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Bacteria of wet-salted *Mugil Cephalus, Chanos Chanos and Gerres Oyena*(pisces) from Sudanese Red Sea Coast

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SUMMARY

Wet-salted Mugil cephalus is occasionally found on shelf. The present study prepared wet-salted *M. cephalus*, Chanos chanos and Gerres oyena at three salt ratios 15%, 20% and 25%. Preparation covered summer and winter season. Ten bacterial species *Micrococcus* sp. I, *Micrococcus* sp. II, *Micrococcus* sp. III and *Micrococcus* sp. IV; *Staphylococcus* sp.; *Aerococcus* sp.; *Micrococcus* kristainae, *Micrococcus* varians, *Staphylococcus* kloosii and *Stomatococcus micococcus*. In summer samples of *M. cephalus*, *C. chanos* and *G. oyena* at all brine concentrations and storage durations *Micrococcus* sp. I, *Micrococcus* sp. II, *Micrococcus* sp. III, *Micrococcus* sp. I, *Staphylococcus* sp., and *Aerococcus* sp. were found. In winter samples *M. kristainae* was found. The frequency of occurrence of *M. varians*, *S. kloosii* and *S. micococcus* followed no consistent pattern. With respect to fresh samples in winter *M. kristainae* was found in M. cephalus and in *G. oyena*; and *S. kloosii* in *C. chanos*.

Keywords: Bacteria; Wet-salted; Marine; Fish; Sudan

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INTRODUCTION

Fish is an easily digestible animal protein. Lean fish exhibit high quality protein, minerals and vitamins. Oily fish comprises a rich source of omega 3 and 6 fatty acids well appreciated as health promoter [1]. Fish as a food commodity is rich in amino acids, iodine, selenium, calcium, magnesium, zinc, iron and other minerals [2] and a number of vitamins A, B, B-6, B-12, D, E and K [3]. Fish are found in all water bodies. This explains the growing demand on fish as food [4] Both authors discussed historical consumption and future demand for fish and fishery products for the years 2015/2030. Thus its gross chemical and body weight composition are of significance in nutrition and industry [5].

Studies on microbiology of Red Sea coast brined fish received little attention [6]. reported Vibrio nguillarum, V. parahemolatycum, *V. harveyi*, *V. furnissii*, *Photobacterium* damselae and Tenacibaculum maritmum from the Red sea fishes Siganus rivulatus, Mulloidicthys vanicolensis, Hipposcarus harid, Pterois volitans and Picasso trigger. According to [7] the marine fish Valamugil scheli is mainly used in production of the salted fermented fish Saudi Arabia. The fermented product is dominated by 6 *Staphylococcus* spp. and 5 *Bacillus* spp. [8] studied the effect of wet salting method on the quality of salted fish products of *Ophiocephalus striatus*. The objective of this work is to study the chemical composition of fresh and wet-salted *M. cephalus*, Chanos chanos and Gerres oyena.

MATERIAL AND METHODS

Fish samples

Highly fresh of Mugil cephalus (n=60), Chanos chanos (n=60) and Gerres oyena (n=60), were purchased from Port Sudan Central Fish Market. Specimens were kept chilled till processed in the laboratory.

Wet-salting

Wet-salting of fish was performed by immersing in brine solution of the desired brine concentration (15%, 20% and 25%). Adequate numbers of tight plastic buckets were used to keep separately the different species at different salt by weight concentration during summer. The same experiment was repeated during winter.

Microbial studies

The standard methods of [9] were used for culturing bacteria

in Nutrient Agar and Blood Agar media. Identification and viable counts followed the same author.

Statistical analysis

Paired t-test was used to compare the bacterial count of each species with respect to season. Simple regression analysis was used to correlate total bacterial count and brining time for each species and season[10].

