Quality Preservation in Salted Fermented Debs sp. (*Lebeo sp.*) During Storage Period

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Abstract: Fish becomes spoiled within 12 hours at tropical regions when a complicated series of chemical and bacterial changes triggered by high temperature, take place within the fish. Spoilage begins as soon as the fish dies and processing should therefore be done quickly to prevent the growth of spoilage bacteria. Salted fermented Debs sp.(*Lebeo sp.*) was assessed for its proximate and microflora composition in order to establish its nutritive and technological usefulness. A decrease in chemical composition of fermented species was observed. The magnitude of change between fresh and treated materials during storage was differing significantly. The dominant species of bacteria which was isolated and identified from both the fresh fish and salted *Lebeo sp.* was *Staphylococcus.* The number of microorganisms increased rapidly during the first fermentation days and then it began to decrease.

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Key words: Salted fermented fish, *Lebeo* species, nutritive value, microbiological changes, and storage.

1. Introduction:

Fish is one of the most perishable food and its preservation is usually accomplished by combination of different techniques. Contamination with spoilage microorganisms is almost unavoidable because fish is a very good culture media. Therefore, good fish preservation techniques must prevent microbial spoilage of fish without affecting its quality and nutritional value (**Ghaly *et al.*, 2010**).

Spoilage of fish can be due to rapid autolysis by the fish enzymes, and because of less acid reaction of fish flesh that favors microbial growth (**Yohanna *et al.*, 2011**). Fish, in general, usually spoil more rapidly than other muscle foods; the spoilage process (Rigor mortis) will start within 12 hrs of their catch in the high ambient temperatures of tropics (**John, 1994**). Fish preservation methods include, salting, drying, chilling, smoking and freezing. Post-harvest losses of fish catch on processing include material, as well as value and nutritional losses. Preservative methods must be applied to the fish even on the fishing boat. The need for efficient processing of landed fishes for maximum yields with best quality should be emphasized (**Ali *et al.*, 1996, Turan *et al.*, 2007**). Fermentation is considered an easy and low-energy preservation methods for meat that results in distinctive products that have an important part in the diet of people making them (**Margy, 1992**). Such fermented meats contribute both nutritional value and pleasure to meals. The employment of fish fermentation differs from place to the other. In Asia, fermented foods are popular and well liked by the general population and so widely used, that the daily diet of the people would not be complete without them (**Sundhaghul *et al.*, 1975, Bhumiratana, 1980, Del Rosario, 1980, Huss and Valimarson, 1990., Olympia *et al.*, 1992**). Salting and drying fish in Africa are accompanied by fermentation, but the period is short (a few days) **FAO (1981)**. **Watanabe(1982)** stated that the fermented fish products of Senegal are highly salted and semi-dried fish products with an obnoxious odour. **Toury *et al.*(1970)** reported that the Guedi is reported as fermented dried fish product popular in Senegal, unsalted fresh fish is piled together for about 24 hrs in the open air. During this period the fish undergoes fermentation by its own enzymes and endogenous bacteria. Then it is eviscerated, sometimes the big species are filleted to shorten the period of drying and soaked in salty sea water in wooden buckets. This water is changed once a week when it has become too dirty. Finally the fishes are spread out on straw mats to dry in the sun for 2 to 4 days. This study is designed to concentrate on salting and fermentation of fish and attempts to carry out exhaustive investigation on Debs sp. (*Lebeo sp.*), leading hopefully to achieve a promoted status to be placed at the disposal of practitioners who enter competition on quality.

2. Material and Methods

This study was conducted at the Fisheries Research Center, Ministry of Science and Technology,

University of Khartoum and Veterinary Research Center, Soba. Sudan.

Sample Collection

Samples of fresh fish, namely, Debs sp. (*Lebeo sp.*), were obtained from El Khartoum market (Mawrada). The samples were transported immediately (early in the morning) to the laboratory at Elshagra Fisheries Research Center. The microbiological and chemical analysis were immediately carried out on fresh sample. The salt used in the processing of the experimental methods was obtained from Khartoum market.

During processing of salted fish in the laboratory, samples were withdrawn at random for salt concentrations (25%) from different containers of replication for microbiological and chemical analyses. Sampling was carried out every four days for the first 2 weeks and then was taken monthly until 6 months.

