Assessing the Microbial Quality of Tahini (Sesame Paste) in Lebanon

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***Abstract*-The microbiological quality of tahini produced by several manufacturers in Lebanon was evaluated. Forty two tahini samples were collected randomly from retail markets throughout the country with production dates ranging from October 2015 to August 2017. The majority of the samples were from companies that are international exporters of the product. Six of the obtained samples were from a traditional tahini manufacturer. All samples were assessed for the total aerobic plate count, the presence and enumeration of *Staphylococcus aureus*, yeasts and molds, *Salmonella*, coliforms and *Escherichia coli*. Spread plate methods were used for detection and enumeration. The following results were obtained: the aerobic plate count of the samples ranged between $1x10^2$ CFU/g and $6.2x10^5$ CFU/g with an average of $6.9x10^4$ CFU/g. *S. aureus* count ranged between <20 CFU/g and $9.2x10^3$ CFU/g with an average of $4.7x10^2$ CFU/g. Yeasts and molds were present at counts ranging from <10 CFU/g to $1.5x10^5$ with an average of $2.3x10^4$ CFU/g. Total coliform counts ranged between <30 CFU/g and $3.4x10^5$ CFU/g with an average of $3.4x10^4$ CFU/g. *E. coli* was present in 43% of the samples (18 out of 42), while *Salmonella* was confirmed present in 17% of the samples (7 out of 42). When compared with Lebanese standards, many of the samples showed unacceptable quantities of microbial contamination and this was not impacted by the storage time, nor the processing method.**

Keywords-Tahini; Sesame Paste; Microbiology; Lebanon.

I. INTRODUCTION

Tahini is a well-known Middle Eastern condiment made from toasted ground hulled sesame seeds. The paste has gained popularity all over the globe as a result of its health and culinary benefits [1]. In 2014, the Middle East and Mediterranean tahini market was estimated to be at a value of US$783.9 Million, with forecasts of a further escalation by 2020. Lebanon has been an important exporter of tahini, and is home to many key players in the market [2].

The importance of tahini comes from the fact that it is used commercially and at a household level as an ingredient in many cultural delicacies. These include products that have gained international popularity, such as Hummus, and mtabal betejen (roasted eggplant and tahini) [3]. The paste is also used as a sauce for meats like shawarma, and as a sauce for fish, also known as tarator. Tahini also makes up about 50% of halawa, a sweet made up of tahini, sugar, citric acid and *Saponaria officinalis* root extract [4].

Tahini is of high nutritive value. It is rich in lipids, proteins, carbohydrates, niacin, thiamin, and some minerals like calcium, and phosphorous [5].

The traditional way of tahini processing in Lebanon includes: sorting the seeds to remove dark or imperfect seeds, followed by soaking the seeds in salt water. This helps settle impurities and dirt at the bottom and ease the peeling process. The seeds that are floating on the surface of the water are then collected, peeled and washed. The next step involves roasting the seeds, followed by the stone-grinding phase, which brings out the oil in the sesame and turns it into a paste.

Many tahini manufacturers, however, rely on a fully automated process. Instead of soaking the seeds in salt water, they are passed into a centrifuge that separates any impurities. The sesame then enters a washing machine, followed by a drying machine and then a roaster. The roasted sesame is cleaned once again and sorted by color. The accepted seeds then undergo grinding, are homogenized and then finally pasteurized at a high temperature for several hours to get rid of any potential bacteria [6][7].

After production tahini is stored at room temperature and has a shelf life up to 2 years [8]. It is typically consumed directly and does not require any further processing. Therefore, it should be free from any pathogenic bacteria upon packaging. The raw sesame itself should also be free from microbes, so as not to increase the risk of contamination. [9] However, despite the development of a hazard analysis critical control point (HACCP) plan for the manufacturing of tahini [10], in recent years, sesame paste has emerged as a product of concern, with many of the end products containing *Salmonella*, *Staphylococcus aureus* , *Escherichia coli*, and a number of other hazardous microbes. In addition tahini has a low water activity (~0.16) as well as low pH (~5.9) [10], conditions that permit the growth of many foodborne microorganisms [11].