RESULTS

Bacterial species encountered and their frequency of occurrence is given in (**Tab. 1**.) The table showed that:
Ten bacteria species were recorded during this study and abbreviated alphabetically. These with their abbreviations are: *Micrococcus* sp. I (A), *Micrococcus* sp. II (B), *Micrococcus* sp. III (C) and *Micrococcus* sp. I (D); *Staphylococcus* sp. (E); *Aerococcus* sp. (F); *Micrococcus* kristainae (G), *Micrococcus* varians (H), *Staphylococcus* kloosii (I) and *Stomatococcus micococcus* (J) [11].
In summer samples of *M. cephalus, C. chanos* and *G. oyena* at all brine concentrations and storage durations the following bacteria species were found: *Micrococcus* sp. I, *Micrococcus* sp. II, *Micrococcus* sp. III, *Micrococcus* sp. I, *Staphylococcus* sp., and *Aerococcus* sp. The following four species *M. kristainae, M. varians, S. kloosii* and *S. micococcus* were not encountered in summer samples [12].
In winter samples of *M. cephalus, C. chanos* and *G. oyena* at all brine concentrations and storage durations *M. kristainae* was found. The frequency of occurrence of *M. varians, S. kloosii* and *S. micococcus* followed no consistent pattern. *Micrococcus* sp. I, *Micrococcus* sp. II, *Micrococcus* sp. III, *Micrococcus* sp. I, *Staphylococcus* sp., and *Aerococcus* sp., were not encountered during winter in the three species [13].
With respect to fresh samples in winter *M. kristainae* was found in *M. cephalus* and in *G. oyena*; and *S. kloosii* in *C. chanos*.
The 10 *bacteria* sp. found were not pathenogenic [14].

Total bacterial count

The descriptive statistics of total bacterial count **(Tab. 2.)** for summer and winter brined *M. cephalus*, *C. chanos* and *G. oyena* showed that:
No bacteria species were encountered in fresh samples of *M. cephalus* and *C. chanos* during summer and winter.
For samples of *M. cephalus* the total bacterial count decreases with increasing brine concentration in summer but increases during winter.
With respect to *C. chanos* and *G. oyena* no consistent pattern in total bacterial count in summer and winter brined samples.
The highest mean total bacteria count was 1,411,000 for *M. cephalus* prepared during winter at 25% brine concentration.
The lowest mean total bacteria count was 72,333 for *M. cephalus* prepared during summer at 25% brine concentration [15].

Tab. 1. Frequency of occurrence of bacterial species with respect to fish species, season, brine concentration. Present (+), Absent (-).

Brine con.	Bacteria spp.									
	A	B	C	D	E	F	G	H	I	J
Mugil cephalus (Summer samples)										
0.0% (Fresh fish)	-	-	-	-	-	-	-	-	-	-
15%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
20%,	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
25%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
Mugil cephalus (Winter samples)										
0.0% (Fresh fish)	-	-	-	-	-	-	1 +	-	-	-
15%	-	-	-	-	-	-	2 +	-	-	-
20%	-	-	-	-	-	-	3 +	1 +	1 +	1 +
25%	-	-	-	-	-	-	3 +	-	3 +	1 +
Chanos chanos (Summer samples)										
0.0% (Fresh fish)	-	-	-	-	-	-	-	-	-	-
15%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
20%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
25%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
Chanos chanos (Winter samples)										
0.0% (Fresh fish)	-	-	-	-	-	-	-	-	-	1 +
15%	-	-	-	-	-	-	2 +	1 +	2 +	-
20%	-	-	-	-	-	-	4 +	-	1 +	2 +
25%	-	-	-	-	-	-	3 +	-	3 +	-
Gerres oyena (Summer samples)										
0.0% (Fresh fish)	1 +	-	-	-	-	-	-	-	-	-
15%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
20%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
25%	6 +	3 +	3 +	3 +	3 +	3 +	-	-	-	-
Gerres oyena (Winter samples)										
0.0% (Fresh fish)	-	-	-	-	-	-	1 +	-	-	-
15%	-	-	-	-	-	-	3 +	-	2 +	1 +
20%	-	-	-	-	-	-	3 +	-	2 +	1 +
25%	-	-	-	-	-	-	1 +	1 +	4 +	-

Tab. 2. Mean±SE of total bacterial count for summer and winter prepared brine fish.

