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MICROBIOLOGICAL AND CHEMICAL PROFILES OF RETAIL FALAFEL SANDWICH IN JORDAN

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ABSTRACT

Microbiological contamination of food poses a significant risk to public health, as a popular ready-to-eat food in the Middle-East, falafel sandwiches require no processing. When ingested, their microbiological integrity is extremely important to the population's health. The aim of the present research was to evaluate the microbial load of falafel sandwich and its basic components, which is an important indicator of hygiene and safety; to that effect, we tested 120 samples from different restaurants in Amman, Jordan (30 falafel sandwiches, 30 tahini salad, 30 hummus, and 30 falafel). The collected samples were transferred to the laboratory in the ice box and tested for microbiological and chemical analysis (pH and titratable acidity). Appropriate media were used in the enumeration: Plate Count Agar, De Man, Rogosa, and Sharpe agar (MRS), Violet Red Bile Lactose Agar, and Baird-Parker Agar for mesophilic aerobes, lactic acid bacteria, coliforms, and *Staphylococcus aureus*, respectively. In all samples, the average pH was ≥ 5 and the average titratable acidity (as citric acid) was ≥ 0.55%, thus permitting the growth of many microorganisms. The samples were assessed for aerobic plate count (APC) and the counts of coliforms (CC), lactic acid bacteria (LABC), yeasts and molds (YMC), *Staphylococcus aureus* count (*S. aureus*), as well as for the presence of *Salmonella enterica (S. enterica)* and *Listeria monocytogenes (L. monocytogenes).* Average APC of 'falafel' sandwich, 'tahini' salad, 'hummus', and falafel were 6.4, 6.3, 5.8, and 2.9 $\log_{10}$ CFU/g respectively; average CC was 2.3, 2.8,1.9, and 0.6 $\log_{10}$ CFU/g, respectively; average LABC was 5.6, 5.5, 5.3, and 2.4 $\log_{10}$ CFU/g, respectively; average YMC was 4.2, 3.8, 3, and 0.7 $\log_{10}$ CFU/g, respectively; average *S. aureus* was 2.09,1.68,0, and 0.3 $\log_{10}$ CFU/g, respectively. *S. enterica* and *L. monocytogenes* were not isolated from any sample. This might be due of the exposure to high temperatures during the frying process, 'falafel' samples had the lowest microbial load. The study revealed through these microbial counts, that hummus and tahini salad are most likely to introduce microorganisms to falafel sandwich.

Key words: falafel sandwich, tahini salad, hummus, coliforms, lactic acid bacteria

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INTRODUCTION

Ready-to-eat food (RTF) is a meal or part of a meal sold to the consumer immediately or later to be directly consumed and does not require further processing, such as cooking or another procedure to eliminate potentially dangerous microbes. Sometimes RTF requires heating only. As well as being easy to prepare, it is usually available in a number of public places [1,2,3,4]. Falafel sandwich is one of the most accessible RTFs in Jordan and most of the Arab countries. Commonly known as “ta`amiyya” in Egypt and Sudan, falafel is typically a fast food or street snack that has recently gained international spread, especially among vegans [5].

Currently, this sandwich is usually prepared in front of the customer just before consumption and consists of a loaf of flat rounded Arabic bread in which the hummus (chickpea dip) is placed, the falafel patties are mashed with a knife, and then tahini salad is added, with or without shatta (fermented chopped red pepper), to be provided to the customer for direct consumption usually with a soft drink or tea.

Moreover, falafel sandwiches which are often made directly by hand, are likely to be highly linked to outbreaks of foodborne infections due to a lack of adherence to personal hygiene by food handlers, and food hygiene [6,7]. Furthermore, RTF could be contaminated during all stages of preparation from raw material to finished product. One of the most important components of the falafel sandwich is the tahini salad consisting of tomatoes, cucumbers, and tahini, which is not subject to any heating or cooking process [8,9,10]. Eating foods and drinks that contain physical, chemical, or biological hazards (bacteria, viruses, or parasites) could cause infection with foodborne diseases [9,11]. Recent microbiological studies on different RTF in Saudi Arabia, Egypt, and some African countries have revealed high bacterial counts and a high incidence of foodborne bacterial pathogens in such food [12,13,14,15].

