

Histamine in Tuna Sandwiches and Hygienic Status of Some School Food Vendor Premises in Tripoli city, Libya.

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Abstract: Tuna sandwiches are prepared either in school's premises or at home and sold to pupils/students during break at schools in Tripoli city- Libya. These sandwiches are nutritious meal for pupils/students however, they are also recognized a major public health risk if they are not prepared and served under good hygienic and sanitary conditions. The present study was conducted to investigate levels of histamine in random samples of tuna sandwiches collected from school food vendor premises and assess the hygienic and sanitary status of these premises as well as the sandwich handlers. Histamine in tuna sandwiches ranged 0.052 – 0.485 mg%. The results were below the maximum allowed level established in Libyan standard 10 mg% and Food and Drug Administration 5 mg%. Despite the low histamine contents obtained in the tuna sandwiches samples. However, inspection results of the school's food vendor premises and sandwich handlers showed weak points that need to be rectified which are: sandwiches must be kept in a proper clean container and held under refrigeration until time of serving to pupils/students. The sandwich handlers must have a valid health certificate before allowed to work in such premises and wear gloves, hair cover and appropriate custom for work.

Keywords: Tuna sandwiches, histamine, hygiene status, sandwich handlers, vendor.

1. Introduction:

Canned tuna fish are consumed widely in Libya in form of tuna sandwiches in homes and schools. According to 2010 statistics, annual consumption of canned tuna in Libya is estimated at 31,680 tons (Anonymous, 2014). Fish and fish products have received the greatest attention in terms of biogenic amines content. Such fishes contain red muscles which are rich in free histidine varying from 1g/kg in herring to as much as 15g/kg in tuna that may be converted to histamine by the decarboxylase process if exposed to poor handling conditions (Shalaby, 1996). Scombroid poisoning results from consuming fish or fish products containing large quantities of histamine, it is usually a mild illness with variety of symptoms including headache, giddiness, rash, stomachache, swallowing difficulties, low blood pressure, nausea, vomiting and diarrhea, flushing, tingling and itching of the skin (Lehane and Olley, 2000; Valiollah et al., 2012).

Levels of histamine can rise in canned tuna meat after opening cans as a result of abuse handling and storage. Anonymous (2005) reported higher levels of histamine sufficient to incidence histamine poisoning after keeping opened canned tuna meat at room temperature for 24 hours or at 33°C for 6-8 hours, he concluded that tuna sandwiches and canned tuna meat after opening should be stored at 4°C or less and consumed as soon as possible.

Tuna sandwiches are sold to pupils and students during break at schools in Tripoli city, Libya. These sandwiches are prepared either in school's premises or at home and brought to schools for sale. Tuna sandwiches provide a source of inexpensive, convenient and nutritious meal for pupils and students at break time, in contrast to the potential benefits, it is also recognized to be a major public health risk if they are not prepared, stored, and offered under good hygienic and sanitary conditions.

The codex adopted revised basic texts on food hygiene and recommended their wide use and understanding by governments, regulatory authorities, food industries, all food handlers and consumers to ensure that food is safe and suitable for human consumption. The general hygienic requirements and practices to be followed by the vendors were also recommended for translation by the relevant authorities into code of practice by fully taking into account local conditions including specific factors that are relevant to each operation (Codex, 2009).

The main objectives of this study were: 1) to determine level of histamine in tuna sandwiches prepared and sold in vendor premises in a random sample of schools in Tripoli city, 2) and assess hygienic and sanitary status of these vendor premises as well as handlers of these sandwiches.

2. Materials and Methods

2.1- Study Plan

The study was conducted between march and April / 2016 on 19 tuna sandwiches vendor premises located in primary and preparatory schools within Tripoli city border. These schools were selected randomly and grouped according to geographic border of 4 municipals which are Suk Alguma, Tripoli center, Abusleem, and Hai Al Andulas.

The selected schools were visited randomly and without previous arrangement. The food premises within each school and the food handlers working in them were evaluated in terms of hygienic practices and sanitary status by using inspection check list form prepared for this purpose (Fig. 1).

Tuna sandwiches samples were collected at the time of premises inspection, kept in polyethylene bags and transferred to the laboratory in ice box. Upon arrived they were kept in deep freezer until time of analysis for histamine content.

