



Microbiological and chemical quality of a traditional salted-fermented fish (Hout-Kasef) product of Jazan Region, Saudi Arabia



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ABSTRACT

The Hout-Kasef is traditional salted fermented fish product of natural fermentation of salted mullet fish of coastal area of Jazan region of Saudi Arabia. The present study was carried out to investigate the microbiological and chemical characteristic of Hout-Kasef. A total of twenty-four salted fish samples were purchased from fish market in Jazan and Abu-Arish at different times of the year. The microbial studies of salted-fermented fish revealed a total bacterial count ranging from 2.81 to 4.72 Log₁₀ CFU/g, yeast and mold counts ranging from 0.48 to 3.14 Log₁₀ CFU/g, total staphylococci count 2.71–3.85 Log₁₀ CFU/g, halophile bacteria count 3.26–5.14 Log₁₀ CFU/g, and coliforms count <1 Log₁₀ CFU/g. However, pathogenic bacteria such as *Listeria monocytogenes*, *Vibrio* spp., *Campylobacter* spp. and *Yersinia* species were not detected. The major bacteria species isolated and identified from the salted fermented fish were *Bacillus Subtilis*, *Bacillus mycoides*, *Bacillus licheniformis*, *Bacillus pumilus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus xylosum*, *Staphylococcus sapropticus* and *Staphylococcus cahmii* subsp *cahmii*. The chemical analysis of salted fermented fish showed high content of moisture (47.96%), protein (25.71%), ash (19.6%) and salt (15.19%) but low contents of lipid (7.25%). The salted-fermented fish also showed high level of total volatile basic nitrogen (78.86 mg/100 gm sample) and thiobarbitric acid number (32.32 mg malonaldehyde/kg) with a pH value of pH 6.3. Finally, this study showed the presence of gram positive and gram negative bacteria in the fish product. The predominant microorganisms found were *Bacillus* and *Staphylococcus* spp. The fish product had high content of salt and TVB-N levels.

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1. Introduction

Jazan region is located in the southwestern part of the Kingdom of Saudi Arabia. The land extends along the Red Sea coast for more than 300 km with more than 100 islands scattered along the coastal area. Jazan is a strategically important sea-port and considered as one of the fishery resources in the Kingdom. There are many fish species found at Jazan region such as Emperor (*Lethrims miniatus*), Spanish mackerel (*Scomberomorus commerson*), Mullet (*Valamugil scheli*) and Jacks (*Alepes djeddaba*). The Mullet (*Valamugil scheli*) species is mainly used in production of the salted fermented fish locally known as Hout-Kasef. In the past this method

was used to preserve the fish however, now the salted fermented fish is consumed during certain time of the year (*Eid Al-Fiter and Eid Al-Adha*) with special foods. Fresh fish is one of the most highly perishable food products. The fish quality deteriorates rapidly during handling and storage that limits the shelf life of the product (Sallam, 2007). The salting process of fish is one of oldest methods in preservation of fish, and is still used in many parts in the world. Traditional fermented fish products are basically salt fermented products, depending on the amount of salt added; the products can also be classified into high salt (more than 20% of total weight), low salt (6–8%) and no salt products. The process of salting fish is influenced by many factors such as weather, size and species of fish and the quality of salt used (Kristin Anna, 2010). Salting preserves by lowering the moisture content of the fish to the point where bacterial and enzymatic activities are retarded, moreover chloride ions are toxic to some microorganisms (Wheaton and Lawson, 1985).

The aim of this study was to study and identify the micro flora associated with salted fermented fish and to determine the proximate composition, salt, total volatile basic nitrogen (TVB-N)

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content, thiobarbitric acid number (TBN) and pH, of the salted fermented fish product of Jazan.

2. Materials and methods

2.1. Preparation of salted fermented fish

The whole, fresh fish is cleaned, gutted and covered with salt and arranged in alternate layers either in wooden boxes and then left to ferment on fishing boat for month or more, depending on ambient temperature. Liquid exudates from the fish during fermentation time are allowed to drain off.

2.2. Sample collection

Twenty-four salted fermented fish samples were purchased from fish market in Jazan and Abu-Arish at different times of the year. Samples were transferred to the Laboratory at King Saud University in Riyadh for analysis.

2.3. Microbiological analysis

Samples for microbiological analysis were prepared by homogenizing 10 g of fillet-salted fermented fish in 90 ml of 0.1% peptone water (Oxoid) using stomacher lab – Blender 400 (Seward Medical, London, U.K) for 30 s at a normal speed. Decimal dilutions were prepared using sterile 0.1% peptone water solution. 1 ml of the homogenate was used for enumeration of microorganisms. Counts were done by using pour plate and spread techniques as described in the Compendium of Methods for Microbiological Examination of Foods (APHA, 2001) and Food and Drug Administration (FDA, 1998).

