BIOCHEMICAL, MICROBIAL AND SENSORY CHANGES OF SALTED GAMBUSIA FISH (*Affinis affinis*)
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ABSTRACT

The Gambusia fish (*Affinis affinis*) is a small freshwater species and it is not normally used for human consumption. So, the main objective of this work is to utilize from gambusia fish for salted fish production. Moisture content was 73.42% of whole fish and then reduced clearly in salted samples. A gradual loss was observed in moisture with extends storage periods for all treatments. In addition, crude protein and lipid content (on dry wt.) reduced in all treatments based of mainly on salt concentration. Concern pH, its value was associated gradually with increase of salt concentration. During storage, TVB-N and TMA were progressively increased in all treatments especially trial 10% salt. Beside, TBA value in raw whole fish was 2.88 mg malonaldehyde/kg sample. After salting, its value was progressively increased during first two months of storage, and then reduced in all treatments. Initial bacterial load of raw whole fish was 3.69 log cfu /g, markedly increased after salting in all trials examined. Furthermore, initial HBC was increased in both trials 10% and 15% salt, respectively and reduced in both 20% and 25% salt, respectively. With regard to sensory evaluation, whole gambusia salted by high salt concentrations had got high scores compared to those contained low salt concentrations.

Keywords: Gambusia, salting, chemical composition, biochemical criteria, microbiological aspects, sensory evaluation.

INTRODUCTION

Salting is one of the oldest techniques for fish preservation, and is essentially intended to increase the shelf life of the product depressing water activity by means of dehydration and salt uptake by the fish muscle. However, the current demand for salted fish is driven more by the flavour of the product than for preservation purposes (Mujaffar and Sankat, 2005). The salting process that could be used for obtaining salted fish products can be classified according to the control mechanism: kinetic and thermodynamic. There are salting processes with kinetic control, with the end of the salting process fixed at a certain time, which is dependent on the salting procedure (pile, brine or kench salting) (Barat, *et al.*, 2003), temperature (Del Valle and Nickerson, 1967), pressure (Barat *et al.*, 2004) and the raw material characteristics such as freshness (Barat *et al.*, 2006), storage method (Røra and Einen, 2003), feed, size, shape (Zugarramurdi and Lupin, 1980), pH, moisture and fat content (Gallart-Jornet *et al.*, 2007). The salting techniques based on this control system include salt injection (Birkeland, *et al.* 2003), addition of a known amount of NaCl and packing the samples under vacuum in plastic bags (Orr, 1967), placing a known amount of NaCl on the product surface and allowing it to dissolve and penetrate, etc. The preservative effect of salting is mainly due to the decrease in water activity (aw) and thus prevention of growth of many spoilage microorganisms along with the
formation of a more membranous surface which further inhibits the growth of microorganisms (Horner, 1997; Leroi and Joffraud, 2000a; Rorvik, 2000). Moreover, chloride ions are toxic for some microorganisms (Leroi et al., 2000b). El-Sherif and Ibrahim (2006) showed that both moisture and ash were clearly increased while both protein and lipid were decreased in all treatments at zero time of salting. During ripening and cold storage periods, moisture content, pH value, total volatile bases nitrogen (TVB-N) and trimethylamine (TMA-N) content were increased while, thiobarbituric acid values were decreased. On the other hand, total bacterial count decreased whereas halophilic bacteria increased. Fuentes et al., (2008) studied kinetic and thermodynamic control of salted sea bass and showed that fish changes were closely related to the square root of time, while for the thermodynamically controlled salting method, the more influential variable was objective NaCl concentration. It was observed that the variables related to fish samples (initial weight, thickness and contact area) affected to the mass transfer in a lesser extent when using thermodynamic control. A more homogeneous product can be obtained by using thermodynamic control, which could provide better control of the safety and sensory aspects. Additionally, the use of a lower amount of salt and the lower influence of other processing variables in the case of thermodynamic control could be further advantages of this salting procedure. The Gambusia fish is a small freshwater species and it is not normally used for human consumption. Therefore, the main objective of this work was to utilize Gambusia fish (Affinis affinis) for salted fish production using different salt concentrations (10, 15, 20 and 25% w/w). In addition, biochemical criteria, microbial and sensory changes of salted gambusia were monitored during storage period.

MATERIALS AND METHODS

Materials:
The average (Mean±SD) of fresh Gambusia Affinis affinis was 5.28±1.75 cm length and 1.63 ± 0.88 gm weight. They were obtained from El-Qanater EL-Khiria Station for fish research, National Institute of Oceanography and Fisheries (NIOF) during harvesting season, 2005. Fish samples were carefully washed drained for a few minutes and divided into four batches based on salt concentrations used (w/w); 10%, 15%, 20% and 25%. The samples were well mixed with finally refined sodium chloride (Bono salt, Saltines Co.) by glove-converted hands. They were packed in polyethylene bags, tightly sealed and put into screw caps–hard plastic containers. After that, all containers were stored at room temperature (from January up to the end of May, 2006).