All visits were done within hour before the break and the sandwiches samples were withdrawn at break time during selling to pupils and students.

2.2- Histamine Analysis

Histamine in tuna meat was analyzed by enzyme – linked immunoassay (ELISA) method, using RIDASCREEN kits R-BiopharmAG Darmstadt (Germany) according to manufacture procedure (Anonymous, 2012).

2.2.1- Sample Preparation:

Tuna meat of each sandwiches were scraped by sharp knife after thawing for 3 hrs. at 4°C. 10 grams of tuna meat were homogenized in a warning blender, 9 ml of distilled water were added to 1 gram of tuna meat homogenate and were mixed well. The mixture was centrifuged for 5 min./ 2500g at room temperature. Aliquante from the supernatant were used for histamine analysis.

2.2.2- Histamine Determination:

One ml supernatant was mixed with 9 ml of distilled water and 200 µl of this solution were diluted with 9.8 ml of distilled water and subsample of 100 µl was derivatized quantitatively by an acylation reagent into N-acylhistamine. After dilution and derivatization, samples and standards containing derivatized histamine and antibody directed against histamine were placed into the wells of the surface of a microtiter plate. Immobilized and free histamine compete in the antibody binding sites. After incubation, washing and removing of unbound material, a peroxidase conjugate directed against the histamine antibody was added into the wells. After the second incubation, the plate were washed again and substrate solution was added and then incubated, which resulted in the development of blue color, then stop solution was added which lead to a color change from blue to yellow.

The intensity of the yellow color was measured photometrically at 450 nm. The absorption obtained is inversely proportional to the histamine concentration in the sample. Histamine standard and blank were run with sample analysis.

3. Statistical Analysis:

The results from the study were analyzed with the statistical package Minitab 16 using descriptive statistics such as minimum and maximum value, means, and confidence interval. One-way analysis of variance (ANOVA) and correlation coefficient between histamine content and some of inspection parameters of food vendor premises were performed. All significant levels were considered at the level of $p < 0.05$.

Name of school:		Municipal:	
I) Premises			
a) Cleanliness of sandwich preparation area:		b) Adequate light:	
<input type="checkbox"/> Clean.	<input type="checkbox"/> Not clean.	<input type="checkbox"/> Exist.	<input type="checkbox"/> Not exist.
c) Availability of clean water and sink:		d) Refrigerator:	
<input type="checkbox"/> Available.	<input type="checkbox"/> Not available.	<input type="checkbox"/> Available.	<input type="checkbox"/> Not available.
e) Serve wrapped sandwiches:			
<input type="checkbox"/> Exist.		<input type="checkbox"/> Not exist.	
II) Sandwiches Handlers.			
a) Holding valid health certificate:		b) Clean general appearance:	
<input type="checkbox"/> Exist.	<input type="checkbox"/> Not exist.	<input type="checkbox"/> Clean.	<input type="checkbox"/> Not clean.
c) Wear working uniform:		d) Wear gloves:	
<input type="checkbox"/> Wearing.	<input type="checkbox"/> Not wearing.	<input type="checkbox"/> Wearing.	<input type="checkbox"/> Not wearing.
e) Hair Cover:		f) Trimming Nail:	
<input type="checkbox"/> Wearing.	<input type="checkbox"/> Not wearing.	<input type="checkbox"/> Clipped.	<input type="checkbox"/> Not clipped.
g) Free from wounds and lesion:			
<input type="checkbox"/> Free.		<input type="checkbox"/> Not free.	

Figure (1): Inspection check list form for school vendor premises.

4. Results and discussion:

The results of histamine content in tuna meat scraped from sandwiches sample sold at schools are displayed in table 1. All tuna meat samples contained histamine and ranged 0.052-0.485 mg%. The results are below the maximum limit established by Libyan standard 10 mg% (LNCSM, 2006), and 5mg% allowable limit suggested by the (FDA, 2001). According to FDA (2010), canned tuna products with histamine level < 10 mg% is considered to be of good quality, the level 30 mg% indicate significant deterioration, whereas level of 50 mg% represent, conclusive evidence of deterioration.

Statistical analysis at $p = 0.05$ did not Show a significant difference between histamine content in tuna sandwiches served at schools among the 4 municipal $p = 0.284$.