The presence of microbes has been attributed to a number of reasons including, the microbial quality of sesame seeds, poor hygiene and sanitation, and improper processing and storage conditions [12]. Outbreaks of *Salmonella* infections have been traced back to tahini, some particularly correlated with Lebanese products [13]. Though some studies have dealt with the microbiological quality of sesame seed products, a collective investigation into tahini products in Lebanon using conventional plating methods has yet to be established. Therefore, the objective of this study will be to detect and enumerate microbial contamination of tahini in Lebanon, while also checking the impact of storage time and processing method on the microbial quality. This paper includes four sections. Aside from the introduction, Section 2

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will include a detailed description of the materials and methods used in the study. In Section 3 we mention the microbial results obtained and whether sample age and the method of processing may have had an impact on the obtained results, which we discuss in accordance to similar studies. In the final section, Section 4, we wrap up our research in a concluding statement and mention some limitations, as well as possible future work in the related area.

II. MATERIALS AND METHODS

A. Sampling

Forty two tahini samples with production dates varying from October 2015 to August 2017 were collected from retailers and producers throughout Lebanon. Six of the samples were obtained from a company that produces tahini via the traditional method (no automated machinery). Sample weights varied between 200g and 900g. All samples were held at room temperature (25^{o}C) and collected in their original packages, which were wiped with ethanol before testing. Using a sterilized rod, the samples were thoroughly mixed. 25 g of each sample were then transferred aseptically into separate sterile plastic bags containing 225 ml of buffered peptone water for homogenization. Homogenization was carried out using a stomacher (Model, 1605 BL Smart) for 2 minutes. Following homogenization ten-fold serial dilutions up to 10^3 were prepared and inoculated on appropriate media.

B. Microbial Analysis

Aerobic plate counts (APC), *Staphylococcus aureus,* coliforms, and yeasts/molds counts were determined for each sample, as well as the presence or absence of *Escherichia coli,* and *Salmonella.*

C. Aerobic Plate Count

APCwas determined according to the procedure specified by Morton R.D [14]. 0.1 ml of each dilution was inoculated and spread onto plate count agar (PCA) (Himedia) and left to dry. The plates were then incubated at 35±1^{0}C for 48 ±2 hours.

D. Staphylococcus aureus

S. aureus was detected and enumerated via surface plating 0.5 ml on Mannitol Salt agar (MSA) (Himedia) and incubating plates at 35±1^{0}C for 48 ±2 hours. Colonies with typical and atypical *S. aureus* morphology were confirmed by catalase and coagulase test. This method is in accordance with that specified by the British standards institution, with a modification of the agar [15].

E. Yeast and Mold

Yeast and mold counts were determined following spread plate inoculation onto Saubaurad dextrose agar (SDA) (Himedia). Plates were incubated at 25 ±1^{0}C for 5 days. This procedure was taken from the United States Food and Drug administration (USFDA) [16] however the proposed agar was substituted with SDA.

F. Total coliforms and Escherichia coli

Total coliforms were enumerated on Eosin methylene blue agar (EMB) (Himedia) [17]. An addition to the procedure determined by Gehm & Heukelekian included pre-enrichment of 1 ml of the samples with 10 ml lactose broth (Himedia) for 48 hours, at an incubation temp of 35 ±1^{0}C. Following the pre-enrichment step, 1 ml of each dilution was surface plated onto EMB agar plates and incubated at 35±1^{0}C for 48 ±2 hours. Plates with typical *E.coli* colonies were confirmed for presence of the bacteria via biochemical IMViC tests (Himedia).

G. Salmonella

For detecting *Salmonella*, the FDA Bacteriological analytical Manual (BAM) procedure was implemented, with some modifications [18]. Pre-enrichment was carried out by suspending 25g of each sample in 225ml of lactose broth (Himedia), followed by incubation at 35±1^{0}C for 24 ±2 hours. 1 ml of each sample was then transferred to 10 ml tubes of selenite F broth (SFB) (Himedia) and incubated at 35±1^{0}C for 24±2 hours. After incubation, 3 mm loopfuls were streaked onto Salmonella Shigella agar (SS) (Himedia) and incubated for another 24 ±2 hours. Typical and atypical colonies for presumptive *Salmonella* were then transferred to Triple Sugar Iron Agar (TSI) (Himedia). Confirmation was carried out via IMViC biochemical tests (Himedia), urease broth (Himedia), and Phenol D broth (Himedia).