There are no published studies looking at the microbial quality of falafel sandwich and the ingredients used in the preparation, although there are some studies that examined the microbes found in falafel sandwich, tahini salad, and hummus individually [12,14,15]. The strength of this study resides in the evaluation of microbiological features of falafel sandwiches sold in markets as a whole and the degree to which tahini salad, hummus, and falafel contribute microbial loads to whole.

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MATERIALS AND METHODS

Collection of samples

One hundred twenty (n=120) falafel sandwich samples were collected from 30 different restaurants and fast-food outlets in Amman, Jordan. Concurrently, individual samples were collected from the sandwich components, tahini salad, hummus, and falafel. (Every two weeks 12 samples were taken, and four sample units were taken from each restaurant for a period of five months from March to July 2022). Each collected sample was placed in its sterile bag and kept in an ice box at a temperature of 10 ^{o}C under aseptic conditions, and transported to the laboratory for microbiological and chemical analysis within 2 hours of collection to determine the hygienic health risk.

Chemical Analysis

The pH and total titratable acidity were determined according to the association of official analytical chemists [16] for each sample unit of a falafel sandwich, tahini salad, and hummus, expressing the results as a percentage of citric acid using the equation:

%Titratable acidity (g citric acid/Kg V x 0.1 x 1000 x 0.064)/m

where:
V is the volume of NaOH consumed (mL)
0.1 is the Normality of NaOH
0.064 is the citric acid conversion factor
m is weight of sample taken

Microbiological Analysis

From each individual sample unit (falafel sandwich, tahini salad, hummus, and falafel) 25g were added to 225 ml of sterile 0.1% peptone water (Oxoid) and homogenized in a stomacher for 2 min. Tenfold serial dilutions were made and then submitted for bacteriological investigation as stated by American Public Health Association [17].

Lactic acid bacteria enumeration was performed as described in International Organization for Standardization [18]. Aerobic plate count and yeasts and molds were done as described by Pamuk *et al.* [19]. *S. aureus* determination were done as described by Alaouie *et al.* [20]. The counts of coliforms were taken according to The Food and Drug Administration (FDA) and Bacteriological Analytical Manual (BAM) [21], The pour plate technique was used in these enumerations, and the

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streak plate method was employed to count S. aureus, the numbers were calculated and expressed as colony forming units (Log_{10} CFU/g).

The presence or absence of *L. monocytogenes,* and *S. enterica* was determined according to Mritunjay and Kumar [22]; Hashemi *et al.* [23].

Aerobic plate count (APC)

The enumeration of the aerobic plate count in different samples unit including falafel sandwich, tahini salad, hummus, and fried falafel was performed using the pour plate technique according to Pamuk *et al.* [19]. Briefly, transfer 1 mL of the inoculum and its successive decimal dilutions into sterile Petri dishes. Then, about 15 mL of cooled (44-47 °C) Plate Count Agar (PCA) (Oxoid CM325; Oxoid Ltd., Basingstoke, Hampshire, UK), was poured onto each plate. The mixture was immediately thoroughly and uniformly mixed. After the agar had solidified, the plates were then inverted and incubated at 35º C ± 1°C for 48 h. The results were given in colony-forming units per mL of sample (Log_{10} CFU)/mL. The plates with between 30 and 300 colonies were counted, and they were calculated and noted.