Brine conc.	Summer season		Winter season	
	Range	Mean±SE	Range	Mean±SE
Mugil cephalus				
0.0% (Fresh)	0.0	0.0	0.0	0.0
15%	13,000-350,000	173,500±56,915	300-400,000	122,550±57,566
20%	11,000-180,000	88,167±32,494	300-1,399,999	334,717±213,658
25%	1,000-150,000	72,333±28,970	0.0-8,000,000	1,411,000±1,319,299
Chanos chanos				
0.0% (Fresh)	0.0	0.0	0.0	0.0
15%	13.00-35,000	160,500±62,599	12,500-2,000,000	686250±297.772
20%	10,000-230,000	1,121,667±41,393	0.0-400,000	169,999±76,463
25%	7,500-200,000	87,250±33,877	0.0-500.000	170,000±78,230
Gerrus oyena				
0.0% (Fresh)	400,000	400,000	500,000	500,000
15%	18,000-350,000	211,333±46,781	14,500-500,000	174,083±78,333
20%	13,000-200,000	123,833±34,574	0.0-4,000,000	868,833±645,777
25%	10,000-250,000	126,667±31,693	0.0-500,000	136,667±78,343

Tab.3. The impact of storage time on total bacterial count.

Season	Salt con.	Bacterial count at different days							
		5	10	15	20	25	30	50	90
Mugil cephalus									
Summer	15%	$3x10^3$	$1.8x10^3$	$1.5x10^5$	$3.5x10^5$	$1.8x10^4$	$1.3x10^4$	$1.2x10^2$	NG
Winter	15%	$3x10^3$	$1x10^5$	$7x10^4$	$1.6x10^3$	$4x10^5$	$6x10^4$	$2x10^2$	$2x10^2$
Summer	20%	$1.8x10^3$	$1.5x10^3$	$1.5x10^5$	$2.5x10^4$	$1.3x10^4$	$1.1x10^4$	NG	NG
Winter	20%	$5x10^3$	$2x10^3$	$1x10^5$	$3x10^2$	$1.3x10^5$	$7x10^3$	$5x10^3$	$4x10^2$
Summer	25%	$1.5x10^3$	$1.3x10^5$	$1.3x10^5$	$1.3x10^4$	$1x10^4$	$1x10^3$	$1x10^2$	NG
Winter	25%	NG	$8x10^6$	$4x10^4$	$6x10^5$	$4x10^5$	$2x10^4$	$2x10^3$	$2x10^2$
Chanos chanos									
Summer	15%	$3.5x10^3$	$3.5x10^4$	$3x10^5$	$2.5x10^4$	$2.4x10^5$	$1.3x10^4$	$1x10^2$	$1x10^2$
Winter	15%	$4.5x10^3$	$8x10^5$	$5.5x10^3$	$12.5x10^2$	$8x10^5$	$2X10^6$	$2x10^4$	$1x10^2$
Summer	20%	$2x10^3$	$3.3x10^4$	$1.8x10^5$	$2x10^4$	$2.3x10^5$	$1X10^4$	$3x10^3$	$2x10^2$
Winter	20%	NG	$7x10^4$	$5x10^4$	$4x10^5$	$4x10^5$	$4x10^4$	$1x10^3$	$3x10^2$
Summer	25%	$1.5x10^3$	$1.8x10^4$	$1.3x10^3$	$1.8x10^4$	$2x10^5$	$7.5x10^3$	$3x10^2$	NG
Winter	25%	NG	$3x10^5$	$3x10^5$	$8x10^4$	9x104	$5x10^4$	$5x10^2$	$3x10^2$
Gerres oyena									
Summer	15%	$3.5x10^3$	$1.8x10^5$	$3x10^5$	$2.2x10^5$	$2x10^5$	$1.8x10^4$	$1.8x10^2$	$1x10^2$
Winter	15%	$3x10^3$	$1.5x10^4$	$4x10^5$	$1.5x10^2$	$5x10^5$	$4x10^4$	$4x10^2$	$3x10^3$
Summer	20%	$2x10^3$	$1.5x10^5$	$2.8x10^5$	$1.8x10^5$	$1.8x10^5$	$1.3x10^3$	$1.3x10^2$	$1x10^2$
Winter	20%	NG	$4x10^6$	$5x10^3$	$2x10^5$	$2x10^5$	$8x10^3$	$5x10^4$	$3x10^4$
Summer	25%	$1.5x10^3$	$1.3x10^5$	$2.5x10^5$	$1x10^5$	$1x10^5$	$1x10^4$	$1x10^2$	NG
Winter	25%	NG	$5x10^5$	$7x10^5$	$3x10^{41.4}$	$2x10^5$	$2x10^4$	$3x10^3$	$2x10^2$

Impact of storage time on total bacterial count

The impact of storage time on total bacterial count **(Tab. 3.)** revealed that:

There is a general trend of decrease in total bacterial count with increase of storage count in the three species and the different brine concentration.