H. Statistical analysis

The data was analyzed using analysis of variance (ANOVA) complete randomized design, and computed via the java software SPSS. Differences among means of the treatments were analyzed using Duncan. Significant differences are determined when $p \leq 0.05$.

III. RESULTS AND DISCUSSION

APC, *S. aureus*, total coliform, and yeast and mold counts, as well as the presence or absence of *Salmonella* and *E.coli* are shown in Table 1. The aerobic plate count of the samples ranged between $1x10^2$ CFU/g and $6.2x10^5$ CFU/g with an average of $6.9x10^4$ CFU/g. *S. aureus* count ranged between <20 CFU/g and $9.2x10^3$ CFU/g with an average of $4.7x10^2$ CFU/g. Yeast and mold was present at counts ranging from <10 CFU/g to $1.5x10^5$ with an average of $2.3x10^4$ CFU/g. Total coliform counts ranged between <30 CFU/g and $3.4x10^5$ CFU/g with an average of $3.4x10^4$ CFU/g. *E.coli* was confirmed present in 43% of the samples (18 out of 42), (presence of *E.coli* was noted when confirmed counts exceeded 10 CFU/g), while *Salmonella* was confirmed present in 17% of the samples (7 out of 42).

Lebanese standards (LIBNOR NL 71 :2012) set the unacceptable limit for APC, yeast and molds, E.coli and Salmonella at 1x104 CFU/g, 1x103 CFU/g, 10 CFU/g, and

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0 CFU/g respectively [19], beyond which microbial content could prove hazardous upon consumption. Standards available from the gulf countries (GSO) set the limit for *S. aureus* in tahini at $1x10^2$ CFU/g [20]. As seen in Table 2, a considerable amount of the samples analyzed contained unacceptable microbial content. 43% of the samples contained unacceptable quantities of APC and *E. coli.*

TABLE 2. MICROBIAL ANALYSIS OF FORTY TWO TAHINI SAMPLES IN LEBANON

	Microbial Analysis CFU/g					
Sample by Manu-facturer	*APC*	*S. aureus*	*Yeast and Mold*	*Total coliforms*	*E. coli*	*Salmo-nella*
A	$3x10^{2a}$	60	$4x10^2$	$1.4x10^4$	+	-
B	$5x10^2$	60	$1x10^2$	$2.2x10^2$	-	-
C	$4x10^2$	60	$1x10^2$	$3.7x10^3$	+	+
D	$6.8x10^2$	<20	<10	$7.30x10^3$	+	-
E	$2.3x10^3$	$2.2x10^2$	<10	$2.1x10^3$	-	+
F	$7x10^2$	60	$6x10^2$	>300	-	+
G	$8.8x10^4$	$3.8x10^2$	$3x10^3$	$3x10^4$	+	-
A	$1x10^2$	$2x10^2$	<10	<30	-	+
B	$1.2x10^4$	$1.8x10^2$	$6.2x10^3$	$2.5x10^4$	+	-
C	$4.5x10^4$	$9.2x10^3$	$4.4x10^4$	$4.8x10^3$	+	-
D	$3x10^2$	60	$4x10^4$	$6.6x10^3$	-	-
E	$6x10^2$	<20	$7x10^2$	$7x10^4$	+	-
F	$1x10^3$	$3.2x10^2$	$1x10^2$	<30	-	-
G	$3x10^2$	40	$4x10^2$	$2x10^3$	+	-
A	$7.5x10^3$	$2.8x10^2$	$1.5x10^3$	$2.5x10^3$	-	-
B	$1.4x10^3$	$1.3x10^2$	$1.9x10^3$	$4x10^3$	-	-
C	$3.2x10^3$	$2x10^2$	$8.3x10^3$	$2.3x10^4$	-	+
D	$6.3x10^3$	50	$3x10^2$	<30	-	-
E	$2.5x10^3$	$1.1x10^2$	<10	$5x10^2$	-	-
F	$2x10^2$	<20	<10	$2.6x10^3$	+	-
G	$1.2x10^4$	60	$1.2x10^4$	$1.2x10^4$	-	-
A	$7x10^2$	60	$2.3x10^3$	$1.3x10^4$	+	-
B	$1x10^2$	<20	$1.5x10^4$	$1x10^2$	-	-
C	$4x10^4$	$1.2x10^2$	$1.5x10^5$	$4x10^2$	+	-
D	$3.1x10^5$	$1.2x10^2$	$7x10^2$	$2.9x10^4$	-	-
E	$2.5x10^5$	<20	$1x10^3$	$1.6x10^4$	+	+
F	$6.5x10^4$	$5.6x10^3$	$1.2x10^5$	$4.3x10^3$	+	-
G	$2.3x10^4$	<20	$5.2x10^4$	$4.1x10^3$	+	-
A	$5x10^4$	40	$1.8x10^4$	$1x10^2$	-	-
B	$1.5x10^4$	$2.2x10^2$	$7.8x10^4$	$2x10^4$	-	+
C	$2.3x10^5$	$1.6x10^2$	$3.8x10^4$	$4x10^4$	+	-
D	$5x10^2$	<20	$6.2x10^3$	$2.2x10^3$	-	-
E	$6x10^3$	80	$3x10^3$	$3.5x10^4$	-	-
F	$2.2x10^3$	$3x10^2$	$1.2x10^4$	$2.2x10^4$	-	-
G	$2x10^2$	<20	$1.9x10^4$	$1.5x10^3$	+	-
A	$5.5x10^4$	$3.2x10^2$	$3.4x10^4$	$3.2x10^5$	-	-
B	$6.2x10^5$	$1x10^2$	$1.2x10^5$	$2.2x10^5$	-	-
C	$3.3x10^5$	$5.4x10^2$	$1.1x10^5$	$3.4x10^5$	-	-
D	$3.3x10^5$	80	$6.4x10^3$	$1.2x10^5$	+	-
E	$1.5x10^3$	40	$1.1x10^5$	$9.3x10^4$	-	-
F	$1.1x10^5$	$2.2x10^2$	$8.4x10^3$	$5.2x10^3$	+	-
G	$1.3x10^4$	<20	$3.2x10^2$	$1x10^2$	-	-
Average counts	$6.9x10^4$	$4.7x10^2$	$2.3x10^4$	$3.4x10^4$	18/42	7/42