Coliform count (CC)

The detection of total coliforms in different samples was performed using the pour plate technique according to The Food and Drug Administration (FDA) and Bacteriological Analytical Manual (BAM) [21]. Briefly,1mL of each sample dilution was transferred into separate, duplicate Petri dishes. Then about 15 mL of cooled (48 °C) Violet Red Bile Lactose agar (VRBL) (Oxoid CM0485), was poured onto each plate. The mixture was immediately thoroughly and uniformly mixed. After the agar had solidified, the plates were then inverted and incubated at 35°C for 24 to 48 hours. The results were given in colony-forming units per g of sample Log_{10} CFU/g.

Lactic acid bacteria (LAB)

The enumeration of the lactic acid bacteria count in different samples was performed using the pour plate technique according to the International Organization for Standardization [18]. Briefly, 1mL of each sample dilution was transferred into Petri dishes. A volume of 100 µL of cooled de Man, Roosa, and Sharpe agar (MRSA, Oxoid, UK) was poured onto each plate. After the agar had solidified, the plates were then inverted and incubated at 30ºC for 48 to 72 hours. The lactic acid bacteria count was expressed as colony-forming units per mL of sample (Log_{10} CFU/g).

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Detection of *Staphylococcus aureus* (*S. aureus)*

S. aureus was detected and enumerated using Baird-Parker agar (BP, CM 275 Oxoid Ltd, Hampshire, England), supplemented with egg yolk tellurite emulsion (50 ml/L, Oxoid SR54) and incubating plates at 35 ºC for 48 h. The results of typical colonies were observed with morphology as dark colonies with a clear zone were confirmed by coagulase-positive on rabbit coagulase plasma (C14389), catalase, and fermentation test.

Yeasts and molds count (YMC)

Yeasts and molds count were determined by using plate count agar (PCA) supplemented with 100mg/L of chloramphenicol and incubated at 25 ºC ±1C for 5 days.

Detection of *Salmonella enterica (S. enterica)*

Pre-enrichment in non-selective broth: Twenty-five grams of examined samples were homogenized in 225 ml of lactose broth in a sterile blender jar and incubated at 37°C for 24 hours. Enrichment in selective broth: One ml of the inoculated Pre-enrichment culture was inoculated into a 10 ml Rappaport Vassiliadis broth (CM0866) tube, then the tube was incubated at 43°C for 24 hours. Selective Plating: A loopful from selective enriched broth was streaked onto the surface of previously prepared Xylose lysine Desoxychoclate agar (XLD) (CM0469 Oxoid Ltd, Hampshire, England) and the plates were incubated at 37°C for 24 hours. Plates were examined for suspected Salmonella colonies which appeared as red with black centers.

Detection of *Listeria monocytogenes (L.monocytogenes)*

Twenty-five grams of each sample was aseptically weighed using a sterile spatula and blended for 1 min with 225 mL of Half-Fraser broth (CM 0895, Himedia) as a pre-enrichment broth and incubated at 30 °C for 24 h according to Mritunjay and Kumar [22]. Afterward, 0.1 ml of the culture was sub-cultured into Frazer broth (Oxoid) and incubated at 37 °C for 48 h. Thereafter, the homogenate was streaked onto two plates of *Listeria* selective agar (Oxoid), and incubated for 48 h at 37 °C.

STATISTICAL ANALYSIS

The results of two replications were analyzed by analysis of variance using the system package Statistical Analysis System [24]. A Tukey's test was used to assess significantly different means. Analysis of variance (ANOVA) was carried out and a t-test was used to compare the means of results of microbial enumerations.

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RESULTS AND DISCUSSION

Chemical Analysis

Titratable acidity and pH are related ideas where it was noted that there is a decrease in the titratable acidity with increases in the results obtained in pH. However, titratable acidity is related to product flavor, and the pH is directly related to the proliferation of microorganisms in food [25]. Furthermore, the pH can be impacted by a number of factors, including the amount of organic acid present, temperature, the food's original pH, and the buffering power of the food [26]. However, as Bangar *et al.* [27] noted, pH, temperature, and their interactions significantly impact the formation of organic acids. Therefore, organic acids, such as propionic, formic, acetic, and lactic acids, inhibit the growth of bacteria that cause spoilage and lower pollution emissions.