Analytical methods:
The moisture, crude protein (TN×6.25), total lipid and ash content were analyzed using standard methods (AOAC, 2000). Biochemical indices; pH, total volatile base nitrogen (TVB-N) and Thiobarbituric acid (TBA) values were determined (Pearson, 1991), Trimethylamine–Nitrogen (AOAC, 2000). For microbiological analysis, 10 g of sample were transferred aseptically to
90 ml of sterile 0.1% (w/v) peptone water. Serial decimal dilutions in 0.1% peptone water were prepared and duplicate 1ml samples of appropriate dilutions were poured on selective agar plates. Plate count agar (PCA, Oxoid) and PCA contained 10% salt for total viable count (TVC) halophilic bacteria count, respectively.

**RESULTS AND DISCUSSION**

**Chemical composition**

The chemical composition of raw Gambusia fish is shown in table (1). Whole fish was composed (on wet wt) 73.42% moisture, 14.59 crude protein, 6.56% lipid and 4.81% ash content. In addition, the values biochemical attributes for whole fish were 6.36 pH, 23.10 mg TVB-N /100 g sample and 2.88 mg malonaldehyde/kg sample as TBA number. Beside, TVC and halophilic bacteria were 2.69 and 2.30 log cfu/g sample, respectively. The compositional parameters of raw Gambusia are quite similar of male capelin-by products except ash content (2.3%) (Gildberg, 2001). High lipid content in whole fish is due to presence its viscera.

**Table (1): Proximate composition and quality attributes of whole gambusia fish (on wet weight basis).**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>%</th>
<th>Criterion</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>73.42 ± 0.05</td>
<td>pH</td>
<td>6.36 ± 0.08</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14.59 ± 0.62</td>
<td>TVB-N (mg/100 g)</td>
<td>23.10 ± 0.99</td>
</tr>
<tr>
<td>Lipid</td>
<td>6.56 ± 0.79</td>
<td>TMA-N (mg/100g)</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>Ash</td>
<td>4.81 ± 0.13</td>
<td>TBARS(mgMalonaldehyde/kg)</td>
<td>2.88 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TVC (log cfu/g)</td>
<td>3.69 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Halophilic bacteria (log cfu/g)</td>
<td>2.30 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means of triplicate determined ± SE.

The compositional parameters of raw and salted Gambusia stored at ambient temperature for five months are shown in fig. (1). Moisture was reduced clearly in all batches after salting. Total loss of moisture at the beginning of storage was ranged between 6.96–17.05% compared to original its content (73.42%).
Fig. (1). Moisture content of salted gambusia.

Fig. (2). Crude protein content of salted gambusia.

Fig. (3). Lipid content of salted gambusia.

Fig. (4). Ash content of salted gambusia.
A continuous loss was observed in moisture with extends storage periods for all batches. However, fluctuate was found in trial contained high salt concentration at the end of storage. This phenomenon may be due to salting illustrated two distinct phases, the first, there is a loss of water and therefore a loss of weight, and the second, the brine is sucked into the tissues so that weight of fish may return to something like its original value. In general, there is a loss of moisture but return to like its original value was not found. Concern crude protein content, it was decreased in all treatments based mainly on salt concentrations studied (fig.2). Therefore, the decreasing rate in protein was ranged between 8.30–24.34% of all trials at the start of storage period compared to initial protein content (54.89% dry wt.). A gradually loss was continued in protein with extend storage time and increase salt concentration. This loss is due to the breakdown of fish proteins which produce TMA, DMA, etc as a result of fermentation process. Lipid content was decreased in all treatments during storage; the loss in its content at the first time of storage was ranged between 30.43-35.05% compared with to its initial content (24.68%) (fig.3) and continued up to the end of storage. This change in lipid may be due to fat oxidation that take place in further reactions, particularly with amines and decomposition products of protein to produce changes in texture and taste of final product. On the other hand, ash content was sharply increased after salting and its value was fluctuated in all studied trials (fig. 4). Moreover, the same trend was found in salt content for all treatments (fig. 5). This increase in both ash and salt were attributed mainly to rise of salt penetration and loss moisture content. The increasing rate in ash was between 117.18-162.43% at zero time of storage. In addition, the salt content was (wet wt.) ranged between 6.08-10.06% at the first storage and then increased with prolong storage period. The obtained data of salted
samples during storage periods are in accordance with those reported by Abo-Raya (1979); Rashad (1986); Ibrahim, (1994); Sarhan, (2003) and El-Sherif and Ibrahim, (2006).

Biochemical criteria:

The biochemical criteria of salted Gambusia stored at ambient temperature for five months are illustrated in fig. (6, 7, 8 and 9).