Treatment

Fresh fishes were weighed, washed, eviscerated, washed again and transferred to baskets to dry up. Then they were weighed again to obtain the actual weight, which will be treated with salt. Fishes were then divided into 3 groups each one were put in container (small plastic barrels with lids) an equal weight of 3 kg. Each group was thoroughly treated with commercial salt, each group were treated with 25% salt (w/w) (3kg of fish +0.75kg of salt). In each group, salt was applied by brushing and rubbing of the fish surface, the gill chamber and the inner lining of eviscerated abdominal cavity.

Chemical Analysis

Preparation of the Sample

The samples of fresh and treated fish were minced through a meat mincer, and then mixed several times to be homogenized before analysis. The methods of **A.O.A.C. (1990)** were used to determine the crude protein (C.P), ether extract (E.E), ash content, dry matter (D.M), crud fat and moisture content of the sample.

pH Measurement

The pH was read using digital pH-meter (model Jenway 3015). The pH-meter was calibrated using standard buffer solutions of pH = 4 and pH = 7. Two grams of sample was minced with 9 ml distilled water and was transferred to test tub. The pH was taken as a mean of 3 readings.

Determinations of Minerals

All minerals (Phosphorus, Iron, Copper, Calcium, Sodium and Potassium) studies of fresh and salted fish were determined according to the methods of A.O.A.C. (1990).

Total Viable Bacterial Count

Cruickshank *et al.* (1975) methods is used to count the total viable bacteria (TVB). The inoculum is deposited as drops from a calibrated dropping pipette. Each drop, 0.02 ml in volume, is allowed to fall from a height of 2.5 cm onto the medium, where it spread over an area of 1.5-2 cm diameter. Each of six plates receives one drop of each dilution in separate numbered sectors. Counts are made in the drop areas showing the largest number of colonies without confluence (up to 20 or more), the mean of the six counts gives the viable count per 0.02 ml of the dilution.

Isolation and Identification of the Colonies Culture

The samples were cultured into the surface of the following media (Nutrient Agar (N.A), Mannitol salt agar, potato dextrose agar, and Blood Agar (B.A)). These plates were incubated for 24-48 h.at 37˚C after which they were examined for characteristic colonies and presence of haemolysis. Suspected isolates were identified morpholoically, culturally and biochemically according to **Cruickshank *et al*., (1975)**.

Statistical Analysis

The data obtained were analyzed as a completely rando,mized design (ANOVA one and two way) and the means were tested for significance using Duncan Multiple range test as described by Statistical Package for Social Science (SPSS Softword (Vearsion 11).

3. **Result and Discussion**

The chemical composition of fresh sample and salted fermented Debs sp. (*Lebeo sp.*), is showed in tables 1 and 2, respectively, a decrease was observed in moisture, ash, protein, ether extract of all fermented fish during storage period. In a similar study carried by **Abbey *et al*.(1994)** reported a gradual decreases in moisture, fat and protein content in salted fermented products.

The moisture content of the treated samples varied between 61.30 ±0.99 during 4 day of storage and decreased to 33.06 ±0.29 on 5 months of the storage, and it was 77.90±0.374 in fresh samples. A significant difference ($p<0.05$) was observed between fresh and fermented salted samples. The moisture values of fermented fish in the present study were closed to values reported by **Abdullhi (2000)**, **Asiedu and Sanni *et al.* (2002)** who obtain 77.8 % for naturally fermented *Enam Ne-Setaaky*, a

West African fermented fish. But the findings of this study disagreed with the 50%-56% moisture content reported by **Sanni *et al*. (2002)** on *Lanhowin* (a fermented fish from Benin). The variation in moisture content of the samples could be due to variable fermented methods, time and amount of salt used for the curing.

