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Fate of *Staphylococcus aureus* in Experimentally Prepared Fermented Fish Sauce (Mehiawah)

المآلات المحتملة لميكروب المكورات العنقودية الذهبية في المهياوة المحضرة مختبريا

Abdul Ameer Al-Laith

عبد الأمير الليث

Department of Biology, College of Science, University of Bahrain

P O Box 32038, Building S41, Sakhier Campus, Kingdom of Bahrain

E-Mail: allaith@sci.uob.bh: aallaith@batelco.com.bh

ABSTRACT: In a previous study, we have shown that home-made Mehiawah, a fermented fish sauce commonly consumed in the Gulf States and traditionally prepared from dried *Sardinella gibbosa* (Indian sardines), contained high counts of *Staphylococcus aureus*, as well as detectable level of staphylococcal enterotoxins. The current study further confirms these findings It also reports the possible fate of inoculated *S. aureus* in experimentally prepared Mehiawah using four different mehiawah treatments. A coagulase-positive *S. aureus* isolate was used as an inoculum. Fermentation was allowed to proceed for two weeks in the presence or absence of natural flora of lactic acid bacteria (LAB). Counts of *S. aureus*, LAB, and total aerobic (APC) were monitored. The fate of inoculated *S. aureus* (10^7 CFU/ml) in the presence and the absence of naturally occurring LAB showed similarity at early stage of fermentation but differ at later stage. By the end of two-week fermentation period, count of inoculated *S. aureus* was about one log higher (5.5) in the absence of LAB compared to in the presence (4.5). pH of all treatment, whether LAB were present or not and as well as in the sterilized Mehiawah, declined from about 5.75-5.9 to between 3.4- 3.9). Physiochemical analyses of commercial samples and the survival pattern of *S. aureus* suggest that Mehiawah may possess health hazards, especially under the conditions that prevail in home-made practice.

Keywords: *fate, Fermentaion, fish sauce, Mehiawah, Staphylococcus aureus.*

المستخلص: المهياوة عبارة عن منتج غذائي شعبي محضر بتخمير نوع من سمك السردين المجفف، وتشتهر به العديد من دول الخليج العربية وغرب إيران. وقد أشرنا في دراسة سابقة إلى أن بعض عينات المهياوة الموجودة في الأسواق والمحضرة منزليا قد تحتوي على أعداد مرتفعة من ميكروب المكورات العنقودية الذهبية (*Staphylococcus aureus*). كما قد تحتوي على كميات محسوسة من سموم هذا الميكروب. وتؤكد الدراسة الحالية النتائج السابقة، كما تبين المآلات المحتملة لميكروب المكورات العنقودية عندما يتم استنباته في أربعة ظروف تجريبية مختلفة في عينات المهياوة المحضرة مختبريا. ففي هذه الدراسة تم استخدام سلالة ميكروب المكورات العنقودية الذهبية إيجابية لإنزيم الكواجوليز معزولة من المهياوة واستنبتت في المهياوة في وجود وفي عدم وجود ميكروبات حمض اللاكتيك (BAL)، ورصدت عملية التخمر لمدة أسبوعين جرى خلالها تعيين أعداد البكتيريا الكلية، وأعداد ميكروب المكورات العنقودية وأعداد بكتيريا حمض اللاكتيك، وقياس الرقم الهيدروجيني. وقد أتضح أن مآلات ميكروب في وجود أو عدم وجود بكتيريا حمض اللاكتيك يبدو متشابها في المرحلة الأولى للتخمير، لكنه يختلف في المراحل اللاحقة. كما أتضح أن أعداد ميكروب المكورات العنقودية يزداد في

غياب بكتيريا حمض اللاكتيك بمقدار لوغاريتم واحد تقريبا (5.5)، عن أعداد الميكروب في وجود بكتيريا حمض اللاكتيك (5.4) عند نهاية التجربة. أما الرقم الهيدروجيني فقد انخفض في جميع المعاملات من 7.5- 9.5 إلى 4.3-9.3 ، سواء في وجود أو عدم وجود الميكروبات المعنية. وتظهر الدراسة أن المهياوة المحضرة منزليا في ظروف غير صحية ربما تكون مصدرا لخطر صحي.

