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EFFECT OF *Lactobacillus acidophilus* 1 M METABOLITES ON MICROBIAL AND BIOCHEMICAL QUALITY CRITERIA OF FROZEN BASA FISH (*Pangasius bocourti*) FILLETS

Salha G. Desouky[1] and S. M. Ibrahim[2]

[1] Faculty of Science, Department of Botany and Microbiology, Suez Univ., Egypt.

[2] Fish Processing Technology Lab., National Institute of Oceanography and Fisheries, Egypt.

ABSTRACT

The present work was planned to investigate the effect of different *Lactobacillus acidophilus* filtrate levels on microbial and biochemical quality criteria of imported basa fish (*Pangasius bocourti*) fillets and heavy metals concentrations i.e. lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn) and iron (Fe). Fish fillets were immersed in cold distilled water containing (v\v) 1%, 1.5% and 2% metabolites for 2 min. Each treatment was packed in polyethylene bags, put in foam dishes and all treatments were stored at -18°C for 30 days. Results showed that frozen contained low residue levels of cadmium (Cd 0.06), copper (Cu 0.42), zinc (Zn 3.0) and iron (Fe 4.98) but lead not detected. Also, basa fillets contained (on wet weight basis) high moisture (81.76 %) and low protein (11.24%), high lipid (4.99%) and ash (1.88%) contents. During frozen storage, *Lb. acidophilus* filtrate levels decreased total plate count (TPC), psychrophilic, proteolytic and lipolytic bacteria in all treatments compared to control samples. Besides, the highest reduction rate in total volatile bases nitrogen (TVB) ranged 27.50–38.70 % in treatment 1% metabolites extract than other ones in particularly control samples under the same conditions. In case of trimethylamin (TMA), it was 29.73% in treatment 2% at first day and 33.33% in treatment 1.5% after 15 days storage. The high reduction rate in thiobarbituric acid (TBA) was 28.42% in treatment 2% at first day while it was 29.30% in treatment 1.5% after 15 days storage. In conclusion, the concentrations of Pb, Cd, Cu, Zn and Fe were lower than the maximum permissible limit and hence safe for human consumption. Bio-preserved techniques of fish fillets using *Lb. acidophilus* are controlled the change rate of bacterial and chemical quality aspects.

Keywords: Basa fish fillets, heavy metals, microbial and biochemical quality.

INTRODUCTION

Catfishes comprise 38 families and include more than 3000 species, representing an important component of the global fish fauna (Sullivan *et al*., 2006). Tra and Basa (*Pangasius bocourti*) have been the two major catfish varieties in recent years, and the production of these *Pangasius* has increased as a result of the national open market economy and exportation to international markets. The Tra is more prevalent than the Basa because the Tra matures earlier than the Basa (Sudova *et al.,* 2007). Sutchi catfish (*P. hypophthalmus*) produced in the freshwater basins of Vietnam, available on the Italian market as frozen or thawed fillets were studied for their nutritional quality and safety aspects (Orban *et al.,* 2008). *Pangasius* meat has high nutritive qualities and excellent sensory properties. The fish can be filleted easily due to the absence of intra-muscular pin bones. Tender flesh, sweet taste; absence of fishy odor and spines, delicate flavor and firm texture when cooked are the attributes that favor consumer preference for *Pangasius*. Frozen catfish fillets popularly known as 'Basa' forms the mainstay of export of fishery products from Vietnam to US and Europe. There is a great potential for development of convenience products such as fish fillets, fish fingers, fish cutlets, fish balls, fish wafers, fish pickles, smoked fish, canned fish and fish curry in retort pouches from *Pangasius* (Silva *et al*., 2002; Ninan *et al*., 2011; Rathod and Pagarkar, 2013).