The pH was decrease during storage period and the values of all fermented sample were below 7 from 1 month until 6 months, it was 7.40 ±0.1 for fresh fish (Tables 4 and 8). The pH values for all treated samples after 4 days of processing were below 7. Similar values of pH were reported on momone (6.5) **Sanni *et al*.(2002)** and Pedah Siam a fermented fish processed in Thailand **(FAO,1992)**. The pH values was not found to be statistically significantly ($p<0.05$) especially during early time of processing and this affected by the addition of sodium chloride either during storage time for salted Debs sp. This observation are in keeping with other researchers **(Gokoglue, *et al*., 1994; Duman *et al*., 2007 and Bahri *et al*., 2006)**, However, the pH values obtain in this study disagreed with those reported for Lanhouin, where the pH values were above 7. a gradual decrease of pH values of fish or other food during fermentation is well documented **(Coulin *et al*., 2006, Paludan-Muler *et al*., 2002)**. The level of ether extract remained basically stable during the 8 days of fish processing .The level of ether extract remained basically stable during the 8 days of fish processing. This was expected since the enzymatic curing process acts mainly on proteins **(Chang *et al*., 1992)**. There were significant differences ($p<0.05$) in the ash during 4 months of the storage,

The protein content in fresh sample was 18.67 0.46 while that of treatd samples ranged between 17.40± 0.39 during 4 days of storage to 10.45 2.00 on 5 month of storage period (Tables 1 and 2). The crude protein of fresh fish ranges between 14-20% and higher levels are obtained during winter season **(Clucas and Ward, 1996)**. The fermentation does not adversely affect the crude protein content of fishery product during early time of storage in Debs sp., this in accordance with the results reported by other outhers **(Sanni *et al*., 2002; Anihouvi *et al*., 2006)**. The results indicated that there was a weak protolytic activity during the salted fermented fish processing. This result is due to the fact that fermented salted Debs sp. was obtained after 3 days of fermentation and the texture of treated samples was not significantly affected by the fermentation compared to fermented fish such as Norvegian rakefisks, suedish surchomings and Vietnamese fermnted fish (Essuman, 1992, Nwabueze and Nwabueze, 2010).

The magnitude of biochemical changes between fresh and treated materials during storage was shown in table 4. **Abu Gideiri *et al*. (2004)** found a significant change in some chemical constituents of salted fish (*O.niloticus*). **El-Sebahy and Metwalli (1988)** found a decrease in the level of crude protein and a significant difference of minerals content recorded (P, Ca and Na) during storage.

In this study a significant difference obtain during 1 and 2 month of storage (Table no.3) In the salted fermentated samples, Ca (6.83 ±0.1 %) and P(1.36±0.30%) contents were in accordance with the Ca and P values reported by **Petenuci *et al*. (2008)** in the tilapia (*Oreochromis niloticus*). The variation observed in the minerals content could probably be due to some microorganism capable of using them during their metabolism such as nitrogen and phosphorous cycles.

The load in bacteria species of the samples is summaries in table 5 for fresh fish and table 8 for treated samples. The species of bacteria isolated of fresh fish in different part of the body were *Staphylococcus gallinarum*,, *Stomatococcus*, *Staphylococcus equorum*, *Escherichia coli*, *Staphylococcus caprae*, and *Staphylococcus caseolyticus*, (Table 5),their number ranged between 756.67 ±1.21 in the muscle to 3813.33 ± 0.692 in the gut. The number of bacteria increases during 4 to 12 days of storage time and then decreases until the end of experiment (Table 7). The species isolated from treted samples were *Staphylococcus gallinarum*, *Staphylococcus auricularis*, *Micrococcus.lylae*, *Staphelococcus caseolyticus* and *Staphelococcus saprophyticus*. **Abu Gideiri (2004)** found that all number of microorganisms increased rapidly during the first fermentation days and then began to decrease. Micoroflora was changed during storage period of *aji-no-susu* **(Kuda *et al*., 2009)**.

The salt actson the muscles, viscera, microorganisms andenzymes developing microorganism which increase the fermentation process, lowering the pH and making the product resistant to the development of putrefying bacteria **(Oetterer and Pescado, 2003)**.

The fermentation may contribute positively to the falvour development of the product. Microorganism product of fermented fish produced amines, ammoniac, organic acids, responsible for the characteristic odour of fermented fish products **(Anihouvi *et al*., 2006; Mensah, 1997)**. some microorganism of treated salted fish enhance nutritive value of the product (Beddows, 1985). In Asia some microorganism used maily to enhance fish product **(Zakhia and Cua, 1991)**.