Variations were noticed in the results of testing for pH and acidity (Table 1 and Figures 1 and 2). Many factors can influence these parameters in hummus and tahini salad, including lemon juice (or citric acid) and the residues of bicarbonate used in boiling of chickpea. In all samples, the average pH was ≥ 5 and titratable acidity (as citric acid) ≥ 0.55%; indicating that the pH of hummus and tahini could permit the growth of many microorganisms, including foodborne pathogens. Results of pH of hummus sample are in agreement with those recorded by Yamani and Dababseh [12] and results for tahini salads are in agreement with findings of Olaimat *et al.* [28].

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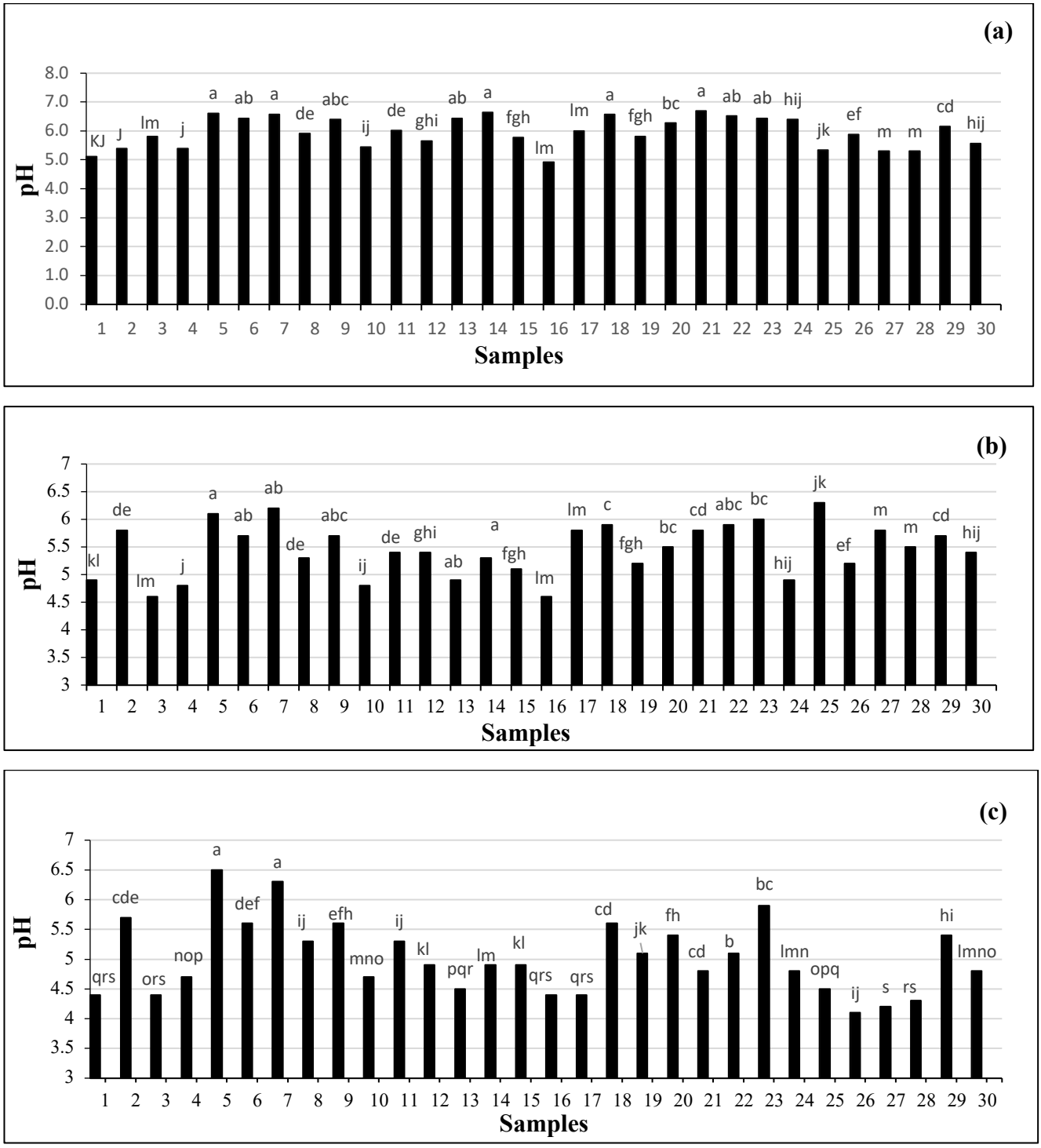