2.3.1. Total aerobic bacterial count

Total aerobic bacterial count was determined using the pour plate technique. Plate Count Agar (Oxoid, CM463) was used as medium. Plates were incubated at 32 °C for 48 h.

2.3.2. Total coliform count

Total coliform count was determined on violet red bile agar (Oxoid) using pour plate technique and an overlay with the same agar after solidifying. Plates were aerobically incubated in inverted positions at 37 °C for 24–48 h.

2.3.3. *Staphylococci* spp.

Staphylococci spp. were determined on Baird Parker Agar (Oxoid CM 275) supplemented by egg yolk tellurite at 37 °C for 24–48 h. Typical black colonies with zones around and atypical black colonies were considered as *Staphylococcus* spp.

2.3.4. Yeasts and molds

Yeasts and molds were enumerated on acidified potato dextrose agar (Oxoid). Plates were incubated aerobically upright at 25 °C for 5 days.

2.3.5. Halophilic bacterial count

Halophilic bacterial count was done using Plate Count Agar medium (Oxoid-PCA) with 10% NaCl. Plates were incubated at 37 °C for 3 days.

2.3.6. The presence of pathogenic bacteria

The presence of pathogenic bacteria such as *Vibrio* spp., *Campylobacter* spp., and *Listeria monocytogenes* was checked using

methods described in Food and Drug Administration (FDA) Bacteriological Analytical Manual (1998).

2.3.7. Microbial identification

Representative colonies were selected from plate counts according to shape and color. Selected colonies were isolated by transferring and streaking on nutrient agar until culture purity was established and further examined by Gram stain.

Characterization and identification of staphylococcal species was carried out using API ID 32 Staph and API CH50 kit was used for identification of bacillus species (API system–BioMeurix, Marcy l'etoile, France).

2.4. Chemical analysis

2.4.1. Proximate composition

The proximate analyses of 24 salted fermented fish samples were carried out in duplicate. The moisture, crude protein (Nx6.25), ash, and fat contents were determined according to AOAC (1990).

2.4.2. Determination of salt

The percentage of salt was determined according to the procedure described by Pearson (1973). One gram of sample was weighed accurately and mixed with 50 ml of distilled water. The mixture was titrated with 0.1 N silver nitrate using 0.5–1 ml of 5% potassium chromate to the first appearance of a slight orange color against the yellow color of the indicator. The percentage of NaCl in the sample was calculated as follow: 1 ml of 0.1 AgNO₃ = 0.005845 g of NaCl.

2.4.3. Determination of total volatile basic nitrogen (TVB-N)

The determination of TVBN was conducted according to the procedure of Pearson (1973). 10 g of sample was blended with 50 ml of fresh tap water; the blender was washed with 250 ml of fresh tap water into the distillation flask and 1–2 g of MgO was added to the mixture. TVB-N was released by boiling the mixture with magnesium oxide, which prevented volatile acids from distilling over into the boric acid. The distillate of volatile nitrogen was received in 25 ml of boric acid 2.0% then titrated by 0.1 N sulfuric acid. The result was calculated as follow: TVB-N (mg N/100 g) = Titration (ml of 0.1 N acid) × 14.

2.4.4. Determination of thiobarbituric acid number (TBA)

The method of Pearson (1973) was used for determining the TBA in salted fish. Ten gram of sample was mixed with 50 ml of water in a mechanical blender then the mixture washed by 47.5 ml of water into a suitable distillation flask and 2.5 ml of 4 N HCl was added to bring the pH to 1.5. After distillation about 50 ml in 10 min exactly from the boiling time commences. Five milliliter of distillate was boiled with 5 ml of TBA reagent (0.2883 g of thiobarbituric acid in 100 ml of 90% acetic acid) for about 35 min exactly. The absorbance was recorded at 538 nm and the number of TBA was calculated as follow:

TBA number (mg malonaldehyde/kg sample) = 7.8 × absorbance.

2.4.5. Measurement of pH

The pH of the samples, was measured using a digital pH-meter (JenwayPH10 flested UK), after calibration using standard buffer solutions of pH 4 and pH 7.