كلمات مدخلية : *مآل، تخمير، صلصة السمك، مهياوة، ستافيلوكوكس أوريس (المكورات العنقودية الذهبية).*

INTRODUCTION

Indigenous fermented food products are considered by many nations as cultural and national heritages. They are highly regarded for their peculiarity and palatability. Mehiawah (also written as mehyawah) is one of the most popular traditional fermented fish sauce in the coastal areas of the Gulf Cooperation Countries (GCC, which includes Bahrain, Kuwait, Oman, Qatar, Saudi Arabia, and United Arab Emirates) as well as the western coast of Iran. Mehiawah preparation is thought to be as a way of utilization of inferior type of fish, and as a bio-preservation as well. It is a sauce-like suspension apparently brought to the Arab countries of the Gulf by Iranian immigrants (Al-Jedah and Ali, 1999). Traditionally, it is eaten as a spread on locally baked thin bread.

Although, commercial production of Mehiawah started lately in Bahrain, Mehiawah is still largely produced as a home-made commodity from either fresh or dried *Sardinella gibbosa* (Indian sardines) locally known as *Matoot*. Fermentation process involves the addition of high proportions of salt, starchy grains, and mixed spices. Mehiawah prepared from fresh fish goes through a preparatory step that lasts 2-3 months and results in fish maceration and hydrolyses to yield a paste-like product which is further mixed with spices to produce the sauce. When dried fish is used, it is grounded and directly mixed with salt and other ingredients (Al-Jedah, *et al.* 1999). Though the essential components of home-made Mehiawah are same, their proportions as well as the methods of preparation vary considerably. An outline of some of these methods, as well as the nutritional values of this commodity, were previously reported (Al-Jedah, *et al.* 1999). The end product of such fermentation, which may be categorized as a hydrolyzed/ fermented product, is a protein-rich commodity with acidic and salty characteristics.

Products of fermented fish, as well as the process of production, vary and depend on the nature of the product, the ingredients used, and the geographical location (Al-Jedah and Ali, 1999). Microbiology of fermented fish products have been reported by many researchers (Paludan-Müller, *et al.* 2002; Thapa, *et al.* 2004).

Fermentation of Mehiawah depends on the action of lactic acid bacteria (LAB) carried over by the ingredients normally used for its preparation (Al-Jedah, *et al.* 1999). Several microbiological studies pertaining the quality and safety issues of Mehiawah appeared on the literature. Musaiger and Jaidah (1991) reported that Mehiawah collected from the market place was free from pathogenic. Al-Jedah, *et al.* (1999) reported that home-made and commercial samples of Mehiawah were free from vegetative pathogens. However, and to the contrary, a study by Allaith, *et al.* (2000) had shown that samples of home-made Mehiawah contained varying count of *S. aureus* ranging from between 10^2 to 10^7 CFU/ml. Only 21% of the samples had no detectable count of *S. aureus*. These authors also showed that some tested home-made preparations exceeded the recommended acceptable limit for *S. aureus* for some pre-cooked fishery products (10^2-10^3 CFU/ g) (Stannard, 1997; ICMSF, 1986), and indicated that *S.aureus* levels in some samples were enough to produce staphylococcal enterotoixns (SETs). In that study, the majority of isolated *S. aureus* colonies were positive for thermostable nuclease (TNase). Furthermore, the presence of varying amount of SETs in most of the sample using reverse passive agglutination assay, was evident, an indication of the prior presence of pathogenic *S. aureus* (Allaith, *et al.* 2000).

The food pathogen *S. aureus* is a common cause of food poisoning in Bahrain, and most of the cases are not reported. Though widely spread in nature, *S. aureus* is not normally isolated from fresh marine fish (Shewan, 1976; FDA,

1992). Its presence in ready-to-eat-foods, such as Mehiawah, may indicate either pre- or post-processing contamination. Since Mehiawah is not a heat-treated sauce, contamination by *S. aureus* during or post-preparation, particularly under poor-hygienic and poorly supervised conditions that are ordinarily practiced during home-made preparation, may represent a potential hazard for the public health. On the other hand, the chemo-physical characteristics of Mehiawah, such as relatively high salt content and low pH in particular, weigh against such potential threat. This study was undertaken to investigate the behavioral growth and the fate of inoculated *S. aureus* during the fermentation of laboratory prepared autoclaved and non-autoclaved Mehiawah in the presence and absence of LAB. lactic acid bacteria.