The pH values were slightly reduced in all treatments after salting. pH values were ranged between 6.01- 6.14 at zero time of storage. In addition, the lowest values in pH of studied treatments stored were observed at the
second month of storage. Reduction level in pH value was associated gradually with increase of salt concentration. This decrease in pH value could be attributed to the formation of organic acids resultant growth of microorganisms and exogenous hydrolytic enzymes. With extend of storage period; pH values were increased in all trials investigated. Moreover, the highest value in pH (7.07) was found in trial 10% salt than others. The rise of pH is probably due to autolysis occurred in fish proteins and increase of alkaline VBN during the fermentation period. Similar observations were reported by Abo-Raya (1979); Ibrahim (1994); Sarhan (2003); El-Sherif and Ibrahim (2006). Concern TVB-N content, it was reduced to range 15.40-21.0 mg /100 g after salting. Therefore, the reduction rate in TVB-N content was based on salt concentration used. This refer to high concentration minimized reduced the microbial growth, enzymatic activity and autolysis occurred in fish proteins. During storage, TVB-N was progressively increased in all treatments especially trial 10% salt. It could be found that low salt level caused a progressive increase in TVB-N content more than high level. This increase may be due to microbial activity and breakdown in fish proteins as affected by storage period extend. Similar observations were reported by Abo-Raya (1979); Ibrahim (1994) and El-Sherif and Ibrahim (2006). On the other hand, TMA-N content was increased (ranged from 1.09 to 1.22 mg /100 g) in all trials after salting. During storage up to the second month, its value was reduced in all treatments except treatment 10% salt (6.13mg /100g). This increase may be due to a reaction between TMAO and lactic acid producing TMA and acetic acid (FAO,1992). After that, its value fluctuated according to each treatment. This fluctuation occurred in TMA value of investigated treatments during ripening and storage periods was reported by Ibrahim, (1994) and El-Sherif and Ibrahim (2006). With regard to fat rancidity, TBA value was progressively increased during the second month of storage, and then reduced in all treatments with storage period extended. This increase observed in TBA value is referring to fat oxidation which takes place and other organic compounds in fish tissues and sod. Chloride may be enhanced both oxidation and hydrolysis or encourage the growth of lipolytic bacteria (Quaglia et al., 1989; Ibrahim, 1994; Sarhan, 2003; El-Sherif and Ibrahim, 2006).

**Microbiological profiles:**

Bacterial load was markedly suppressed after salting in all treatments examined (fig. 10). During storage period up to two months, a slightly increase in TBC in all treatments was found. After that, TBC reduced during storage periods extended. On the other hand, HBC was increased in both trials 10% and 15% salt, respectively while, its count was reduced in both 20% and 25% salt, respectively (fig.11). During storage, HBC was stabilized in all trials except trial 15% salt and fluctuated. In addition, HBC was not detected in some periods of storage. In general, increase in bacterial count may be due to the release of water and soluble from tissues and then the surrounded media was diluted to be more suitable for growth HB and salt tolerance bacteria (Rashad,1986; Ibrahim,1994; El-Sherif and Ibrahim, 2006).
Sensory evaluation
Fig. (12) illustrates mean scores had been given for salted gambusia throughout different storage periods. Generally, salted fish with high salt concentrations have got high scores more than ones.

Conclusion
According to the results obtained, proximate composition, biochemical attributes and microbial aspects of whole gambusia fish can be
utilized for salted fish production. Besides, shelf life for salted whole gambusia is varied based mainly upon salt concentration.

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التغيرات الكيميائية والميكروبية والحسية لسمك الجامبوزيا المملح
سيد مكاوى إبراهيم و شعبان عبد الحليم عبد المجيد الشريف

يعتبر سمك الجامبوز مركبا بارزا من الأسماك الغير اقتصادية لصغر حجمه. يساعد بشان تحسين الأسماك المنتجة في العالم، على الحصاد على عينات سمك الجامبوز في الحقول على محطة إنتاج سمك الجامبوز. تم الأخذ في الحساب على طيف أسماك الفرد، وذلك مرتبطًا بالفترة الزمنية والحدود العمرية. بالإضافة إلى ذلك، تمحول الفترات الزمنية الفردية بطور متوازن، يشير إلى عدم التخلص من المواد الفردية. والتحاليل الكيميائية الفردية من الأسماك الفردية، التي تم تحليلها، تشير إلى عدم التخلص من المواد الفردية أثناء الخضوع لفترة زمنية محددة. وخلال فترات التخزين، قد تتم تقليل بعض التغيرات في التركيب الكيميائي، ومعايير الجودة الكيميائية والميكروبية والحساسية خلال فترات التخزين المختلفة. وأوضحت نتائج الدراسة الأتي:

حدث نقص واضح بمعدل 6.2% في المحتوى الرطلي 8.9- 8.85% في البروتين (وزن جاف)، 3- 3.5% في اللبانات (وزن جاف). بينما حذت زيادة بمعدل 12.8 في حمض كيماوي (وزن جاف). في هذا العمل، نلاحظ تأثيرات كبيرة في الفترات الزمنية المستخدمة في تغذية الأسماك الفردية. ويدعو على عدد من الإجراءات لتقليل هذه الظاهرة. وبناء على هذه الدراسة، يمكن استغلال سمك الجامبوز في الحقول على منتج منتج مملح للصين. وضعت له تسع فئة درجة عالية خاصة بالعناصر التي تحدث على تراكيب مملح مرتفعة (20% و 25%).