Table 1: Levels of histamine in samples of tuna meat sandwiches collected from school food vendor premises.

Municipal	No. of school visited	Histamine values (mg%)			Confidence Interval
		Min	Max	Mean	
Suk Alguma	5	0.074	0.485	0.360	±0.088
Tripoli center	5	0.250	0.481	0.344	±0.056
Abusleem	7	0.052	0.401	0.269	±0.070
Hai Al-Andulas	2	0.250	0.340	0.301	±0.052

The low level of histamine obtained in this study could be resulted from the low levels of histamine in commercial canned tuna used in these sandwiches and/or to the short period of time between preparation and selling which is not more than 2 hours especially in schools where sandwiches were prepared at school premises before break.

The range of histamine content in tuna sandwiches found in this study were less than the range reported by Kung et al. (2009) in 43 tuna sandwiches samples obtained from store chain in Taiwan in which the range of histamine content were non detected to 5.2 mg%. Anonymous (2005) studied levels of histamine in 20 tuna sandwiches samples which were sampled from different major retail outlets and restaurants in the month of May 2005 in Hong Kong. The results showed that histamine levels in tuna sandwiches samples were below the maximum level in codex standard. However, further experiment showed that high levels of histamine sufficient to cause histamine poisoning were detected in the tuna fish samples that were kept at room temperature for 24 hours, or at 33°C for 6-8 hours. The study concluded that the public and the trade are therefore advised to observe personal and food hygiene to prevent cross contamination. Tuna sandwiches and opened canned tuna should be stored at 4°C or below and be consumed as soon as possible. The trade is also advised not to store/ display the tuna sandwiches at room temperature for more than 2 hours.

On spot inspection showed that 87% of food premises in schools sampled in this study had clean area for preparing sandwiches, while 52% of these premises had enough light, 96% had clean water and sink for washing hands and utensils, 74% had refrigerator and 65% wrap sandwiches before display and sale (Table 2).

Table (2) % of food vendor premises in schools within each municipal complying to hygienic and sanitary parameters.

Municipal Parameter	% Complying				% of total
	Suk Alguma	Tripoli center	Abusleem	Hai Al Andulas	
I) Premises					
a) Cleanliness of sandwich preparation area	86	83	100	75	87
b) Adequate light	43	33	83	50	52
c) Availability of clean water and sink	100	100	83	100	96
d) Refrigerator	86	50	67	100	74
e) Serve wrapped sandwiches	43	67	67	75	65
II) Sandwiches Handlers					
a) Holding valid health certificate	57	17	33	75	43
b) Clean general appearance	100	100	100	100	100
c) Wear working uniform	29	33	0	0	17
d) Wear gloves	14	67	17	0	26
e) Hair Cover	43	83	33	50	52
f) Trimming Nail	86	33	100	100	78
g) Free from wounds and lesion	100	100	83	100	96

These results indicated that all schools visited had a weak points that need to be corrected in order to satisfy the basic hygienic requirements or standard for food vending premises. The weak points that need to be given a priority for correction are the way the sandwiches are displayed and served, because most of the premises keep and display the sandwiches in carton boxes that were used for handling eggs or banana. Such conditions and practices are likely lead to cross contamination of sandwiches with pathogenic bacteria that represent risk to human health. Also serving sandwiches without wrapping is another weak points that

should be aware of it in order to avoid contamination of these sandwiches. The percent of the food premises having weak light intensity were within the municipal jurisdiction border of Tripoli center, Suk Algumaand and Hai Al Andulas respectively in ascending order (Table2).

In Bangkok, Thailand, it was found that contamination of food handler's hands and utensils ranged from 18 to 69% depending on the availability of adequate and safe water supply (Dawson et al., 1996). In our study, all schools sandwiches vending premises had safe water supply except the schools located within the municipal jurisdiction council area of Abusleem where only 8% of schools had a safe water supply.