MATERIALS AND METHODS

Physico-Chemical Analysis

Five samples of commercially available Mehiawah were bought from the local markets, at Manama (3 samples) and Muharraq (2 samples), and subjected to various physicochemical analyses. Water activity was estimated by measuring the water vapor pressure of commercially available Mehiawah at room temperature using YSI model 91HC Dew Point Hygrometer (Yellow Springs Instruments, Ohio, USA). Each sample was sealed in a closed container, and the probe was inserted above the sauce to measure the equilibrium relative humidity. Vapor pressure of pure water was used as a reference, and the water activity (a_w) was estimated as ERH/100. pH and redox potential were measured using Jenway pH meter (Jenway, UK) after 10x dilution with distilled H_2O. Sodium chloride was estimated according to the modified Volhard Method by the titration of appropriately diluted Mehiawah with silver nitrate using potassium chromate as an indicator (AOAC, 1995). Protein was determined after H_2SO_4 digestion using micro-Kjeldahl method followed by spectrophotometric determination of liberated ammonia using phenol/ nitroprusside/ hypochloride procedure (Keeney and Wilson, 1982).

Mehiawah Recipe and Preparation

Prior to the preparation of Mehiawah, an advice was sought from several women working in the field of fish fermentation. One recipe, practiced by an old and expert woman, was selected based on its clarity and commonality. Ingredients used to prepare Mehiawah were obtained from Muharraq markets and are listed in Table 1. Amount of each ingredients is usually based on one kg of the dried matoot (*S. gibbosa*).

The dried Mehiawah mix was prepared by the experienced old woman according to the above mentioned recipe, while the laboratory preparation was handled by a technician familiar with preparation of Mehiawah. Steps of preparation included: thoroughly rinsing of dried *Matoot* under running water, followed by air-drying for 3 days at room temperature. Mustard seeds were slightly roasted over light heat, while spices (caraway, cumin and fennel seeds) were mixed together, and than similarly roasted. Roasting step was performed to ensure effective seed dryness. Spices were then grounded and mixed with the dried Matoot in proportions mentioned in Table (1). Upon completion of these steps, the grounded mix was taken to the laboratory in plastic bags, placed into a pan, and warm distilled water was added (in a dried mix to water ratio of; 1 kg: 8 L). Salt was added to give a final concentration of 3%. Ingredients were aseptically hand-mixed using large stainless steel spoon, and distributed into 250-ml capped glass microbiological bottles. Each bottle received 50 ml of the liquefied mix. These bottles were subjected to the treatment scheme described on the following section.

Experimental Design.

Two bottles of freshly prepared Mehiawah mentioned above were assigned to each of the following four treatments: treatment 1: Mehiawah was autoclaved and not inoculated with of *S. aureus* to serve as a control; treatment 2: Mehiawah was not autoclaved and not inoculated with *S. aureus*, to estimate the aerobic total plate count (APC), as well as to examine the growth of naturally occurring *S. aureus* and LAB; treatment 3: Mehiawah was autoclaved and inoculated with *S. aureus*, to examine the fate of added *S. aureus*

Table 1. Local and English, scientific names and the quantities of ingredients typically used to prepare Mehiawah. Weight of matoot (dried sardines) is usually taken as the basis to measure other ingredients [a].

Local name	English name	Scientific name	Part used	Quantity[b]
Matoot (Dried)	Indian sardines	*Sardinella gibbosa*	Whole	1 kg
Khandal, Khardal	Mustard (dark brown)	*Brassica nigra*	Seed	2 kg[b]
Jeljalan, Kazbarah	Caraway	*Coriandum sativum*	Seed	0.25 kg[b]
Sannoot, Cammon	Cumin	*Cuminum cyminum* L.	Seed	One large tablespoon[b]
Hilwa	Fennel	*Foeniculum vulgare* Mill	Seed	One large tablespoon[b]

[a] *According to this recipe, sodium chloride is added to give a final concentration of 3% (270 g), whereas enough warm water is added to make a liquefied slurry and to bring the volume to 9 liters.* [b] *plant part used were seeds.)*

during the course of fermentation in the absence of any potential competitor; and treatment 4: Mehiawah was not autoclaved and inoculated with *S. aureus* in order to examine the effect of the indigenous bacterial flora on the growth of added *S. aureus*. Two replicas were used for each treatment. Treatments 1 and 3 were autoclaved at 121°C for 20 min, whereas treatments 2 and 4 were not autoclaved. .

