Microbial Safety and Quality and Protein Integrity of Shrimp Sold in Shops in Riyadh, Saudi Arabia

MOSFFER M. AL-DAGAL

Food Science Department, College of Agriculture, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia

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ABSTRACT

The microbial safety and quality were assessed of shrimp from fish shops located in five separate zones in Riyadh City, Saudi Arabia. Protein hydrolysis of the shrimp was determined as a function of microbial growth by using an isoelectric focusing technique. A resuscitation culture step also was assessed and compared with direct plating on selective media for detection of the coliform group and fecal enterococci. The results of this study indicated high (6.23 to 7.23), moderate (4.10 to 5.94), and low (3.49 to 3.94 log CFU/g) ranges of psychrotrophic counts in 23.3, 60, and 16.7% of the samples, respectively. Statistical analysis showed significant ($P < 0.05$) differences in the psychrotrophic counts among the zones tested, with the highest counts being in the western and central zones, and the lowest counts in the northern zone. Similar results were obtained with coliform, fecal enterococci, and staphylococci counts. Use of the resuscitation step resulted in significantly ($P < 0.05$) higher recovery of coliforms and fecal enterococci. Band intensities of the sarcoplasmic proteins, as shown in the gel electrophoresis, were faint in samples with higher counts and comparatively intense in the ones with lower counts, indicating a clear relationship between product degradation and microbial load.

Key words: Shrimp, seafood, Riyadh, microbial quality, microbial safety, protein integrity

The amount of shrimp consumed in Japan, the countries of Europe, and the United States is estimated to be more than 10,000,000 kg per year (13). In Saudi Arabia (SA), industrial shrimping started in 1963 on the East Coast (9) and thereafter in Jizzan (southwestern SA). The increasing amount of seafood, including shrimp, consumed is represented by the spreading of fish sales outlets in big cities such as Riyadh. The leading company in this field is The Saudi Fisheries Company (SFC) which started shrimp (and other seafoods) production in 1981 and had more than 47 sales outlets at the end of 1993. In the period from 1981 to 1993, the SFC produced and processed more than 20,000 tons of shrimp (16, 18).

Maintaining the quality of shrimp is important not only because it is a perishable product, but also because of the increasing demand for this product in the Saudi Arabia market. The primary sources of contamination that affect shrimp safety and quality, especially on the East Coast, are sewage, industrial wastes, and agricultural drainage (15).

The deterioration of shrimp quality sold in any of these shrimping regions or transported to major cities like Riyadh can be attributed to the delay in postharvest refrigeration and/or mishandling during transportation, storage, and processing. Quality determination of this valuable product can be assessed microbiologically by enumerating spoilage microorganisms (e.g., psychrotrophic count), chemically by measuring some compounds (e.g., trimethyl amine, total volatile base, and alpha-amine nitrogen), and physically by assessing protein integrity using gel electrophoresis and/or water-holding capacity (1, 12).

Studies conducted on Saudi Arabia's shrimp have been limited to its ecology and chemical contamination, especially in the Ad-Dammam area (East Coast). This study, therefore, was intended to determine the quality and safety of shrimp sold in Riyadh City as well as to compare the psychrotrophic counts with protein electrophoresis as indicators of product deterioration.

MATERIALS AND METHODS

Shrimp samples

Fresh raw shrimp was purchased from one wholesale outlet (center) and in four (north, south, east, and west) other regions in Riyadh City. The central and western areas are older and more crowded than the others, especially the northern zone where the shops are new and the population is much less crowded. In addition, several shops in the western region seem to get their supply from the wholesale market (central zone). The samples (6 samples from each region, each weighing 1 to 1.5 kg) were prepared (peeled and washed) in the sales outlets as if they were to be sold for ordinary consumers. Cleaned samples were immediately put into an insulated container along with ice surrounding the bags of samples and rushed to the laboratory within 20 to 30 min. Microbiological tests were conducted as soon as the samples reached the laboratory. Samples for the isoelectric focusing assay were kept frozen at $-40^\circ$C until used. General notes concerning the sanitation conditions and personal hygiene in the sales outlets were collected when purchasing the samples.
Microbiological assays

Psychrotrophic plate counts were conducted by using the method outlined by Cousin et al. (3). One hundred and eighty milliliters of 0.85% saline were added to 45 g of peeled shrimp placed in sterile stomacher bags (Model 400, Seward Medical, London, UK) and the mixture was homogenized for 2 min using the Stomacher 400. Appropriate further dilutions were made from this mixture for the different microbiological assays. Portions (1.0 ml) of the selected dilution were mixed with 13 to 15 ml of warmed tryptic soy agar (TSA) (Oxoid Ltd, Basingstoke, Hampshire, UK) which was poured into duplicate petri dishes. After solidification of the agar, the plates were incubated for 7 to 10 days at 7°C.