Medical examination of food handlers as per FAO and WHO (Anonymous, 1997), is necessary to ensure that peoples with communicable diseases are excluded from food handling. Results from the study showed that the % of school that hire or assign teacher as a volunteer to work in these food premises holding valid health certificate ranged 7 to 33%. The 7% were reported in schools within Tripoli center municipal and 75% were within Hai Al Andulasmunicipal. The overall % of schools that had food handlers with valid health certificate did not exceed 45% (Table2). According to (Ferron et al. 2000 and Muinde and Kuria,2005), the hands of food vendors are usually the most critical means of transmitting pathogens from contaminated places and items and hence could result in cross contamination upon contact with food. Particularly, in the case where vendors use the same hands to handle money from consumers, as this can further aggravate the situation due to possible accumulation of dirt on the money.

In addition 78% of the school visited had sandwich handlers with clean, short and well trimmed finger nails, 52% of schools had sandwich handlers with hair restraints in the form of head scarf, while 26% of the schools inspected had sandwiches handlers wearing gloves during preparing and handling sandwiches (Table2). Rane (2011), reported that Salmonella, non-typhi salmonella, Campylobacter and *E. coli* can survive on finger tips and other surfaces for different periods of time and some instances even after hand washing. It therefore behooves on food vendors always to keep their finger nails short and clean to prevent them from serving as a vehicle for transmission of pathogens (Lues et al., 2006).

The results concerning sandwich handlers with hair restraints reported in this study were corroboration with those reported by Muinde and Kuria (2005) and Musa and Akande (2003) and Abdalla et al. (2008) who reported a relatively low level of hair protection by food vendors. The world health organization (WHO) has however asserted that as a practice, the use of aprons and hair restraints by food vendors has more to do with food aesthetics and stimulating consumers assurance than food safety (Rane, 2011).

Statistical analysis showed a correlation ($r = -0.66$) between histamine content in tuna sandwiches and the % of schools had clean area for preparing the tunas sandwiches and the % of schools display wrapped sandwiches ($r = -0.45$). These statistical findings confirm the importance of applying good hygiene practices in such premises. Maintain high food safety levels in school food services is very important because any incidence can affect a high number of students (Monney et al., 2013).

5. Conclusion:

Despite the results of low levels of histamine in tuna sandwiches reported in this study, however the results of food vendor premises inspection found a weak points that need to be rectified which are: sandwiches should be kept and displayed in appropriate container instead of used cartons boxes and held under refrigeration temperature until time of serving to students. The sandwich handlers should have a valid health certificate before allowed to work in these premises, they should wear appropriate custom for work, gloves and hair cover.

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Conflict of Interest:

The authors declare no conflict of Interest.

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ملخص

تحظي سندوتشات التونة المحضرة والمباعة ببعض مدارس مدينة طرابلس ليبيا بإقبال كبير من التلاميذ والطلاب. بالرغم من القيمة الغذائية لهذه السندوتشات إلا إنها يمكن أن تشكل خطر على صحة التلاميذ والطلاب لو أعدت وقدمت تحت ظروف غير صحية. عليه فقد صممت هذه الدراسة للتعرف على مستوي الهيستامين في عينات من سندوتشات التونة التي جمعت من بعض مقاصف مدارس مدينة طرابلس – ليبيا، وتقييم الظروف الصحية لتلك المقاصف والقائمين علي إعداد السندوتشات بها من خلال نموذج اعد لهذا الغرض. احتوت كل عينات السندوتشات على الهيستامين وبمدى 0.052 – 0.485 مجم %. وأن مستوي الهيستامين في العينات لم يتجاوز الحد المسموح به والمعتمد من قبل السلطات الليبية 10 مجم % وهيئة الغذاء والدواء الأمريكية 5 مجم %. إلا انه تبين من نتائج الفحص الميداني فيما يخص الشؤون الصحية الواجب مراعاتها عند إعداد هذه السندوتشات بالمقاصف قيد الدراسة وجود نقاط ضعف يتطلب معالجتها وتصحيحها لضمان تقديم سندوتشات آمنة للتلاميذ والطلاب. تتمحور هذه النقاط في ضرورة التقيد بحفظ السندوتشات في حافظات نظيفة وملائمة والمحافظة عليها مبردة لحين تقديمها للتلاميذ والطلاب مع ضرورة عدم السماح للعمال بالعمل قبل الحصول على الشهادة الصحية والزامهم بارتداء الزي المناسب وارتداء قفازات على الأيدي وتغطية شعر الرأس.

الكلمات المفتاحية: سندوتشات التونة، هيستامين، الوضع الصحي، مناوالي السندوتشات، البائعين.