Figure 1: Range and average of pH of samples of falafel sandwich (a), tahini salad (b), and hummus (c) collected from 30 restaurants

Note: Averages with different superscript letters at the top of the columns are significantly different ($p \leq 0.05$) and Averages with same superscript letters at the top of the columns are non- significantly different ($p \leq 0.05$) according to the "ANOVA" test

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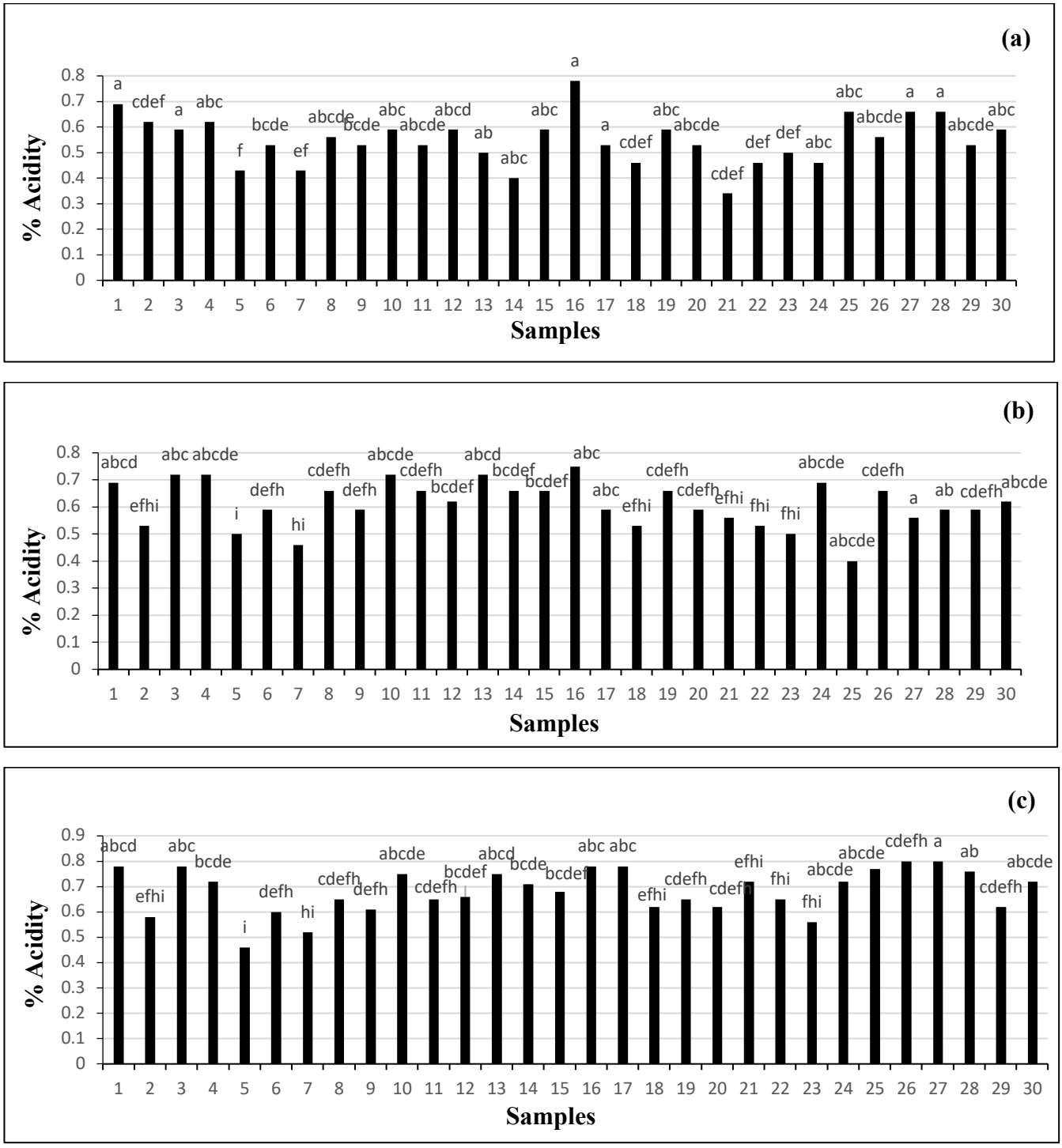