Preparation of *S. aureus* Inoculum

A laboratory *S. aureus* isolate previously isolated from locally prepared Mehiawah was used as an inoculum. Isolation was performed using Baired-Parker (BP) (Oxoid, U.K) medium (Allaith, *et al.*, 2000). A single colony of *S. aureus*, maintained in tryptic soya agar (Oxoid), was used to inoculate nutrient broth. The broth was incubated at 35°C overnight and used as an inoculum. Enumeration of *S. aureus* was performed prior to the inoculation as described below. Three-mls inoculum, containing ~ 10^9 CFU/ml, was transferred into treatment 1, 2, and 3, to give initial counts of about 5-6 x10^7 CFU/ml. All treatments were mixed vigorously and incubated at room temperature (25 ± 1°C).

Time Course Experiments

Enumeration of *S. aureus* during the course of the experiment was performed in BP plates supplemented with fresh egg yolk (5%) and filtered-sterilized potassium tellurite (0.0125%) (AOAC, 1995). Two-ml aliquots from each treatment were aseptically withdrawn at specified intervals. One ml was added to 9 ml of sterilized saline solution (0.9%), mixed, serially diluted, and 0.1 ml aliquots was poured, in triplicate, onto BP agar plates. The plates were than incubated aerobically at 35°C and examined after 24-48 hrs. In this medium, *S. aureus* appears as black or dark gray colonies showing clear halo and/or an opaque zone. Serially diluted aliquots mentioned above were also used for the enumeration of the lactic acid bacteria (LAB). 0.1 ml of the diluents were poured, in triplicate, into MARS agar supplemented with 0.015% L-cysteine-HCl (Mccann, *et al.* 1996), and incubated anaerobically at 35°C using Oxoid anaerobic jar. Counts were taken after incubation for 72 hr. No attempt was made to differentiate the various LAB species. Total aerobic plate count (APC) was estimated in treatment 3 using a standard nutrient agar pour plate procedure. Means of counts of *S. aureus*, LAB, and APC were from triplicate assays of two separate experiments and are reported as colony-forming units (CFU)/ml.

To monitor the pH, the second one-ml aliquots was transferred into 9 ml of distilled water, mixed and the pH was measured as mentioned earlier.

RESULTS AND DISCUSSION

Physical and Chemical Properties of Commercial Mehiawah

Table (2) presents several physio-chemical characteristics of some commercially available home-made Mehiawah bought from the local markets, as well as of experimentally-prepared Mehiawah. Moisture content, water activity and NaCl of the commercial samples varied

considerably. Moisture content ranged from 70 to 83%, while water activity values ranged from 0.84 to 0.97 with an average 0.94. Furthermore, salt content showed considerable variation from as low as 2% to as high as high as 13%. Paludan-Müller, *et al.* (2002) had shown that salt concentration of 9-11% slowed the rate of fermentation in *plaa-som*; a Thai fermented fish product. In addition, the redox potential pointed out to an aerobic condition, thus favoring the growth of *S. aureus* (FDA, 2000). Despite these variations, the a_w and NaCl level remained within the growth limit of *S. aureus*. The limiting growth conditions of *S. aureus* are: the water activity (a_w) (0.83-0.99), pH (4-10), NaCl (maximum 25%), and temperature (7-50°C) (FDA, 2000). *S. aureus* have different limiting conditions for endotoxins production.

Table 2. Physio-Chemical Characteristics of Some Commercially Available Mehiawah and the Limiting Growth Conditions of *S. aureus*.

