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(Original Article)

Effect of Some Natural Antimicrobial Extracts on Stability of Nile Tilapia (*Oreochromis niloticus*) Fish Products During Frozen Storage

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Abstract

Nile tilapia (*Oreochromis niloticus*) fish was used in producing fish fillets and fish balls after treating it with two natural antimicrobials (green tea extract and thyme extract) and storing under freezing conditions for 10 months. The results showed a reduction in moisture content, WHC levels and total acidity for all samples, while % EW, pH, TBA and TVB-N values increased for all samples with the extension of the storage period under freezing conditions. The data showed that the total number of bacteria (log cfu/g) was less than 6 in all treated and untreated samples. Over the period of storage, there was a decrease in the total number of bacteria. Compared to the treated samples, the control samples had the highest total bacterial count. The results showed that Log cfu/g of pdychrophilic bacteria decreased after all treatments and storage periods. According to the results, only three samples-control fillets, control fish balls, and fish balls treated with green tea extract-had bacterial counts of the coliform group at the beginning of the storage time (time zero) less than log 2.5 (cfu/g), and no colonies were found for the remaining storage time.

Keywords: *Natural antimicrobial, Fish products, Nile tilapia*

Introduction

Fish is one of the cheapest sources of animal protein and essential nutrients for humans all over the world, as it is a cheap and important source of protein, vitamins and minerals for the people of many poor countries (Jaclyn *et al*., 2010). In addition, fish is also easy to digest. Fish in Egypt is one of the most important sources of national income. In addition, it is also a safe protein source that provides the nutritional needs of Egyptians. The Egyptian fisheries occupies about 150% of the agricultural land (13 million acres), which includes the seas (the Red Sea and the Mediterranean Sea), many lakes and fresh water sources such as the Nile River with its two branches, canals and banks. Man has created artificial resources such as Lake Nasser and Lake El Rayan, in addition to fish farms located in different regions of Egypt.

Nile tilapia (*Oreochromis niloticus*) is the most popular type of fish. Egypt ranked third in the world in the production of Nile tilapia fish, and it exported $8,637,000 in Nile tilapia in a year. The original home of Nile tilapia is in large parts of Africa. The Nile tilapia fish comes either from the lakes or the Nile River

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or from fish farms, pointing out that most of the tilapia fish in the Egyptian market come from fish farms.

Fish proteins are essential in the diets of countries with high population density and poor communities to overcome low total protein intake (Solomon & Oluchi., 2018, Saleem *et al*., 2019).

However, there are factors that make fish the most perishable animal product such as its high water content, non-protein nitrogen, free amino acids, postmortem pH, and trimethylamine oxide. In a study conducted by Talab *et al*., (2016) on the Chemical compositions and heavy metal contents of Nile tilapia (*Oreochromis niloticus*) from the main irrigated canals (rayahs) of Nile Delta. They found that, the moisture, protein, fat, ash, carbohydrates and calorific values varied between (78.55–80.77%), (16.10–17.88%), (1.10–1.95%), (0.55–1.50%), (0.10–0.94%) and (78.37–89.73%), respectively. As a result of the reaction of polyunsaturated fatty acids (PUFA) with atmospheric oxygen, Malonaldehyde is formed. Which is measured its content by calculating the value of thiobarbituric acid (TBA), which is an indicator of fat oxidation (Ozogul *et al*., (2011). Chomnawang *et al*., (2007) found that as a result of fish spoilage, pH values increase due to the accumulation of some compounds such as trimethylamine and ammonia, which are produced due to the action of body enzymes and bacterial enzymes in fish. Ali *et al*. (2010), mentioned that, during freezing about 10-60% of viable microbes die, and the remaining number gradually increases during storage, which makes us expect the production of (TVB-N) resulting from bacterial activity during frozen storage. The loss or reduction in the ability to hold water also causes the fish and meat industry to lose millions of dollars annually. Huff-Lonergan and Lonergan, (2005) mentioned that one of the most important reasons for maintaining the quality characteristics of raw products is due to the ability of fresh meat or fish muscle to retain moisture. Food safety has become one of the priorities of the times, given the great challenges facing the world of food, especially meat, poultry and fish, with regard to additives that are used either to improve the efficiency of the product or to prolong its refrigeration period to protect food from foodborne pathogens that lead to microbial spoilage, in line with the desire of and consumer taste. During production, processing, delivery and storage, food is subjected to chemical reactions and microbial actions (Kenawi, *et al*., 2011). Microbial contamination and spoilage is the main component of this decomposition because of its negative effects on sensory qualities (odor, flavor, texture, color, etc. (McCarthy *et al*., 2001). Because of consumers' awareness of the risks arising from the use of food additives of artificial origin, the demand for consumption of foods free of those artificial preservatives and replace them with natural products has increased. Recently, the consumption of fast foods and ready-to-eat foods such as fish fingers, fish burgers, sausages and fish balls has been increased due to the development in the lifestyle and nutritional awareness of consumers. It may be fresh, raw or cooked, and can be consumed without further preparation or treatment (Arif *et al*., 2018).