[a] Calculations were based on average of duplicate replications
+, presence of microbe in unacceptable amounts
-, absence of microbe, or present but in acceptable amounts

Almost half of the samples (48%) showed unacceptable quantities of S. aureus, while more than half of the samples were unacceptable for yeast and mold quantities (64%). Meanwhile coliform counts were intolerably high in 98% of the tested samples. Even minute amounts of *Salmonella* are detrimental to one's health and therefore 17% of the samples were found to be hazardous. These results were also consistent with standards set by the FDA for ready to eat foods [21].

TABLE 1. COMPARISION OF SAMPLE RESULTS WITH MICROBIAL STANDARDS

Micro-organism	Unacceptable limits	Unacceptable samples *N*	% unacceptable
APC[a]	$1x10^4$ CFU/g	18	43%
S.aureus[b]	$1x10^2$ CFU/g	20	48%
Yeast and molds[a]	$1x10^3$ CFU/g	27	64%
Total coliforms[a]	$1x10^2$ CFU/g	39	93%
E.coli[a]	10 CFU/g	18	43%
Salmonella[a]	0 CFU/g	7	17%

[a] obtained from LIBNOR standards
[b] obtained from GSO standards

Furthermore, when the microbial content of the samples were determined in accordance with sample age, there seemed to be no significant differences. Therefore the amount of time the product spends on the shelf seemed to have no significant impact ($p>0.05$) on the microbial quality. In a similar study however, the microbial counts of tahini were seen to have decreased after four months [10].

Another factor studied was the processing method, whether by traditional methods or solely automated machinery (modern). Statistical analysis of the data showed

TABLE 3. COMPARISION OF MICROBIAL CONTENT OF TAHINI SAMPLES DEPENDING ON SAMPLE AGE

	Sample age (Months)				
Micro-organism CFU/g	Fresh[a]	x≤ 3	3<x≤6	6<x<12	≥12
N[b]	6	15	15	4	2
APC	$2.3x10^4$	$8.9x10^4$	$7.3x10^4$	$7.3x10^4$	$6.7x10^3$
S. aureus	90	$7.9x10^2$	$4.6x10^2$	58	$1.6x10^2$
Yeast & molds	$1.4x10^4$	$2.8x10^4$	$3x10^4$	$8.3x10^3$	$4.1x10^4$
Total coliforms	$8.3x10^4$	$6.6x10^4$	$2.5x10^4$	$4.7x10^2$	$1.5x10^4$
E. coli	4^c	4^c	9^c	0^c	1^c
Salmonella	0^c	5^c	2^c	0^c	0^c