	Commercial		Experimental**
Parameter	**average***	**range***	**Average (±SD)**
a_w	0.98	0.84-0.99	ND
pH	3.39	3.1-3.9	6.75 (3.85)
Moisture (%)	77.4	70-83	76.2 (0.42)
NaCl (%)	5.2	2-13	3.1 (0.14)
Redox Potential	+ 93.4 mV	+56 to +125 mV	+ 164 (8.5)
Protein (%)	5.28	3.4-8.4	5.2 (0.11)

* *Average and range of 5 different samples of Mehiawah obtained from local market.*

** *Experimentally-prepared Mehiawah, average of 2 readings. Analysis was performed in the autoclaved Mehiawah prior to inoculation with Staphylococcus aureus.*

Variations in the physio-chemical characteristics of home-made Mehiawah probably reflected the different preparations used by different practitioners. They also suggest that Mehiawah, at present time, may not be considered as a standardized product. During the survey for Mehiawah recipe (data not shown), the major sources of variation found, and may directly affect the fermentation process, were: the amount of dried Matoot (may vary by up to 50% decrease from the recipe shown in Table 1), the source of complex carbohydrate added (wheat may replace mustard), the inclusion of acidic component such as dried lemon or dried lime peels, at initial stages of preparation, replacement of commercially available common salt with Persian or crude non-refined sea salt.

In addition, Mehiawah is a protein-rich product. Chemical analysis of amino acid content of similar Korean products indicate the presence of various amino acids in a free form (Chang, *et al.* 1994). Hence, in the absence of any potential competitor, such as LAB, the inhibitory effect in Mehiawah for *S. aureus* is probably due to the acidity, a condition which is not attained until the passage of several days, enough to allow for the growth *S. aureus* and produce toxins if its initial load due to contamination is high.

Growth studies with experimentally prepared Mehiawah

Table 3. presents total Aerobic(APC), *S. aureus aureus* and lactic acid bacteria count during the fermentation of experimentally prepared Mehiawah expressed as log CFU/ml (range).

Treatment 1: autoclaved and un-inoculated Mehiawah

Figures (1) to (3) show the log CFU/ml of APC, of *S. aureus* and LAB during the time course experiments of the treatments performed in this study. In treatment 1, which was used as un-inoculated control, data not shown, the autoclaved Mehiawah was free from *S. aureus* LAB and other aerobic bacteria.

Treatment 2: not-autoclaved and not inoculated Mehiawah

The initial normal count of *Staphylococcus aureus* was relatively low (10^3 CFU/ml). By day 3, *Staphylococcus aureus* decreased to below the detection limit (< 10 CFU/ml). Within the same period, LAB count increased exponentially without a sign of lag period, and by the days 2-3 the total count of LAB reached almost 10^7 CFU/ml from an initial 10^3 CFU/ml. Total LAB count remained

Table 3. Total Aerobic (APC), *Staphylococcus aureus* and Lactic Acid Bacteria Count During the Fermentation of Experimentally prepared Mehiawah Expressed as Log CFU/ml (range).

	Log CFU/ml								
Treatment/ parameter	**Days of Fermentation**								
	0	**1**	**2**	**3**	**4**	**6**	**9**	**12**	**14**
Total plate count									
1. Autoclaved & not inoculated	None	-	-	-	-	-	-	-	-
2. Not autoclaved & not inoculated	3.70 (3.63-3.85)	6.84 (6.35-6.96)	6.92 (6.85-7.1)	7.60 (7.46-7.88)		7.08 (6.70-7.35)	7.19 (6.95-7.51)		7.11 (6.78-7.38)
S. aureus									
1. Autoclaved & not inoculated	None	-	-	-	-	-	-	-	-
2. Not autoclaved & not inoculated	3.04 (2.91-3.42)	1.70 (1.62-1.95)	< 0.3	ND	-	-	-	-	-
3. Autoclaved & inoculated	7.74 (7.60-7.91)	7.48 (6.92-7.94)	7.17 (6.63-7.74)	7.26 (6.71-7.88)	7.28 (6.33-7.77)	7.18 (6.66-7.63)	7.38 (7.22-7.62)	7.34 (6.87-7.85)	7.38 (7.10-7.61)
4. Not autoclaved & inoculated	7.74 (7.65-7.88)	7.19 (7.00-7.37)	6.95 (6.87-7.08)	6.78 (6.73-6.91)	7.07 (6.94-7.20)	7.19 (6.95-7.30)	6.70 (6.62-6.88)	5.81 (5.65-5.94)	5.40 (5.2-5.60)
Lactic acid bacteria									
1. Autoclaved & not inoculated	None	-	-	-	-	-	-	-	-
2. Not autoclaved & not inoculated	2.93 (2.87-2.97)	1.0×10^5	7.0×10^6	1.5×10^7	2.1×10^7	2.0×10^7	2.3×10^7	2.15×10^7	2.4×10^7
3. Autoclaved and inoculated	ND	ND	ND	ND		ND	ND	ND	ND
4. Not autoclaved & inoculated	2.88 (2.76-2.91)	3.90 (3.86-3.98)	5.30 (5.05-5.45)	5.84 (5.78-5.95)	7.0 (6.56-7.32)	6.54 (6.90-7.10)	7.45 (7.30-7.67)	7.31 (7.26-7.49)	7.32 (7.20-7.54)