Figure 2: Range and average of titratable acidity (as citric acid%) of samples of falafel sandwich (a), tahini salad (b), and hummus (c) collected from 30 restaurants

Note: Averages with different superscript letters at the top of the columns are significantly different ($p \leq 0.05$) and Averages with same superscript letters at the top of the columns are non- significantly different ($p \leq 0.05$) according to the "ANOVA" test

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Microbiological Testing

Microbial load 1.9, and 0.6 log_{10} CFU/g respectively; average LABC was 5.6, 5.5, 5.3, and 2.4 log_{10} CFU/g respectively; average YMC was 4.2, 3.8, 3, and 0.7 log_{10} CFU/g, respectively; average STC was 2.09,1.68,0, and 0.3 log_{10} CFU/g, respectively. Falafel stands out among the products of this study since patties are deep-fried in vegetable oil, which destroys most of the microorganisms originally present in the falafel mix. Consequently, the low microbial load in the falafel samples could be a result of cross-contamination and/or direct handling by the workers. This applies to the bread used in the preparation of the sandwich since it is not uncommon for the workers to touch the falafel and the bread with bare hands.

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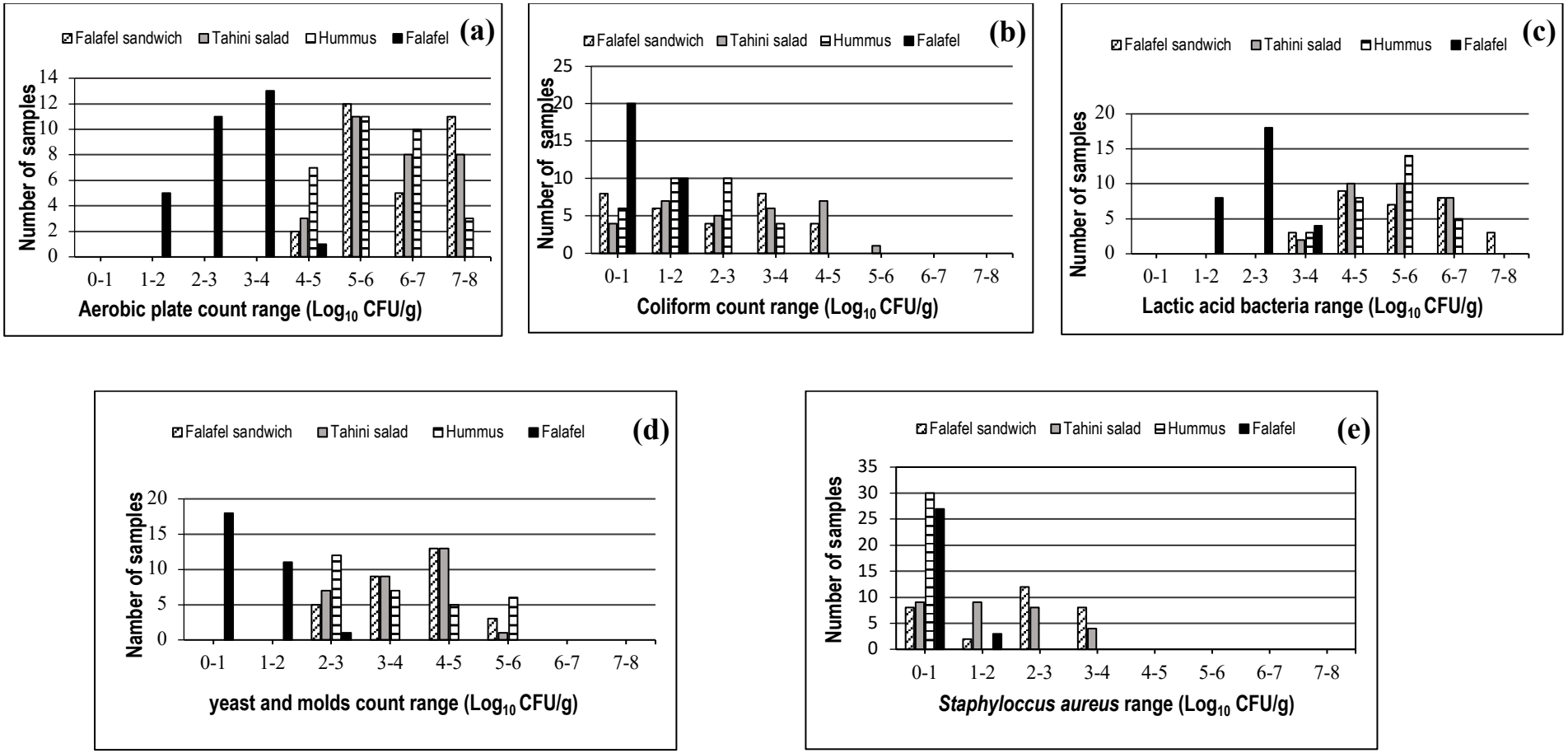

Figure 3: Range of aerobic plate count (a), coliforms count (b), lactic acid bacteria (c), yeasts and molds (d), and Staphylococcus aureus count (e) of falafel sandwich, tahini salad, hummus, and falafel samples collected from different restaurants in Amman, Jordan