[a] Fresh samples include samples tested directly after production
[b] number of samples within each sample age group
[c] number of samples positive for presence of microbe

TABLE 4. COMPARISION OF MICROBIAL CONTENT OF TAHINI SAMPLES DEPENDING ON PROCESSING METHOD

	Tahini Processing Method	
Microorganism CFU/g	Traditional[a]	Modern[b]
N[c]	6	36
APC	$2.3x10^4$	$7.6x10^4$
S. aureus	90	$5.4x10^2$
Yeast & molds	$1.4x10^4$	$2.5x10^4$
Total coliforms	$8.3x10^4$	$3.8x10^4$
E.coli	4	14
Salmonella	0	7

[a] No automated machinery
[b] Solely automated machinery
[c] number of samples within each processing method group

in Table 4 also showed no significant differences ($p>0.05$) between the samples. Therefore the processing method does not appear to be an influencing factor in regards to microbial counts.

The obtained results indicate that some tahini produced in Lebanon is hazardous and could pose life threatening consequences. In addition, about 35 percent of tahini produced in the country is exported, specifically to the USA, EU, Australia and the GCC. The products tested in this study include some of the country's major producers and exporters. The fact that Lebanese tahini has had incidents where *Salmonella* was detected, has reduced the quantities for export, especially to the USA [22]. Therefore the results indicate that Lebanese tahini could also have threatening consequences to health on a global scale, or to the country's profits from tahini exports. Similar studies on the microbial quality of sesame seed products have been carried out and similar results were obtained. Tahini samples taken immediately after production from 14 plants located in Amman, Jordan were found to contain significant results of APC, coliforms, *S. aureus*, and yeasts and molds [8]. This is not the first time *Salmonella* has been identified in tahini. In a comparable study in Saudi Arabia, *Salmonella* was apparent in 20% of the samples studied [23]. *Salmonella* was also identified in other sesame seed products [24]. In Turkey, a study done on the microbial quality of halva (halawa), also showed that *Salmonella* dominated in many of the samples [8].Tahini has a low water activity and is therefore considered to be a low risk food. However it is a ready to eat product and there are many critical points during production that can expose the product to contamination if good manufacturing processes are not implemented. Contamination may occur from the use of contaminated water during washing or soaking, cross contamination during processes that are open to air, for example, grinding or filling, or bad hygiene conditions within the factory [7]. Therefore it is strongly recommended that tahini manufacturing companies strictly adhere to the implementation of good manufacturing processes to ensure safe microbial counts. Other studies have even shown that contamination could come from the soil or sesame seeds themselves [25], and so thorough investigation of the microbial quality of sesame seeds used for tahini production in Lebanon could also be assessed in the near future.

This is the first collective study in Lebanon that determines the quality of tahini produced in the country by studying the microbial quality of the products via conventional plating methods, while also considering the sample age as well as the processing methods as possible impact factors.

Although the paper does only consider Lebanese products, it is worth noting that all the studied samples are from companies that export tahini worldwide, making the problem a global concern. Also, other major worldwide exporters (e.g., Turkish, Jordanian, and Saudi Arabian companies) also carried out similar studies on the tahini quality in their respective countries [10][23][8], and hence, this study is to complement the others. Furthermore the discovery of contaminated tahini products in other countries, (Jordon, Turkey, and Saudi Arabia) [10][23][8] motivated us to test the quality of tahini in Lebanon.

IV. CONCLUSION

The results of this study provided an evaluation into the microbial quality of forty two tahini products manufactured in Lebanon and showed that some products are unacceptable in accordance to local and international standards. The results were also determined to be irrespective of the sample age, or processing method. Limitations include unequal sample sizes for the different factors studied (sample age, processing method) due to limited resources, and the randomization procedure. Currently, more samples are being assessed for microbial contamination in order to provide more accurate results. Further testing will be required to determine the source of contamination in order to contain it. Furthermore, the study could be a basis for further research (19) aimed at eradicating high microbial counts in tahini without impacting the overall physiochemical quality.

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