Total coliform counts were determined in the shrimp samples using a violet red bile agar (VRB, Oxoid) plating method. From the sample homogenate prepared for the psychrotrophic count, 1-ml portions of the desired dilution were dispensed into duplicate petri dishes and mixed with about 10 ml of tempered (45 to 48°C) VRB agar. After solidification, the plates were overlaid with 5 ml of VRB agar. Another set of plates was used for a resuscitation step in which the diluted shrimp sample was mixed with 5 to 6 ml of TSA and incubated for 2 h at 35°C. After this step, 10 to 12 ml of VRBA were overlaid with the TSA. All of the plates were incubated at 35°C for 24 h (5, 7).

Fecal enterococci (formerly known as streptococci) were enumerated in a way similar to the coliform group. Portions (1 ml) of the sample mixture were mixed with 15 ml of KF-streptococcus agar (KF-SA, Oxoid), to which 1 ml of 1% triphenyltetrazolium chloride (TTC) was added just before mixing. For the resuscitation step, the sample was plated first using 5 to 6 ml of TSA and then overlaid with 10 to 12 ml of KF-SA after incubation for 2 h. All of the plates were incubated, after solidification, for 48 h at 35°C. Colonies with a deep red color were counted as enterococci (5, 6).

Staphylococci were enumerated using Vogel-Johnson agar (Oxoid). The shrimp homogenate was plated as previously stated for the other microbiological assays. The plates were incubated for 48 h at 35°C and colonies with a black precipitate were counted and considered presumptive pathogenic staphylococci (4, 10).

Isoelectric focusing

This technique was applied to determine the hydrolysis of shrimp sarcoplasmic protein as a function of microbial growth.

a. Preparation of ultra-thin-layer polyacrylamide gels.

Isoelectric focusing of shrimp proteins was performed according to the procedure of Radola (14) with some modification. Total monomer concentration was 6% and methylene bis-acrylamide was 3% of the total monomer. Then 40% (wt/vol) amphotolyte, pH range 3.5 to 10 or 2.5 to 6, were added to form the gel with final concentration of 1:20 parts (vol/vol). Ten percent glycerol was added to compare the consistency of the gel (20).

b. Shrimp sample preparation for sarcoplasmic protein extraction.

About 5 g of the shrimp were blended with distilled cold water and centrifuged 15,000 rpm for 15 min. The supernatant was used for isoelectric focusing.

c. Ultra-thin-layer isoelectric focusing of shrimp samples.

The ultra-thin-layer isoelectric focusing was carried out in a Pharmacia flat bed chamber, Multiphor II type, equipped with EPS 3500 XL power supply (Pharmacia, LKB, Sweden). A cooling solution at 4°C was circulated through apparatus from a Multi Temp II constant temperature circulator (Pharmacia). The electrode solutions were 25 mM aspartic acid and 25 mM glutamic acid at the anode, and 2 N ethylenediamine containing 25 mM arginine and 25 mM lysine at the cathode. For the 10 by 5 cm gel the power setting was limited to 2,000 V and 100 W; the endpoint value was 600 V - h. After prefocusing at 400 V, about 10 μl from the supernatant of shrimp samples were directly applied on the gels with a syringe (Syringe Perfection, SGE, Australia). Immediately after focusing, the gels were transferred to a 20% (vol/vol) solution of TCA for fixation of protein and removal of carrier ampholytes (5min). The gels were stained with 0.2% (wt/vol) solution of Serva (Heidelberg) violet 49 in universal solution (ethanol, distilled water, and acetic acid).

RESULTS AND DISCUSSION

The psychrotrophic counts were high (6.23 to 7.23 log CFU/g) in about 23% of the 30 samples. However, 60% of the samples were in the range of 4.10 to 5.94 log CFU/g, and more than 16% ranged from 3.49 to 3.94 log CFU/g. Although some samples had unacceptable psychrotrophic counts, that does not mean the shrimp had decomposed (17), especially if the contamination of shrimp was accidental during the last stages of preparation for sales. This fact emphasized the importance of applying methods other than microbiological ones to determine the extent of product deterioration. On the other hand, an unacceptable smell was noted in some samples with moderate (4.10 to 5.94 log CFU/g) counts. The obvious interpretation of such a finding could be that the spoilage depends not only on the history of microbial contamination, but also on the type of biochemical activity of the contaminating microorganism, which in turn is affected by the microflora of the shrimping waters.