Staphylococcus sp. was generally present in all the samples and participates in the technological

processing. These bacteria species could contribute to keeping the quality of such products, and inhibit some pathogens. The decreases in the total viable count of bacteria could be relateted to salt concentration added (25%) weight of fish.

Table No. (1): Chemical composition of fresh samples Debs sp. (*Lebeo sp.*)

	Parameters												
	C.P	C.F	E.E	Ash	D.M	Moist	P	pH	Ca	Fe	Na	k	Cu
Sp.	18.37[a]	1.19	1.47	0.67	21.74	77.90[a]	1.57	7.4	8.57[a]	55.33b	176.57b	5.10	5.03
± SE	0.4583	0.716	0.833	0.19	0.392	0.374	0.10	0.1	0.18	0.58	0.221	0.21	0.20
Sig.	*	NS	NS	NS	NS	*	NS	NS	*	*	*	NS	NS

In this and subsequent tables means within the same column followed by different superscript are significantly different ($p<0.05$).

* Significant at 5% level NS =not significant.

Legend:
C.P. = Crude protein Ca = Calcium k= potassium C.F. = Crude fat Fe = iron sp.=species E.E. =Ether extract
Na =Sodium D.M.=Dry matter Cu= Cobalt P = phosphorus SE= Standard error

Table No. (2): The effect of salt, fermentation and time on C.P, C.F, E.E, Ash, D.M and Moisture content of Debs sp. (*Lebeo sp.*) during storage period.

Days	C.P	C.F	E.E	Ash	D.M	Moisture
	25% ± SE Sig.	25% ± SE Sig.	25% ± SE Sig.	25% ± SE Sig.	25% ± SE Sig	25% ± SE Sig.
4D	17.40 0.39 NS	0.67 0.96 NS	1.23 0.29 NS	15.67 0.15 NS	38.67 0.99 NS	61.30 0.99 NS
8D	15.57 0.73 NS	0.76 0. 88 NS	1.23 0.51 NS	14.30 0.76 NS	38.83 0.94 NS	61.26 0.97 NS
12D	15.93 0.23 NS	0.36 0.40 NS	1.00 0.40 NS	13.43 0.62 NS	54.78 0.80 NS	45.21 0.80 NS
1M	14.63 0.14 NS	0.43 0.12 NS	1.36 0.11 NS	13.63 0.19 NS	38.27 0. 29 NS	61.72 0.29 NS
2M	11.92 0.33 NS	0.30 0.50 NS	0.90 0.23 NS	12.54 0.18 NS	41.81 0.98 NS	58.22 0.25 NS
3M	11.23 0.13 NS	0.26 0.16 NS	0.73 0.50 NS	12.55 0.24 NS	52.28 0. 31 NS	47.72 0.31 NS
4M	11.03 0.35 NS	0.16 0.16 NS	0.46 0.50 NS	13.70[a] 0.34 *	58.66 1.48 NS	41.33 0.15 NS
5M	10.45 0.20 NS	0.16 0.11 NS	0.23 0.50 NS	12.18 0.15 NS	66.93 6.20 NS	33.06 0.29 NS
6M	- - NS	- - *	- - NS	S 0.11 NS	S - NS	S - NS

Table (3): The effect of Salt, fermentation and time on P, Ca, Fe, Na, K, and Cu. of Debs sp. (*Lebeo sp.*) during storage.

