of the group fed on diet containing flavor with cheese chips showed the highest BWG as compared to the other groups. There were significant changes between the group fed on basal diet containing 1% tomato flavor and the other groups. There is no significant differences among G3, G4 and the control group.

The observed effect of on feed intake and body weight (Table 1) in this study was agreed with that reported by (Eddoakset al., 2005) that the; like any fat, is rich in calories. However, most people eat to satiety. In our Early Arthritis Clinic, a cohort of 33 RA patients taking at the rate of 15 ml/day immediately before or during a meal did not increase their mean weight over 1 year; there was a nonsignificant mean change of -0.4 kg from baseline to 1 year. Metabolic studies suggest the LC n3 PUFAs present in can reduce adipocyte numbers and the contribution of adipose tissue to body mass.

Table (1): Effect of basal diet containing flavor supplemented on Feed intake (FI), Feed efficiency ratio (FER) and Body weight gain (BWG):

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Mean of feed intake (g/day)</th>
<th>FER Mean±SD</th>
<th>BWG (g/28 days) Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G1) Control group</td>
<td>14.321 ± 1.05 *</td>
<td>0.191 ± 0.011 *</td>
<td>42.27 ± 1.2 *</td>
</tr>
<tr>
<td>(G2) Cheese chips 10%</td>
<td>12.213 ± 0.91 *</td>
<td>0.101 ± 0.01 *</td>
<td>47.86 ± 1.34 *</td>
</tr>
<tr>
<td>(G3) Tomato chips 10%</td>
<td>12.67 ± 1.02 *</td>
<td>0.091 ± 0.07 *</td>
<td>41.91 ± 2.02 *</td>
</tr>
<tr>
<td>(G4) Cheese flavor 1% (G4)</td>
<td>13.14 ± 1.11 *</td>
<td>0.089 ± 0.08 *</td>
<td>41.1 ± 3.14 *</td>
</tr>
<tr>
<td>(G4) Tomato flavor 1% (G4)</td>
<td>12.642 ± 2.11 *</td>
<td>0.191 ± 0.12 *</td>
<td>38.2 ± 1.31 *</td>
</tr>
</tbody>
</table>

Means in the same column with different litters are significantly different (P ≤0.05).
Effect of basal diet containing flavor supplemented on some organs weight

Table (2) represents the effect of feeding of basal diet contained tested material on liver, heart, kidney and spleen weight.

The control’s liver weight was 3.15 ± 0.17, there is no significant difference between group fed on basal diet containing 10% chesse chips and group fed on basal diet containing tomato flavor. Also, there is no significant changes between G3 and G4. Whereas, there were significant differences between group fed on control diet and the other groups which was the lowest one.

In case of heart weight, there is no significant difference between group fed on basal diet containing tested materials. While, control group showed significant differences with the tested groups. For kidney and spleen weights, it could be noticed that there is no significant difference between tested groups and control group.

Table (2): Effect of basal diet containing flavor supplemented on some organs weight.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Parameters</th>
<th>Liver Mean ±SD</th>
<th>Heart Mean ±SD</th>
<th>Kidney Mean ±SD</th>
<th>Spleen Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G1) Control group</td>
<td></td>
<td>3.11 ± 0.17</td>
<td>0.30 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>(G2) Chesses chips10%</td>
<td></td>
<td>4.58 ± 0.13</td>
<td>0.37 ± 0.03</td>
<td>0.60 ± 0.09</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>(G3) Tomato chips10%</td>
<td></td>
<td>3.97 ± 0.14</td>
<td>0.35 ± 0.04</td>
<td>0.59 ± 0.2</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>(G4) Chesses flavor1%</td>
<td></td>
<td>3.92 ± 0.19</td>
<td>0.33 ± 0.05</td>
<td>0.58 ± 0.03</td>
<td>0.19 ± 0.09</td>
</tr>
<tr>
<td>(G5) Tomato flavor1%</td>
<td></td>
<td>4.26 ± 0.04</td>
<td>0.36 ± 0.02</td>
<td>0.59 ± 0.02</td>
<td>0.20 ± 0.01</td>
</tr>
</tbody>
</table>

Means in the same column with different litters are significantly different (P ≤0.05).

Effect of basal diet containing flavor supplemented on lipid profile.

Table (3) illustrated the effect tested materials on the serum total cholesterol, triglycerides, HDL and LDL levels of rats.

Data in table (3) showed that, total cholesterol and triglycerides levels (mg/dl) increased significant (P≤0.05) for rats fed on diet contained chesse chips as compared to the other groups.

Total cholesterol and triglycerides decreased significantly (P≤0.05) when rats fed on basal diet as control group. The statistical analysis showed a significant changes in total cholesterol between G3,G4 and control group.
For HDL-c, the group (2) was the lowest one, there is no significant difference between groups 3, 4 and control group. Groups 2 and 5 were significant with the other groups.

In case of LDL-c, group (2) was the highest group while group (3) was the lowest group. there is no significant difference between groups 3, 4 and control group.

In accordance with the present results, Wafeka, (2010) demonstrated that low-dose diets improve lipid metabolism by modifying the expression of lipid metabolism-related genes in the liver and increasing fecal cholesterol excretion, while combining fatty acids in flavor is the main cause to increase plasma cholesterol and triacylglycerol concentration in women. However, fatty acids decreased the oxidative stress and the pleiotropic effect of statins seemed to be not enough to counterbalance this result. Our data also suggested that the mechanism by which fatty acids interfere in oxidative stress can be associated with antioxidant enzymes expression and activity.