Table 1 shows the means and ranges of psychrotrophic counts in the five different zones. An analysis of variance indicates significant (P < 0.05) differences among them. Samples from the north section of Riyadh had the lowest mean. This outcome could be attributed to good handling in the newly built fish shops. Although a large number of customers were noticed in the central zone, it ranked second to the highest in psychrotrophs. Obviously, this was due to the very poor sanitary conditions during display (floating shrimp in ice drainage water) and during cleaning. In some shops, the shrimp was also sold in big steel containers in the open rather than being in confined and refrigerated cabinets. The highest range (5.02 to 7.22 log CFU/g) of psychrotrophs was found in the western region. This finding could be explained best by the fact that most of the small shops in this area were supplied from the wholesale (central) market in which the shrimp already had been mishandled.

In relation to microbial growth and its partial effect on the shrimp protein, Figure 1 shows sarcoplasmic protein

<table>
<thead>
<tr>
<th>City zone</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>4.54</td>
<td>3.491-5.000</td>
<td>0.628</td>
</tr>
<tr>
<td>Center</td>
<td>4.96</td>
<td>3.620-6.803</td>
<td>1.243</td>
</tr>
<tr>
<td>South</td>
<td>4.93</td>
<td>4.146-6.244</td>
<td>0.978</td>
</tr>
<tr>
<td>East</td>
<td>4.75</td>
<td>3.671-5.910</td>
<td>0.897</td>
</tr>
<tr>
<td>West</td>
<td>6.05</td>
<td>5.019-7.217</td>
<td>0.909</td>
</tr>
</tbody>
</table>

* Means followed by different letters are significantly different.
patterns in gels with a pH gradient of 3.5 to 10. According to the microbiological results, gels 1 to 5 illustrate good-quality shrimp samples in which the psychrotrophic counts ranged from 3.49 to 3.94 log CFU/ml, while gels 6 to 10 represent low-quality shrimp samples in which the psychrotrophic counts ranged from 6.37 to 7.22 log CFU/g. All tested samples gave the same major patterns with isoelectric points in the pH range 2.5 to 6 (anode region) and very faint bands in the upper part of the gel (cathode region) with isoelectric points in the pH range 6 to 9. However, remarkable differences can be seen in the intensity of the stained bands: protein patterns from good-quality samples (1 to 5) had very intense bands while protein patterns from low-quality samples had less intense bands, indicating hydrolysis in the sarcoplasmic protein. The results reflect degradation of the sarcoplasmic proteins of the shrimp during handling and cold storage.

The same samples were separated on a 2.5 to 6 pH gradient in order to distribute the anodic bands along the complete length of the gel (Figure 2). The pattern obtained in this narrow pH gradient confirmed the observation from the first set of gels.

Total coliform counts of the shrimp samples from the different zones of the city are shown in Table 2. The coliform count pattern was similar (considering the direct plating using VRBA) to the psychrotrophic counts. Shrimp from the western zone had the highest mean count, followed by the central zone, and the lowest mean count was found in the northern zone. Fecal enterococci count means were highest in shrimp from the central zone (and in the western zone in the case applying the resuscitation step) and lowest in the northern zone, which is another indication of the better quality of the shrimp sold in this area.

A study to modify the fecal streptococci (enterococci) standards in India of 100 CFU/g of frozen shrimp suggested a proposed standard of 1,000 CFU/g. This recommendation was based on a study that showed 64% of test samples exceeding 100 CFU/g of fecal enterococci when samples were plated on KFS agar (11). Applying a resuscitation culture step, as was done in this work, could have resulted in a higher percentage, because freezing causes more injury to bacterial cells than refrigeration. Statistical analysis comparing the two methods of coliform (and fecal enterococci) enumeration showed a significant \( P < 0.05 \) difference. Because of this finding, it is highly recommended to use the resuscitation step when plating such microorganisms under similar conditions in order to reduce the chance of underestimating the numbers of contamination indicators.

The means and ranges of staphylococci counts in the different city zones are shown in Table 2. As with the other microbial tests, the mean counts were the highest in the western zone, followed by the central zone, and the lowest mean counts were obtained in the samples from the northern zone.
zone. Other studies also have indicated the importance of staphylococci as a predominant entity in fresh (2, 8) and frozen (19) shrimp. From our observation during purchasing the samples and in other studies (19), it appears that shrimp is most likely to be contaminated with staphylococci by the handlers in the different stages of preparation after harvesting.

In conclusion, this study indicates poor-quality shrimp in Riyadh City, especially in the wholesale market (central zone) and at the shops of the western region. To improve the quality of such a valuable product, shrimp always should be kept cold with crushed ice in closed refrigerated cabinets or inside clean insulated boxes. Also workers should receive proper training in sanitation and personal hygiene. They should handle this product under good conditions. These measures become more and more important during the summer season, when the temperature can reach 45°C.

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REFERENCES