Concerning heavy metals, Industrial and agricultural discharges such as coal and oil combustion, phosphate fertilizers, plastics and pesticides are considered the major sources of heavy metal pollutants of water. Fish absorbed heavy metals from water through the gills, skin and digestive tract. The heavy metals of the most wide spread concern to human health are lead, copper, mercury and cadmium (Kris-Etherton *et al.,* 2003; Chen *et al.,* 2007; Din *et al.,* 2008; Bhouri *et al.,* 2010). Elnimr (2011) found that Basa fish contained (µg/g) phosphorus level (4.06), lead (0.79), zinc (0.62), cadmium (0.12), manganese (0.07) and mercury (0.004). Cadmium and lead levels were higher than permissible safety level of human use (0.1 ppm).

With regard to fish bio-preservation method; *Streptomces sp.* NIOF1 metabolites, especially at concentration of 15% played effective role in lowering the biochemical and microbiological changes, extending shelf-life of fish stored under low temperature (Daboor and Ibrahim, 2008). Also, Ibrahim and Desouky (2009) investigated the effect of antimicrobial metabolites produced by *Lactobacillus casei* DSM 120011 (A) and *Lactobacillus acidophilus* 1M (B) individually and in combination on quality attributes of tilapia (*Oreochromis niloticus*) fillets during frozen storage. They showed that the use of metabolites improved the biochemical quality criteria, microbial aspects and safety of frozen fish fillets. Therefore, the present work was planned to determine the nutritive value of basa fillets, determine of some heavy metals concentrations; lead, cadmium, copper, zinc and iron and also to investigate the effect of different LAB filtrate levels (1.0, 1.5 and 2.0% v\v) on microbial and biochemical quality criteria of imported Basa fish (*Pangasius bocourti*) fillets during different frozen storage for 30 days.

MATERIALS AND METHODS

Culture

The bacterial strain was obtained from the collection of Food Science Department, Faculty of Agriculture, Ain Shams University, Egypt.

Extraction of Antimicrobial Metabolites

Lactobacillus acidophilus 1M was propagated in 100 ml MRS broth for 48 hrs at 32°C in duplicate. Extraction of the antimicrobial metabolites was carried out by centrifugation at 10,000 rpm at 4°C for 20 min, and then the pH of the extracts was adjusted to 7.0 by using 1M NaOH to avoid the effect of organic acids. The supernatants were filtered through a 0.2 µm pore-size cellulose acetate filter (Schillinger and Luke, 1989).

Testing of the antibacterial activity of the metabolites

The antibacterial activity of *Lactobacillus acidophilus* 1 M was tested by using different concentrations (1-1.5 and 2 %) were added to 100 ml of each specific medium, then used in pour plate method to demonstrate the role of bacterium in lowering (TPC) psychrophillic, proteolytic and lipolytic bacteria which contaminated fish fillets.

Fish fillets

The imported Basa fish (*Pangasius bocourti*) fillets were purchased from local market during 2014. They were transferred using icebox at the Laboratory of Fish Technology, El-Qanatir El-Khiria, National Institute of Oceanography and Fisheries within 2 hours. Fish fillets were immersed in cold distilled water containing (v\v) 1%, 1.5% and 2% concentrations of metabolites for 2 min. Each treatment was packed in polyethylene bags, put in foam dishes and all treatments were stored at -18°C for 30 days.

Analytical Methods

Bacteriological Analysis

10 g sample of fish flesh was placed in 90 ml of sterile saline (0.85% NaCl) and well shacked for 2 min. then1 tenfold serial dilution was prepared. One ml of 10^{4} was tested in pour plate method for estimation of total plate count (TPC), *Psychrophyles* on nutrient agar medium, proteolytic bacteria on milk nutrient agar (Nester *et al*., 1998) and finally, lipolytic bacteria on butter-oil nutrient agar (Harrigan and McCanc, 1990). Total plate count (TPC) incubated for 24 h, proteolytic bacteria for 48 h and lipolytic bacteria for 7 days at 37˚C and psychrophyles at 4˚C for 24 h.

Heavy metals concentrations in fish muscles

The concentrations of Pb, Cd, Cu, Zn, and Fe were determined in raw frozen fish fillets samples according to APHA (1999). Digested samples, blanks and standard solutions were analyzed by Atomic Absorption Spectrophotometer using atomic absorption spectrophotometer, Shimadzu AA-6800, and the results were expressed as µg/g..