Wafeka, (2010) reported that flavor as cheese increased bad lipoprotein to increase fatty acids which increased total cholesterol.

**Table (3): Effect of basal diet containing flavor supplemented on lipid profile.**

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>Lipid Fraction</th>
<th>Lipid Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total cholesterol Mean ± SD</td>
<td>Triglyceride Mean ± SD</td>
</tr>
<tr>
<td>(G1) Control group</td>
<td>82.29 ± 6.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.4 ± 2.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G2) Chesse chips10%</td>
<td>189.78 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>139.4 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G3) Tomatochips10%</td>
<td>85.3 ± 4.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.3 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G4) Chesse flavor1%</td>
<td>83.04 ± 5.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.3 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>(G5) Tomato flavor1%</td>
<td>90.3 ± 1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.7 ± 3.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in the same column with different litters are significantly different (P ≤0.05).

**Effect of basal diet containing flavor supplemented on blood glucose.**

Data presented in table (5) show the effect of feeding substitutes on blood glucose of rats.

It could be observed that, the mean value ±SD of glucose of group (2) significantly increased, as compared to normal rats, it was being 140.14±0.02 and 80.05±2.11 mg/dl, respectively. There were a significant increase in the glucose levels in the other groups as compared to normal group. There is no significant difference between groups 3, 4.
Table (4): The effect of basal diet containing flavor supplemented on blood glucose.

<table>
<thead>
<tr>
<th>Animal Groups</th>
<th>GlucoseMean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(G1) Control group</td>
<td>80.05±2.11</td>
</tr>
<tr>
<td>(G2) Chesses chips 10%</td>
<td>140.14±0.02</td>
</tr>
<tr>
<td>(G3) Tomato chips 10%</td>
<td>100.13±0.21</td>
</tr>
<tr>
<td>(G4) Chesses flavor 1%</td>
<td>99.11±0.20</td>
</tr>
<tr>
<td>(G5) Tomato flavor 1%</td>
<td>125.79±0.72</td>
</tr>
</tbody>
</table>

Means in the same column with different litters are significantly different (P ≤0.05).

**Histopathological examination of Liver:**

Microscopically, liver of rats from group 1 revealed the normal histological structure of hepatic lobule (photo 1). On the other hand, liver of rats from group 2 revealed Kupffer cells activation and focal hepatocellular necrosis associated with mononuclear inflammatory cells infiltration (photo 2). Meanwhile, liver from group 3 showed vacuolar degeneration of hepatocytes (photo 3). Moreover, liver from group 4 revealed steatosis of hepatocytes (photo 4). Examined sections from group 5 showed congestion of central vein and slight vacuolation of some hepatocytes (photo 5).

Photo (1): Liver of rat from group 1 showing the normal histological structure of hepatic lobule (H & E X 400).
Photo (2): Liver of rat from group 2 showing Kupffer cells activation (H & E X 400).

Photo (3): Liver of rat from group 3 showing vacuolar degeneration of hepatocytes (H & E X 400).
Photo (4): Liver of rat from group 4 showing steatosis of hepatocytes (H & E X 400).

Photo (5): Liver of rat from group 5 showing congestion of central vein (H & E X 400).
References


دراسات بيولوجية على العلاقة بين بعض المواد المضافة للأطعمة الخفيفة والسعرات المأخوذة من الطعام المتناول في الفنكر

أ.د/ حميدة أحمد هلال، أ.د/ نهاد رشاد الطحان، سارة لوي عبد العليم عبد الملك يونس

أماة التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة المنوفية
باحثة ماجستير قسم التغذية وعلوم الأطعمة - كلية الاقتصاد المنزلي - جامعة المنوفية

المستخلص العربي:
تهدف هذه إلى دراسة تأثير النكهة الصناعية مثل نكهة الطماطم والجبنة تم تقسيم ثلاثين من الفنكر ألبان البالغة، وزنها (115 ± 5 جم)، تم تقسيم الفنكر إلى خمس مجموعات تتغذى على المجموعة 1 (الفنكر التي تتغذى على النظام الغذائي الأساسي 
كمجموعة تحكم. المجموعة 2 (الفنكر التي تتغذى على النظام الغذائي الأساسي مع منتج 10 ٪ (الرقائق بنكهة الطماطم). المجموعة 3 (الفنكر التي تتغذى على النظام الغذائي الأساسي بنسبة 10٪ من المنتج 2 (الرقائق بنكهة الجبن). المجموعة 4 (الفنكر التي تتغذى على النظام الغذائي الأساسي مع 1٪ من الرقائق بنكهة الطماطم. المجموعة 5 (الفنكر التي تتغذى على نظام غذائي أساسي برقائق 1٪ بنكهة الجبن. وتبين النتائج زيادة علامات الدم الحيوية لوظائف الكبد، ووظائف الكلي (البوري)، التي تمت ارتفاع مؤشراتها بواسطة رقائق الجبن و الطماطم. زادت نكهة الطماطم في الوزن النهائي للجسم وتناول الطعام اليومي الذي تناقصت بواسطة رقائق الجبن والطماطم وكذلك نكهة الطماطم والجبنة.

الكلمات المفتاحية: وزن الجسم - رقائق الجبن - نكهة الطماطم - وظائف الكبد - وظائف الكلي