3. Results and discussion

3.1. Microbiological analysis

Results of microbiological analysis are given in Tables 1 and 2. The microflora of salted–naturally fermented fish consisted of various species of micro-organisms such as aerobic, halophile, and staphylococcal bacteria, yeasts and molds. The microbial load of Hout-Kasef samples range from 2.81 to 4.72 with an average $3.77 \log_{10}$ CFU/g for the total aerobic bacterial count, from 3.61 to 5.14 with an average $4.32 \log_{10}$ CFU/g for Halophile bacteria count, from 2.71 to 3.85 with an average $3.23 \log_{10}$ CFU/g for Staphylococcal count, and from 0.48 to 3.14 with an average $1.33 \log_{10}$ CFU/g for Yeasts and molds Count. Total Coliform count was found less than $1.0 \log_{10}$ CFU/g. No *Vibrio* spp., *Listeria monocytogenes*, *Yersinia* spp. and *Campylobacter* were detected in all samples. The findings of this study were comparable to those of salted Grey Mullet and Kass, smoked Atanatic herring and fermented salted Mugil cephalous fish (Fesskh) (Achinewhu et al., 2004; Patir et al., 2006; Ahmed et al., 2010 and Adegunwa et al., 2013). The absence of coliforms and other pathogenic bacteria could be attributed to the salt added during fermentation, proper hygiene and good manufacturing practices during processing of fish. The identification and distribution studies of microorganism associated with salted fermented fish (Hout-Kasef) showed that *Bacillus* spp and *Staphylococcus* spp. were the predominant genera. The *Bacillus* isolates were identified as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus mycoides* and *Bacillus pumilus*; whereas *Staphylococcus* species consisted mainly of *Staphylococcus lentus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus sapropticus*, *Staphylococcus xylosus* and *Staphylococcus cahonii subsp cahonii*. (Table 2). Results obtained in this study are in agreement with those obtained by Thapa et al. (2004), who reported that *Staphylococci* and *Bacilli* were the dominant groups of bacteria isolated from ngari, hentak and Tungtag fermented fish products of North-East India. Similarly, Anihouvi et al. (2006, 2007) observed that *Staphylococci* and *Bacilli* were the dominant groups of bacteria isolated from lanhouin and cassava fish traditionally processed fermented fish product in the

Table 1
Microbiological counts of salted fermented fish samples (\log_{10} CFU/g).

Microorganisms parameters	Mean	Minimum	Maximum
Total aerobic bacteria count	3.77	2.81	4.72
Coliforms count	<1	<1	<1
Staphylococci count	3.23	2.71	3.85
Yeasts & molds count	1.33	0.48	3.14
Halophile bacteria count	4.32	3.26	5.14
<i>Vibrio</i> spp.	ND*	–	–
<i>Listeria monocytogenes</i>	ND	–	–
<i>Campylobacter</i>	ND	–	–
<i>Yersinia</i> spp.	ND	–	–

ND* = Not detected in 25 g of sample.

Total number of samples = 24 salted fermented fish.

Each sample was done in duplicate.

Table 2
Staphylococci and *Bacillus* species isolated from salted fermented fish samples.

Bacillus species	Staphylococci species
<i>Bacillus Subtilis</i>	<i>Staphylococcus aureus</i>
<i>Bacillus mycoides</i>	<i>Staphylococcus epidermidis</i>
<i>Bacillus licheniformis</i>	<i>Staphylococcus hominis</i>
<i>Bacillus pumilus</i>	<i>Staphylococcus xylosus</i>
	<i>Staphylococcus sapropticus</i>
	<i>Staphylococcus cahonii subsp cahonii</i>

Republic of Benin. In contrast Koffi-Nevry et al. (2011) reported that the fermentation of Agjuevan a fermented fish from Ivory Coast, was dominated by lactic acid bacteria.

3.2. Chemical analysis

The results of chemical analysis of fermented salted fish (Hout-Kasef) are summarized in Tables 3 and 4. The salt-fermented fish is good source of protein. It contains relatively high amount of moisture ranging from 44.8 to 53.46% with a mean of 47.96%. Results obtained in this study are in agreement with those reported for fermented cassava fish (Lanhouin) and tigger fish (Tirkin) (Anihouvi et al., 2009; Mohammed, 2010), but lower than those reported by Koffi-Nevry et al. (2011) for Adjuevan traditional Ivorian fermented fish products. The protein content of Hout-kasef ranged from 23.44 to 29.56 with a mean value 25.71%. Similar results were reported for Momone, lanhouin and Tirkin fermented fish products (Abbey et al., 1994; Anihouvi et al., 2009; Mohammed, 2010). The ash contents varied between 17.67 and 22.46% with a mean value of 19.60% and salt content ranged from 12.72% to 19.74% with an average 15.19%. The salt content in this study is higher than those reported for fermented fish products (Koffi-Nevry et al., 2011 and Anihouvi et al., 2006), but lower than that reported for Wadi Betok, a traditional fermented fish from South Kalimantan, Indonesia, (Petrus et al., 2013). The fat content varied between 5.46 and 9.82 with a mean of 7.42%. The fat content of salted fermented fish reported here was in agreement with that reported for the Fassiikh fermented fish product in Sudan (Osman et al., 2012), but higher than that of Wadi Betok, a traditional fermented fish from South Kalimantan Indonesia (Petrus et al., 2013). These differences could be attributed to fish species, salt content, environmental conditions, and different processing technologies.