Proximate composition and freshness tests of fish fillets

The moisture, crude protein (Nx6.25), lipid and ash contents were determined (AOAC, 2000). Biochemical Criteria: Determination of total volatile basic nitrogen (TVB-N) was determined (Pearson, 1991). The quantity of TVBN = V (HCl) × N (0.1 HCl) ×100 /14 mg/100 g sample. Trimethylamine nitrogen (TMA-N) content was determined (AOAC, 2000). 2-thiobarbituric acid (TBA) value was determined and the color was read at 538 nm using Spectronic 21 D (Milton Roy) UV-Vis Spectrophotometer (Pearson, 1991). TBA values = Abs. $_{538\ nm}$ × 7.8 mg Malonaldehyde (MA)/ kg sample.

Statistical analysis

All the analyses were done in triplicates and the data were subjected to statistical evaluation using SPSS 16 for Windows and they were expressed as mean +SE.

RESULTS AND DISCUSSION

Changes in bacteriological aspects

According to our data in Fig. 1, the total plate count of bacteria in control sample recorded 328 (cfu) at zero time. The lowering of plate count is due to prolonged storage in low temperature which can inhibit the growth of many bacterial species. Fish caught in very cold, clean waters carry the lower numbers whereas fish caught in warm waters have slightly higher counts. Very high numbers i.e. 10^7 cfu/ cm2 are found on fish from polluted waters. (Morita, 1975). By adding the filtrate of *lb. acidophilus* 1M to immersing solution of fillets, the concentration of 1%lowered the plate count to 71 (cfu), 12 and 0 at zero, 15 and 30 days under frozen storage. By using 1.5% the (TPC) much decreased to (62, 3, and 0 cfu) at 0, 15 and 30 days. Raising the concentration to 2% dramatically decreased the (TPC) to 3, 1 and 0 (cfu) respectively. As shown in Fig. 2 the total count of psychrophiles demonstrating relatively low count in control sample (14 cfu) during 30 days of frozen storage. Many bacteria are unable to grow at temperature below10◦C and even psychrophillis grow very slowly (Andersen *et al.,* 1965). 1% of the filtrate lowered the bacteria to 8 (cfu) at zero time and the bacteria disappeared in fillets after 15 days of storage. Microorganisms growing at low Temperature and exhibit proteolytic activity have been assumed to be responsible for fish spoilage (Shewan, 1974). The result of Fig. 3 illustrated the initial count of proteolytic bacteria in control sample was 70 at zero time then lowered gradually by adding the different concentrations of the filtrate until it was recorded 11 (cfu) in 2% at zero time, then lowered from 46 (cfu) to 7 and from 22 to 2 (cfu) at 15 and 30 days respectively. Two obligatory psychrophillic vibrios MV-3 and MV-6, hydrolyzed proteins and deteriorated refrigerated and frozen fish. Protein hydrolysis was determined after storage at 4 ◦C and -20 ◦C and the results indicated that, the isolates capable of hydrolyzing protein and deteriorating quality of fish under refrigeration or frozen storage (Ibrahim *et al*., 1993). Fatty fish are of course particularly susceptible to lipid degradation which can create severe quality problems even on storage at subzero temperature (FAO 1993). According to our results Fig. 4 demonstrating that the total counts of lipolytic bacteria was much higher in control sample (10^2 cfu) at 0 time, 65 and 38 at 15 and 30 days respectively. The results indicated good antibacterial activity of *Lb. acidophilus* in which it reduced the total count at different concentrations during 15 days of storage and then the activity was lost after 30 days of storage which explain the raising in plate count from 15 to 30 days of storage. The growth pattern of *Lb. acidophilus* NC1M2287 and antagonism against fish borne spoilage bacteria namely, lipolytic and proteolytic bacteria and total plate count estimated for 3 days. The growth of specific spoilage bacteria was decreased by *Lb. acidophilus* in the second day by 4.3 log difference, so it can be used as bio- preservative bacteria to preserve fish meat. (Kannappan and Kannar, 2012).