(Experimental conditions are given in Materials and Methods. Mean (CFU)/ml) from triplicate assays of two separate experiments).

essentially unchanged till the end of the experiment. Such growth was equivalent to a doubling time of 3.6 hrs (218 min). Counts of APC followed similar pattern as LAB, reaching the highest count of 10^7 CFU/ml by day 2, and remaining at that level all over the remaining days of the experiment. During the two-week experimental period, the pH dropped from an initial 6.1 to 3.64. Large decreased of pH coincided with LAB attaining higher count at days 2-4. It is known that low pH, due to lactic acid production and accumulation as a result of the action of LAB in carbohydrate and glucose, also inhibits the growth of LAB.

A previous study of the Thai fish sauce, *plaa-som*, had shown that salt concentration affect the fermentation process and the status of *S. aureus* spp (Paludan-Müller, *et al.*, 2002). At high salt concentration (9-11%), fermentation was inhibited and high count of *S. aureus* spp. was observed, a situation not found at low salt concentration (6-7%).(Paludan-Müller, *et al.*, 2002). In our study, the salt content in Mehiawah was low, thus neither the fermentation process nor the *S. aureus* growth were hindered, and the outcome of the experiment relied on the competition between the LAB and *S. aureus*, as discussed below.

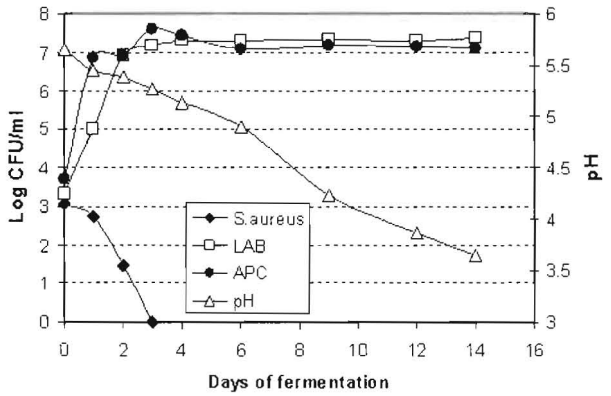

Fig. 1. Treatment 2; Counts of *S. aureus*, LAB, and Total Aerobic Bacteria (APC) and the Change in pH During the Course of Fermentation in Non-Autoclaved and Un-Inoculated Mehiawah. (See Materials and Methods for Experimental Details. Mean (CFU)/ml) of *S. aureus*, LAB, and APC from Triplicate Assays of Two Separate Experiments).

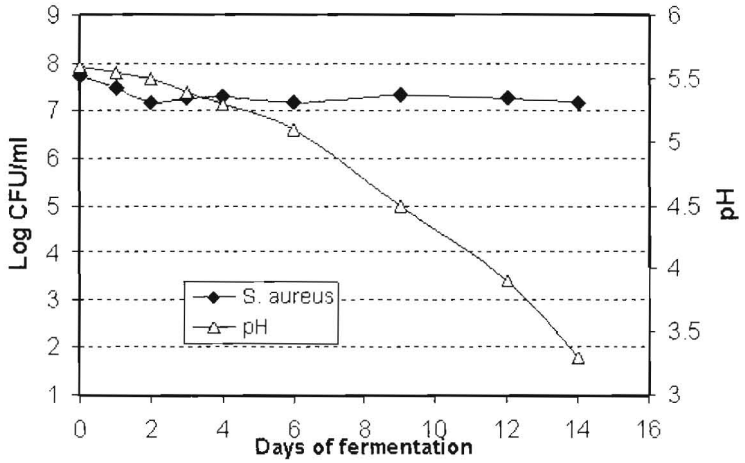

Fig. 2. Treatment 3; Counts of *S. aureus*, LAB, and Total Aerobic Bacteria (APC) and the Change in pH During the Course of Fermentation in Autoclaved and Inoculated Mehiawah. (See Materials and Methods for Experimental Details. Mean (CFU)/ml) of *S. aureus*, from Ttriplicate Assays of Two Separate Experiments).