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Aerobic plate count was high in tahini salad and hummus samples, the count was >5 $\log_{10}$ CFU/g (Figure 3/a). This was also the situation with LAB (Figure 3/c), indicating that LAB was probably the prevalent microorganism contributing to APC; as was the case in hummus samples tested by Yamani and Dababseh [12]. Results of APC of hummus in this study were in agreement with Yamani's and Dababseh's findings [12] in which the average of APC, coliform count, and LAB count in retail hummus samples were 8.3, 8.2, and 5.5 $\log_{10}$ CFU/g, respectively according to the authors these results might indicate a lack of hygienic practices during the production.

With the exception of falafel, coliform count was high in samples of other products (Figure 3b). In some samples, the count was up to 5 $\log_{10}$ CFU/g similar results were found in a study by Yamani and Dababseh [12]. Such coliform count in RTF is generally unacceptable since as an indicator group of bacteria, the presence of coliforms could be accompanied by the presence of enteropathogenic bacteria, viruses, and parasites.

S. aureus count in the samples could also indicate mishandling of the sandwich. However, being≤ 2 $\log_{10}$ CFU/g would not impose a public health risk, since higher counts of S. aureus≥2 $\log_{10}$ CFU/g are usually needed to cause illness [29]. *S. enterica* and *L. monocytogens* were not isolated from any sample; however, this does not mean that falafel sandwich can be guaranteed to be free of enteric pathogens of human origin.