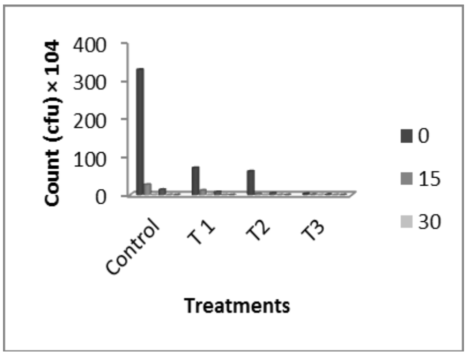

Fig. (1 a): Antibacterial activity *of Lb. acidophilus* 1M against (TVC)

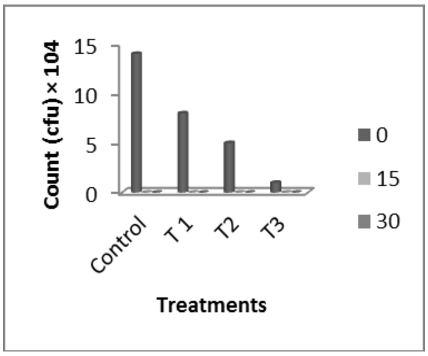

Fig.(1 b): Antibacterial activity of *Lb. acidophilus* 1M against psychrophilic bacteria

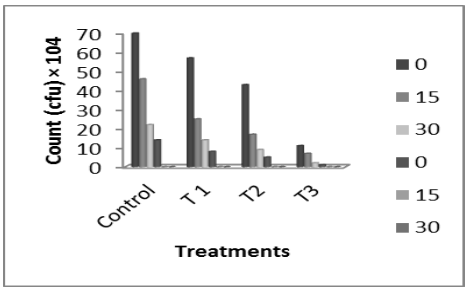

Fig.(1 c): Antibacterial activity of *Lb. acidophilus* 1M against proteolytic bacteria

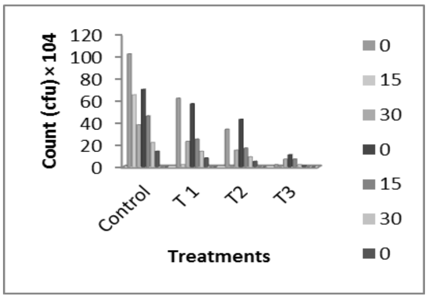

Fig.(1 d): Antibacterial activity of *Lb. acido*philus 1M against lipolytic bacteria.

Fig. (1) Shows the bacterial load of raw and frozen Basa fillets treatments.

Heavy metals concentrations in Basa fillets

Heavy metals accumulate in the tissues of marine organisms and fishes can easily absorb those heavy metals from the waters, food and sediments. Despite the fact that most heavy metals such as mercury, cadmium and lead are toxic and dangerous, there are some heavy metals that are essential in biological systems and must be taken in small quantities such as iron, copper, manganese and zinc (Basa and Usha*,* 2003; Ashraf, 2005 and Zabri, 2009). Table (1) shows the concentrations of some heavy metals in Basa fillets. The levels of Pb, Cd, Cu, Zn and Fe were not detectable, 0.06, 0.42, 3.0 and 4.98 ppm, respectively. Fe level (4.98 ppm) was the most abundant metal in the studied Basa fillets. These concentrations are lower than the maximum permissible limits as set the local and international organizations, where the maximum permissible level (MPL) of Pb (0.1ppm), Cu (20.0 ppm) and Cd (0.1 ppm) and Fe (not detected) in fish for human consumption as reported by (EOSQC, 2005) and FAO/ WHO (2007). Our results with exception of Zn level are lower than those reported by Elnimr (2011) who found the concentrations of Pb, Zn, and Cd were 0.79, 0.62, 0.12 µg/g in Basa fish (*Pangasius hypophthalmus*). Jalal *et al.,* (2013) revealed that all the heavy metals concentrations in the tissues were lower than the maximum permissible limit as recommended by The Malaysian Food Regulation and hence safe for human consumption. In contrast, Javed and Usmani (2011) found that metals bioaccumulated in tissues of *Channa punctatus, Clarias gariepinus* and *Labeo rohita,* exceeded more than the permissible limits. Finally, the permissible limits for Pb, Cd and Zn are 0.1, 0.1, and 50 ppm as set Egyptian Organization for Standardization and Quality Control (EOS, 1998) which are less than the values observed in this study.