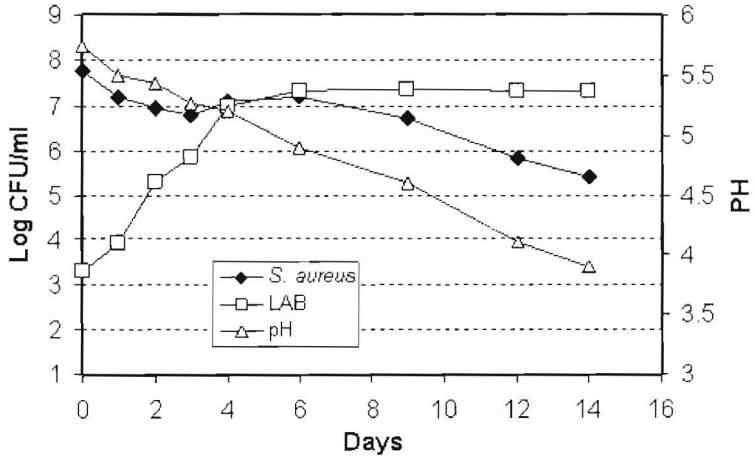

Fig. 3. Treatment 2; Counts of *S. aureus*, LAB, and Total Aerobic Bacteria (APC) and the Change in pH During the Course of Fermentation in Non-Autoclaved and Inoculated Mehiawah. (See Materials and Methods for Experimental Details. Mean (CFU)/ml) of *S. aureus*, and LAB from Triplicate Assays of Two Separate Experiments).

Treatments 3 and 4: Autoclaved-Inoculated and 4 Non-Autoclaved-Inoculated Mehiawah

Treatments 3 (Fig 2) and 4 (Fig 3) were both intentionally inoculated with comparable initial numbers of *S. aureus* of 5.5 x 10^7 and 5 x 10^7 CFU/ml, respectively. Within the first 24 hrs, the number of inoculated *S. aureus* in treatment 3 decreased from 5.5 x10^7 to 3.0 x10^7 CFU/ml, and remained at that level until the end of the experiment. A similar growth pattern of inoculated *S. aureus* was found when it was applied at high load (~ 8.5 $\log_{10}$ CFU/g) to milk before cheese making, the counts of the control showed no significant decreases during 30 days of storage (López-Pedemonte, *et al.* 2006).

During the two weeks period, the pH decreased from an initial value of ~ 5.9 to ~ 3.9. Since, no LAB were present in Mehiawah, the decrease in pH was probably due to the leaching process of free amino acids from ground *Matoot*, and to the effect of exogenous and extracellular proteases produced by the inoculated *S. aureus*. Furthermore, *S. aureus is known* to metabolize complex carbohydrates, as well as some monosaccharide, such as glucose, and produce lactic acid (Axelsson, 2004; Blumenthal, 1972). Though the Initial pH of Mehiawah was within the range of Staphylococcus growth, after about 10 days, the pH dropped significantly in the absence of LAB activity,. At that stage, there was gradual decrease, though slow, in the total count of *S. aureus* reaching ~ 10^5 CFU/ml at the end.

The growth of inoculated *S. aureus* in the presence of naturally occurring LAB, carried over by various ingredients, in the un-autoclaved Mehiawah is shown in Fig 3. During the first few days of inoculation, *S. aureus* behaved similarly as in the case of the autoclaved Mehiawah. However, as the count of LAB started to increase exponentially at day 3 and as the pH decreased, the number of *S. aureus* gradually decreased during the second week of fermentation from 10^7 to 10^4 CFU/ml During the two weeks fermentation period, LAB increased from 10^3 to 10^7 CFU/ml within the first 4 days and remained at that level until the end. The pH decreased .from 5.7 to 3.5, similar to that reported for in other treatments mentioned earlier. It is apparent from Fig 2 and 3 that the growth of natural LAB resulted in speeding up the decrease in pH; i.e. at day 6 of fermentation, the pH value of autoclaved and un-autoclaved Mehiawah were 5.3 and 4.72 respectively.