Under prevailing environmental conditions, especially personal hygiene and temperature control, this sandwich may act as a vehicle for such pathogens.

It could be deduced from Figure 3 that hummus and tahini salad were the major source of microorganisms in the falafel sandwich. Both have in common tahini as a basic ingredient. Tahini is manufactured on a large scale in Jordan and in many other Arab countries. By being a product obtained by grinding mature, roasted, and husked sesame seeds, tahini is characterized by having a high concentration of oil and protein and very low moisture content. Typically, tahini contains > 45%, >19%, and <1.5% sesame oil, protein, and moisture, respectively [30,31].

A study by Yamani and Isa [32] investigating the microbial content of tahini produced in Jordan has shown that Tahini usually contains microorganisms in appreciable counts and these microorganisms are still viable after relatively long ambient storage. *S. enterica* and *E. coli* were not isolated from any of the examined sample but were able to survive when introduced to the product.

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The microbiological quality of the majority of samples of tahini salad used in falafel sandwich preparation was unsatisfactory and that there is room of improvement in this regard. This is commonly the situation with vegetable salads from retail organizations, for example in [19,33,34]. Abadias *et al.* [33] found in 132 RTF mixed salad containing from one to six vegetables from 4 supermarkets in the Lleida area (Catalonia, Spain) that aerobic mesophilic count (AMC) ranged from 5.4-8.5 $\log_{10}$ CFU/g with an average of 7.1 $\log_{10}$ CFU/g; in > 50% of the samples, AMC ranged from 7-8 $\log_{10}$ CFU/g. Furthermore, 1.3% and 0.7% of the samples were positive to *S. enterica* and *L. monocytogenes*, respectively. Pamuk *et al.* [19] found in RTE salad sold in Afyonkarahisar, Turkey, that 55.1 % of the 261 samples were contaminated with > 6 log CFU /g total viable count, and 54% were contaminated with > 4 log CFU/g Enterobacteriaceae.

Calonico *et al.* [34] established that the high unsatisfactory microbial loads as reflected in the APC in retail RTF salads in Italy was mainly due to microbial growth during transport and storage. In Riyadh, Saudi Arabia Khiyami *et al.* [35] found that the total coliform counts in restaurants salad were around 4.3 - 4.9 $\log_{10}$ CFU/g as compared to 3.3 - 3.7 $\log_{10}$ CFU/g of homemade salads.

Frequently, the public health situation could become aggravated when foodborne outbreaks take place due to *Salmonella* [36] *Listeria monocytogenes* [37], and *Escherichia coli* [38]. This situation can be mitigated if food is prepared under conventional hygiene guidelines in which microbial load is acceptable by the Interdepartmental Center for Research and Documentation on Food Safety [39] and Public Health Laboratory Services [40].

CONCLUSION, AND RECOMMENDATIONS FOR DEVELOPMENT

In conclusion, the results from this study revealed that the levels of microbial load that appeared in falafel sandwiches and their basic components did not meet bacteriological quality criteria. This suggests that if food hygiene and personal hygiene requirements were not met, cleaning and disinfection temperature control, and maintenance of cooling chain during storage and transport might be added to the list of factors contributing to these findings. Producing food for the masses in poor environmental conditions causes a threat to public thus the importance of implementing strong guidelines and corrective measures when needed.

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CONFLICTS OF INTEREST

We have no conflicts of interest to disclose.

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Table 1: Range and average of pH and titratable acidity (as citric acid%) of falafel sandwich, tahini salad, and hummus samples collected from 30 restaurants, in Amman, Jordan

	Falafel sandwich		Tahini salad		Hummus	
	pH	Acidity %	pH	Acidity %	pH	Acidity %
Range	4.9-6.7	0.34-0.72	4.6-6.3	0.4-0.78	4.1-6.5	0.4-0.8
Average	6	0.55	5.5	0.61	5	0.68

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