Table (1): Concentrations of some heavy metals (ppm) of frozen basa fillets compared with the previous studies.

Metal (ppm)	Present study	Elnimr (2011)	**Permissible limits
Pb	nd*	0.79	1.0
Cd	0.06	0.12	0.1
Cu	0.42	Not detected	---
Zn	3.0	0.62	---
Fe	4.98	Not detected	---

***: Not detectable. **EOS (1998).**

Proximate analysis of Basa fillets

Basa grow extremely fast, which is the main reason why they are so inexpensive and there is no need for chemical or hormonal stimulants to speed that process (Orban *et al.,* 2008). The proximate composition of frozen Basa fillets was determined as shown in Table (2). Basa fillets were contained (on wet weight basis) high moisture content (81.76 %) and low protein (11.24 %), high lipid (4.99%) and ash (1.88%) contents. High moisture content is due to the sodium tripolyphosphate (E 451) used. This additive, permitted by current regulations, improves water retention by proteins during processing and helps the maintenance of moisture and quality of fillets; in particular by reducing the amount of thaw drip. Moisture content influences the quality of the products. Moreover, moisture content of the final product has economic implications as the retention of moisture by the product increases the gross weight of the product resulting in economic gains (Rao *et al.,* 2013). When comparing our result with the previous studies; Orban *et al*. (2008) found that proximate composition (wet weight) of *P. hypophthalmus* fillets were moisture 80–85%, protein 12.6–15.6%, lipid 1.1–3.0% and ash content 1.03–1.50%. Also, Phan *et al.,* (2009) decided that *Pangasius* fillets are characterized by high moisture levels, low protein, lipid, cholesterol and polyunsaturated fatty acids, high percentage of saturated fatty acids and sodium content probably partially due to the sodium tripolyphosphate used to retain moisture. In other study, Rao *et al.,* (2013) showed the proximate composition of *P. hypophthalmus* fish fillets were rich source of protein (17.24%), moisture 78.2%, fat 2.84% and ash content 1.3%. Therefore, it could be concluded that there are several grades of Basa fillets based on lipid content.

Table (2): Proximate composition of frozen basa fillets compared with the previous studies.

Constituent (%)	Present study	Orban *et al*. (2008)	Rao *et al.* (2013)
Moisture	81.76 ± 2.23	80 – 85	78.20
Crude protein	11.24 ± 1.84	12.6 – 15.6	17.21
Lipid	4.99 ± 0.71	1.1 – 3.0	2.84
Ash	1.88 ± 0.09	1.03 – 1.50	1.30

Quality attributes of bio-preserved Basa fillets during frozen storage

TVB and TMA values are considered quality criteria of protein decomposition while TBA value of a rancidity criterion occurring in basa fillets during frozen storage periods. The TVBN values were 27.64, 20.04, 21.19 and 24.74mg\100 g sample of control, treatments 1%, 1.5% and 2%, respectively at zero time of storage (fig. 2 a). All of these values decreased after 15 while they were increased after 30 days of storage at -18C. However, the TVBN values of basa fillets were less than acceptable value of 30 mg 100 g^{-1} (Connell, 1975 and EOS, 1998). Also, the highest reduction rate in TVB ranged 27.50–38.70 % in treatment 1% metabolites extract than other ones in particularly control samples under the same conditions. Rao *et al.,* (2013) found that TVB values of *P. hypophthalmus* fish fillets were lower than 20 mg\100 g at zero time and they were accepted extent up to 9 days of chilling storage.