Results of total volatile basic nitrogen (TVB-N mg/100 g) and thiobarbituric acid (mg malonaldehyde/kg) and the pH values are given in Table 4. Quality analysis, of salted-fermented fish revealed high contents of TVB-N and TBA. The TVB-N contents in salted-fermented fish ranged from 32.20 to 131.82 with an average value 78.86 mg/100 g. All the present samples of Hout-Kasef contained a high level of TVN-B, at which was above the acceptable limit 30 mg TVB-N/100 g as the upper limits, above which fishery products are considered unfit for human consumption (Lannelongue, 1980; Sikorski et al., 1989). Results obtained in this study are in agreement with those obtained by Anihouvi et al. (2012) who reported high TVB-N value ranging from 264.7 mg/100 g to 389.9 mg/100 g for naturally fermented cassava fish for Lanhouin Production. Similarly, Roy et al. (2014) observed high level of TVB-N (210.92 mg/100 g) in Telesech-fermented fish products of Tripura State in India. The TVB-N is widely used as an indicator of fish spoilage; its increase is related to the activity of spoilage bacteria and endogenous enzymes (Ozogul et al., 2004; Ruiz-Capillas and Moral, 2005). According to Silva et al. (1998), TVB-N level below 2–3.6 mg N/100 g indicates that the fish is fresh, whereas the fish would be rejected for human consumption when the TVB-N level exceeds approximately 50 mg N/100 g.

Table 3
Chemical analysis of salted-fermented fish samples (Based on wet weight %).

Parameters	Mean	Minimum	Maximum
Moisture%	47.96	44.80	53.46
Protein%	25.71	23.44	29.56
Fat%	07.52	05.46	09.80
Ash%	19.60	17.67	22.46
Salt content%	15.19	12.72	19.74

Total number of samples = 24 salted fermented fish.

Each sample was done in duplicate.

Table 4

Total volatile base nitrogen-TVB-N (mg/100 g), Thio-barbituric acid-TBA values (mg malonaldehyde/kg) (Based on wet weight) and pH of salted fermented fish samples.

Parameters	Mean	Minimum	Maximum
TVB-N	78.86	32.2	131.82
TBA	15.32	11.41	19.81
pH	6.39	6.18	6.50

Total number of samples = 24 salted fermented fish.

Each sample was done in duplicate.

In the present study, Thiobarbituric acid (TBA) (an indicator of lipid oxidation) content ranged from 11.41 to 19.81 with an average 15.32 mg malonaldehyde/kg for all Hout-Kasef samples. These values are high and out of the acceptable limits according to Schormüller (1969) and Sinhuber and Yu (1958) who reported the malonaldehyde less than 3.0–5 mg malonaldehyde kg⁻¹ in the fermented fish as the limit of acceptability respectively. The TBA index is a measure of malonaldehyde (MDA) content, one of the degradation products of lipid hydroperoxides, formed during the oxidation process of polyunsaturated fatty acids (Gomes et al., 2003 and Arashisar et al., 2004) proposed the use MDA, a secondary product of lipid oxidation as a suitable indicator of fish meat freshness.

The pH values of the samples were (below 7) it ranging from 6.18 to 6.50 with a mean of 6.39. The pH value of the present study is similar to those reported for *Fesseikh* samples-a traditional fermented fish product in Sudan and *telesech*-a traditional fermented fish product in India (Agab and Shafie, 1989; El-tom, 1989; Roy et al., 2014), but lower than reported by Momone and lanhouin (Anihouvi et al., 2006,2007). However, in Thailand the standard PH requirement for fermented fish known as Pedah siam is 6.0–6.4 with a pH 6.5 or higher being considered as indicative of poor quality (Anihouvi et al., 2012). There is no recommended pH range for Saudi salted-fermented fish products such as Hout-Kasef set yet by Saudi Food and Drug Authority. The high pH values of salted fermented fish products could be attributed to the high level of total volatile basic nitrogen (TVB-N).

4. Conclusion

In conclusion the microbiological studies of natural fermentation of Hout-Kasef, salted fish revealed the presence of gram positive and gram negative bacteria, yeast and mold. The predominant microorganisms were *Bacillus* and *Staphylococcus* spp. No pathogenic bacteria were detected. Chemical analysis of salted fermented fish samples showed high content of moisture, protein, and salt but low fat content. The TVB-N and TBA levels were higher than maximum allowable, indicating the need for controlled fermentation process or the use of selected culture.

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