Days	P			Ca			Fe			Na			K			Cu		
	25%	± SE	Sig.	25%	± SE	Sig	25%	± SE	Sig.	25%	± SE	Sig.	25%	± SE	Sig	25%	± SE	Sig.
4D	1.23	0. 68	NS	7.87	0.11	NS	57.67	0.211	NS	542.67	0.10	NS	6.43	0.44	NS	4.46	0.14	NS
8D	1.17	0. 44	NS	7.63	0.59	NS	59.67	0.21	NS	556.67[a]b	0.12	*	6.46	0.80	NS	4.30	0.16	NS
12D	1.12[a]	0. 67	*	6.83[a]b	0.10	*	39.00	1.52	NS	416.00b	0.10	*	4.13	0.12	NS	3.60	0.11	NS
1M	1.36	0.50	NS	7.10	0.33	NS	50.66	0.20	NS	319.00	0.98	NS	6.23	0.50	NS	4.30	0.154	*
2M	1.03	0.10	NS	7.10	0.50	NS	51.66	0.27	NS	479.33b	0. 98	*	5.60	0.20	NS	4.06	0.16	*
3M	0.76	0. 33	NS	6.86	0.13	NS	44.33	0.26	NS	399.00b	0.11	*	5.76	0.65	NS	3.76	0.83	NS
4M	0.53	0. 33	NS	6.50	0.83	NS	38.00	0.16	NS	310.33b	0.13	*	4.23	0.83	NS	3.13	0.16	NS
5M	0.40	0.33	NS	6.16	0.11	NS	30.66	0.11	NS	264.33b	0.12	*	4.10	0.13	NS	2.80	0.12	NS
6M	S	-	NS	S	-	NS	S	-	NS	S	-	NS	S	-	NS	S	-	NS

D=Day M=Month

Table No. (4): The magnitude of change between fresh and treated materials during storage period of Debs sp. (*Lebeo sp.*).

Samples	Parameters										
	C.P	C.F	E.E	Ash	DM	Moist.	P	Ca	Fe	K	Cu
F.D.	18.37	1.19	1.47	0.67	21.74	77.90	1.57	8.57	55.33	176.57	5.10
T.D.	14.07	0.41	1.25	13.44	70.13	29.86	1.31	7.15	54.33	6.17	4.2

F.D.=Fresh Debs T.D.= Treated Debs

Table No. (5): Total Viable Bacteria count in fresh samples Debs sp. (*Lebeo sp.).*

Species	Parameters				
	Gill	Gut	Muscle	Skin	Whole
D	3176.67	3813.33	756.67	2756.67	990.00
t	3.98	6.92	1.21	5.15	4.76
Sig.	0.15	0.09	0.44	0.12	0.13

D=Debs t=t-test Sig.=Significant

Table No. (6): Bacteria species found in fresh samples of Debs sp. (*Lebeo sp.*).

Gill	Gut	Muscle	Skin	Whole
S. gallinarum + Stomatococcus	*Escherichia coli*	*S. gallinarum + S. equorum*	*S. gallinarum + S. caseolyticus*	*S.gallinarum + Stomatococcus*
S. gallinarum	*S.gallinarum + S. caseolyticus*	*S. gallinarum*	*S. gallinarum + S. schleifor* .	*S. gallinarum*
Escherichia coli + S. caprae	*Escherichia coli*	*Escherichia coli + S. caprae*	*Escherichia coli + S. caprae*	*Escherichia coli + S. caprae*

S=Staphylococcus

Table No. (7): The effect of time storage on , pH, and total viable Bacteria count of Debs sp. (*Lebeo sp.).*

Days	pH			B.count		
	Mean	± SE	Sig.	Mean	± SE	Sig.
4D	7.30	1.93	*	9233.3	0.21	*
8D	7.20	1.92	NS	16754.44	0.14	NS
12D	7.11	0.29	*	2299.22	0.21	NS
1M	6.80	0.33	NS	0.00	0.00	NS
2M	6.50	0.33	NS	2.66	0.27	NS
3M	6.60	0.83	NS	23.83	1.75	NS
4M	6.62	0.50	NS	43.83	0.28	NS
5M	6.50	0.83	NS	10.33	0.53	NS
6M	6.40	-	NS	0.00	-	NS

Table No. (8): Bacterial species found in treated samples during storage period.

Days	Bacteria species
4 D	*Staphylococcus gallinarum + Staphylococcus auricularis*
8 D	*Micrococcus.lylae + Staphylococcus auricularis*
12 D	*Staphelococcus lentus + Staphelococcus caseolyticus*
1 M	*Staphelococcus caseolyticus*
2 M	*Staphelococcus caseolyticus*
3 M	*Staphelococcus caseolyticus*
4 M	*Staphelococcus saprophyticus + Staphylococcus gallinarum + Staphelococcus caseolyticus*
5 M	*Staphelococcus caseolyticus*
6 M	*Staphelococcus caseolyticus*

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