Fig. (2 b) shows the values of TMA in basa pre-treated with different metabolites levels and stored at -18C for 30 days. Its values recorded 0.37, 0.34, 0.28 and 0.26 mg\100 g sample of control, treatments 1%, 1.5% and 2%, respectively at zero time of storage. All of these values decreased after 15 and stabilized after 30 days of storage at -18C. Also, the reduction rate in TMA values was not clearly shown in fillets treated with metabolites and control samples under the same conditions. The high reduction rate in TMA was 29.73% in treatment 2% at 0 day while it was 33.33% in treatment1.5% after 15 days storage. Moreover, the TMA values of basa fillets were less than acceptable value of 10 mg 100 g-1 (EOS, 1988).

The values of TBA in basa pre-treated with different metabolites levels and stored at -18C for 30 days are shown in fig. (2 c). The TBA values were 2.85, 2.62, 2.22 and 2.04 mgMA\kg g sample of control, treatments 1%, 1.5% and 2%, respectively at zero time of storage. All of these values sharply decreased in particular treatment 1.5% after 15 days while they were increased especially control samples after 30 days of storage at -18C. The high reduction rate in TBA was 28.42% in treatment 2% at 0 day while it was 29.30% in treatment 1.5% after 15 days storage. Moreover, the TBA values of basa fillets were less than acceptable value of 10 mg 100 g-1 (EOS, 1998).

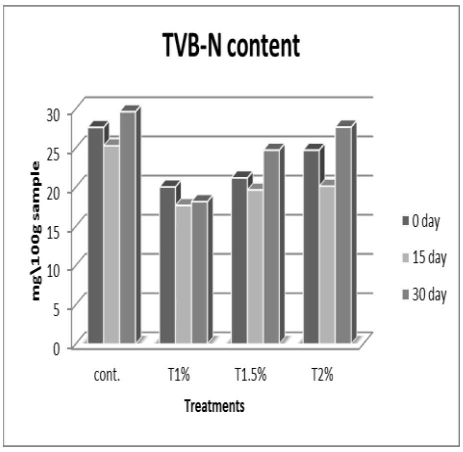

Fig. (2 a): Change inTVB-N content of basa fillets treated with different levels of *L.acidufulus* metabolites.

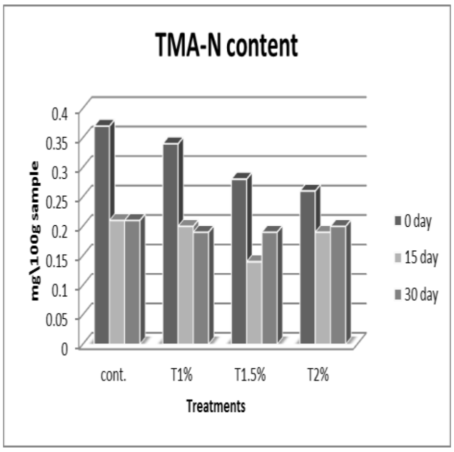

Fig.(2 b): Change in TMA-N content of basa fillets treated with different levels of *L.acidufulus* metabolites.

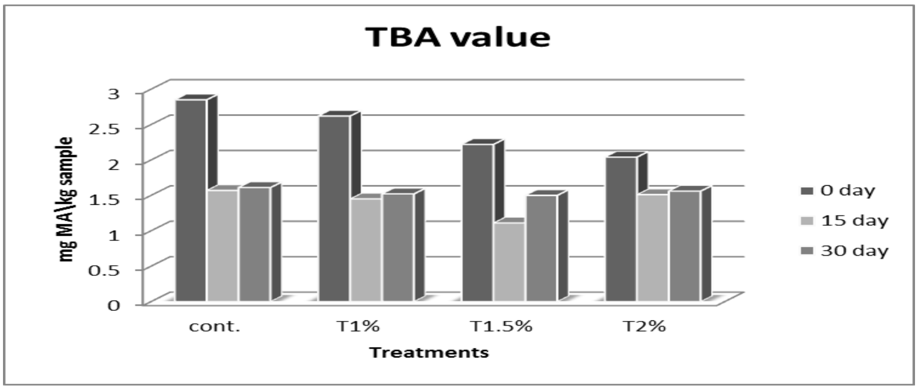

Fig. (2 c): Change inTBA value of basa fillets treated with different levels of *L.acidufulus* metabolites.