The bacterial count during summer was less than winter at days 25, 30, 60 and 90.

The regression analysis of total bacterial count (TBC) and storage time (T) in days revealed weak

correlation ($p<0.40$) only in G. oyena as follows:

At 15%, TBC=324,141 – 4,411T (r=0.047).

At 20%, TBC=216,834 – 2,868T (r=0.024).

At 25%, TBC=167,095 – 2,261T (r=0.035).

DISCUSSION

Stated that spoilage of fresh and lightly preserved fish products is caused by microbial action. They found that highly salted fish products may spoil due to the growth of halophilic bacteria or growth of anaerobic bacteria. Traditionally fermented fish products is the resultant of the action of a wide range of microorganisms including Gram positive and Gram negative bacteria [16]. The present study prepared wet-salted *M. cephalus, C. chanos* and *G. oyena* at three salt ratios 15%, 20% and 25%. Preparation covered summer and winter season. Ten bacterial species *Micrococcus* sp. I, *Micrococcus* sp. II, *Micrococcus* sp., and *Micrococcus* sp. IV; *Staphylococcus* sp.; Aerococcus sp.; *Micrococcus* kristainae, M. \ varians, *Staphylococcus* kloosii and *Stomatococcus micococcus*. In summer samples of *M. cephalus*, *C. chanos* and *G. oyena* at all brine concentrations and storage durations *Micrococcus* sp. I, *Micrococcus*sp. II, *Micrococcus* sp. III, *Micrococcus* sp. I, *Staphylococcus* sp., and *Aerococcus* sp. were found. In winter samples *M. kristainae* was found. The occurrence of *M. varians*, *S. kloosii* and *S. micococcus* followed no consistent pattern. With respect to fresh samples in winter *M. kristainae* was found in *M. cephalus* and in isolated fromEgyptian salted fish

Micrococcus spp., *Bacillus* spp., Proteus vulgaris, *P. mirabils* and Aeromonas liquefaciens [17]. found *Micrococcus* spp., *Pseudomonas* spp., *Aerococcus* spp., and *Vibrio* spp. from the salt-fermented fishery products. studied wet-salted Mugil cephalus. He identified aerobic bacteria 18 strains of *Staphylococcus equorum*, 2 strains of *Bacillus* subtilis subtilis, two strains of *Lactobacillus* sp., and 1 strains *Bacillus subtilis*. He also identified 31 halophilic bacteria included 16 strains of *Staphylococcus* equorum, 10 strains *Staph*. sp., and 5 strains of *Teratogenococcus halophilus*. The 36 identified anaerobes were 23 strains of Clostridium bifermentans, 7 strains of *Clostridium* bifermentans, 3 strains of *Clostridium* sp., one strain of each of *Clostridium* butyricum, *Clostridium* cochlearium and *Clostridium* sp. reported Vibrio nguillarum, *V. Parahemolatycum*, *V. harveyi*, *V. furnissii*, Photobacterium damselae and *Tenacibaculum* Maritmum from a number of marine fish species from Hurghada, Egypt [18]. Reported that the marine Valamugil scheli fish is mainly used in production of the salted fermented fish in *Saudi Arabia*. The fermented product is dominated by 6 Staphylococcus spp. and 5 *Bacillus* spp. Determination of the microbiological quality of salted and sun dried fish product is important for protecting consumer's health studied the characteristics of traditionally dry salted fish product collected from West Nile Region of Uganda and found that *E. coli* can be used to determine and estimate the microbial quality [19]. Found that salt fermentation resulted in significant ($p<0.05$) reduction in the total Staphylococcus spp., *Micrococcus* spp. and yeast mould count for salted freshwater Hydrocynus forskalii. According to studies the fermentation process yielded amines, acetic acid and lactic acid which are responsible for the characteristic odour of the fermented fish and control of spoilage organism's growth. Thus extends the shelf life of the product. Dry and wet salting is one of the oldest methods of fish preservation. It extracts water from the fish flesh to a level that slow down microbial growth and enzymatic activities [20].

ETHICAL MATTERS

Ethical issues pertaining to approval and consent to participate, human and animal rights, consent for publication, availability of data and materials are Not Applicable.

The authors declare no conflict of interest financial or otherwise.

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