Growth rate of *S. aureus* in laboratory medium and food systems as well, is influenced by pH, temperature, NaCl, and nutrient availability (FDA 2000; Dengremont and Membre, 1995; Notermans and Heuvelman, 1983). It is also well known that *S. aureus* is a weak competitor, and could easily be outgrown by vigorous competitor or effector organisms such as LAB. Gianluigi, *et al.*(2004), showed that a pathogenic *S. aureus* strain was inhibited, independently of its inoculum, by *L. plantarum.* Arslan and Uraz (2005) reported that *S. aureus* strain was inhibited by 81% of 32 LAB isolated from milk. Inoculation of the several lactic acid bacteria strains depressed the propagation of *S. aureus* cells and their enterotoxin production during meat fermentation. However, Gomolka – Pawlicka, *et al.* (2004) found that out of 15 LAB spp, only one showed antagonistic effect on studied strains of *S. aureus* both in vitro as well as in meat and raw sausages while five other strains of LAB spp. showed the antagonistic effect in vitro only. In *plaa-som*, a similar fish sauce, when fermentation by LAB was not inhibited, *S. aureus* was no longer a serious a threat.(Paludan-Müller, *et al.* 2002).

S. aureus is capable of producing endotoxins when the cell density reaches beyond 10^7 CFU/ml or g (Wong, *et al.* 2004; Beckers, *et al.* 1985). Beckers, *et al.* (1985) had shown that, in aseptically peeled shrimp, the total *S. aureus* flora increased by 3-4 log units and endotoxins were detected when the number exceeded 10^7 CFU/g. The initial bacterial density of *S. aureus* used for inoculation in the current study was high (10^7 CFU/ml), a situation that may not be encountered in real life situations except under very poor hygienic conditions. In such circumstances, Mehiawah contaminated with *S. aureus* will support both the growth and production of staphylococcal endotoxins (SETs), in the absence of LAB, as evident by the survival of the inoculated bacterium long enough at high level. Attention to probable public health hazards associated with home–prepared foods was previously drawn by Musaigar and Jaidah

(1999).

On the other hand, Mehiawah as a food system may support the growth of both *S. aureus* and LAB, since both the initial pH and the final salt content are within its growth limits. However, LAB produce a variety of inhibitory substances against other competitors including *S. aureus* (Ouwehand and Vesterlund, 2004). Only in the absence of LAB, *S. aureus* may take an advantage and grow to high level with subsequent production of STEs. This situation is not a remote possibility in the case of the production of Mehiawah, since the major source of LAB in this case are the mustard and other spices used for the preparation. Normally these ingredients are subjected to roasting (medium heat treatment) which may result in minimizing the number LAB, hence prolonging the fermentation process, giving access to *S. aureus* to proliferate. Further work is needed to clarify the source(s) of LAB in Mehiawah fermentation.

CONCLUSION

Despite the fact that results of this study is limited to experimentally-prepared fermented Mehiawah, it can be concluded, based on these results, that the home-made Mehiawah, prepared under sub-hygienic conditions with salt content exceeding the normal range of LAB growth, may possess health risks, since the prevailing conditions at the commencement of fermentation, and in the absence of natural competitor and effector organisms of *S. aureus*, allow for the growth of pathogenic *S. aureus* and the probable production of SETs. Based on the finding of this study and others, Mehiawah fermentation and its health status may be advanced by either marinating the salt concentration at high level in the final product (~10%), or using starter LAB, thus speeding up the fermentation process. Furthermore, standardizing the Mehiawah sauce may reduce the current variability among commercially available Mehiawah. Currently, the state of our knowledge on the microbiology of Mehiawah is insufficient and more work should be done. In addition, as a natural fermented food product, consideration should be given so Mehiawah could be effectively utilized as a probiotic since its intake is associated with high count of LAB.

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