Fig. (2): The quality attributes of bio-preserved Basa fillets during frozen storage

CONCLUSION

The concentrations of Pb, Cd, Cu, Zn and Fe in basa fillets were lower than the maximum permissible limit and hence safe for human consumption. Bio-preserved techniques of fish fillets using *Lactobacillus acidophilus* are controlled the change rate of bacterial and chemical quality aspects.

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تأثير التركيزات المختلفة من المستخلص البكتيرى لبكتيريا (*Lactobacillus acidophilus* 1 M) على معايير الجودة الميكروبية والكيميائية لشرائح سمك الباسا (*Pangasius bocourti*)

صالحة جابر دسوقى[١] و سيد مكاوى ابراهيم[٢]

١- كلية العلوم، قسم النبات والميكروبيولوجى، جامعة السويس.

٢- معمل تكنولوجيا تصنيع الأسماك – شعبة المصايد - المعهد القومى لعلوم البحار والمصايد.

يهدف هذا البحث الى دراسة تأثير تركيزات مختلفة من المستخلص المتحصل عليه من بكتيريا *Lactobacillus acidophilus* 1 M علي بعض معايير الجودة الميكروبية والبيوكيميائية لشرائح سمك الباسا المستوردة (*Pangasius bocourti*) ، حيث تم وضع شرائح الأسماك في ماء مقطر مبرد (٤° م) محتوى على تركيزات مختلفة (١ ، ١.٥، ٢ %) من المستخلص المضاد للبكتيريا لمدة دقيقتين ثم تم تعبئة هذه الشرائح في أكياس بلاستيكية معقمة ومحكمة القفل تم وضعها فى أطباق فلينية وخزنت على درجة حرارة - ١٨° لمدة ٣٠ يوم. *كما* تناولت الدراسة التركيب الكيميائى، وقياس تركيز المعادن الثقيلة (الرصاص، والكادميوم، والنحاس، والزنك، والحديد) لسمك الباسا محل الدراسة.

وقد أوضحت النتائج المتحصل عليها مايلى:

- احتوت شرائح سمك الباسا (جزء فى المليون) علي الرصاص (لم يتم اكتشافها)، الكادميوم (٠.٠٦)، النحاس (٠.٤٢)، الزنك (٣.٠)، الحديد (٤.٩٨) حيث ان النسب المسموح بها محليا ودوليا هي للرصاص ١و٠- للنحاس ٠و٢٠- للكادميوم ١و٠ جزء في المليون والحديد لم يتم تحديده)
- كما احتوت على ٨١.٧٦ % رطوبة، ١١.٢٤ % بروتين كلى، ٤.٩٩ % دهون، ١.٨٨ % رماد .
- أدى المستخلص المضاد للبكتيريا محل الدراسة الى خفض العدد الكلي للبكتيريا، وكذلك أعداد البكتيريا المحبة للبرودة، والبكتيريا المحللة للبروتين، والمحللة للدهون مقارنة بالعينة غيرالمعاملة (الضابطة).
- كما أدت المعاملة بالمستخلص الحيوى الى خفض محتوى المركبات النيتروجينة الطيارة بمعدل تراوح من ٢٧.٥٠ - ٣٨.٧٠ % للمعاملة تركيز ١ % مستخلص بكتيرى مقارنة بالمعاملة الضابطة، وبمعدل انخفاض ٢٩.٧٣ % لثلاثى ميثيل امين للمعاملة تركيز ٢ % في اليوم الأول ومعدل ٣٣,٣٣% لرقم حمض الثيوباربتيوريك للمعاملة تركيز ١.٥ % بعد ١٥ يوما من التخزين.

وبناءا على ماسبق فان شرائح سمك الباسا المستوردة تعتبر امنة للمستهلك حيث يحتوى على تركيزات منخفضة من العناصر الثقيلة أقل من المستويات المسموح به دوليا، كما أن الحفظ الحيوي باستخدام *Lb. acidophilus* 1M أدى الى خفض الأعداد البكتيرية وتحسين خواص الجودة الكيميائية لشرائح اسماك الباسا المستوردة.