The objectives of this investigation were to

1-Increase the economical and price value of the fresh Nile tilapia (*Oreochromis niloticus*) by producing fish fillet and fish balls products.

2-Study the effect of adding two natural antimicrobial agents (thyme extract and green tea extract) on the stability of frozen packaged tilapia products stored for 10 months.

Materials and Methods

Thyme and green tea extracts

Thyme plant was obtained from local market of Minia city, Egypt. The thyme leaves were washed and dried in air, then ground to obtain particles of 60 mesh. A hundred grams of thyme powder mixed with 500 ml ethanol solution (50%) in flask. The mixture shook (at 1500 rpm) over night at room temperature, then, centrifuged at 3000 rpm for 15 minutes. The extract was preserved in brown glass, wrapped with aluminum foil, and stored in refrigerator till use (Aboelazab *et al*., 2019).

The green tea was purchased from local market of Minia city, Egypt. The green tea was ground to obtain particles of 60 mesh. Twenty-five grams of the powder mixed with 900 ml of hot distilled water (85 °C) for 24 hours, and frequently shook then, centrifuged at 3000 rpm for 15 minutes. The extract was preserved in brown glass, wrapped with aluminum foil, and stored in refrigerator till use (Luo *et al*., 2020).

Spices (black pepper, cumin powder, paprika, and garlic powder), salt, potato starch, vegetable sunflower oil (Afia brand), and lemon were purchased from the local market of Minia city, Egypt.

Preparation of Nile tilapia fish product

Thirty-five kg of live Nile tilapia fish (*Oreochromis niloticus*) used in this study was obtained from local market of El-Minia, Egypt. The fish were thoroughly washed, gutted, manually made into fillet. Half of the fillet was kept, and the other half mechanically minced by a meat grinder using 4 mm (coarse), then stored refrigerated at 4°C until use. The fish fillet part was divided into three portions. One portion was kept without any treatment as control. The other two portions one of them soaked in thyme extract for one hour at refrigerated condition, while the last portion was soaked in the green tea extract for one hour at the same refrigerated condition.

The second minced part was mixed with other ingredients to form fish balls as shown in table (1) according to the method described by (Chowdhury *et al*., 2017).

The fish ball mixture divided into three portions. One portion left as control with no additives, while the second portion mixed with thyme extract in the ration of (1ml/10 g), and the third portion mixed with green tea extract in the same ratio. The mixing time was 5 min. Each portion (of the fish balls mixture) was divided

into small balls 40±1 g each, and placed in polystyrene (foam) tray with LDPE cheats in between to prevent sticking, and packaged in LDPE bags with no vacuum. All the treatments were stored frozen at -18°C for 10 months as shown in (Figure 1).

Table 1. Formulation of Nile tilapia fish balls product

Ingredient	Percentage (%)
Minced fish	80.0
Vegetable oil	6.0
Potato starch	6.0
Salt	2.0
Black pepper	1.0
Garlic powder	2.0
Cold water ice	1.0
Lemon juice	1.0
Paprika	0.5
Cumin powder	0.5

Chemical composition, pH, total acidity, total volatile basic nitrogen (TVB-N), Thiobarbituric acid (TBA), water holding capacity (W.H.C) and expressible water (EW), Total plate count (TPC), Psychrophilic bacteria, and Coliform bacterial count for the fish products (fillet and fish balls) was conducted according to the methods described by the Association of Official Analytical Chemists (AOAC, 2002), (MHM *et al*., 2017), Alvarez, *et al*. (1992), Tarladgis *et al*. (1969).

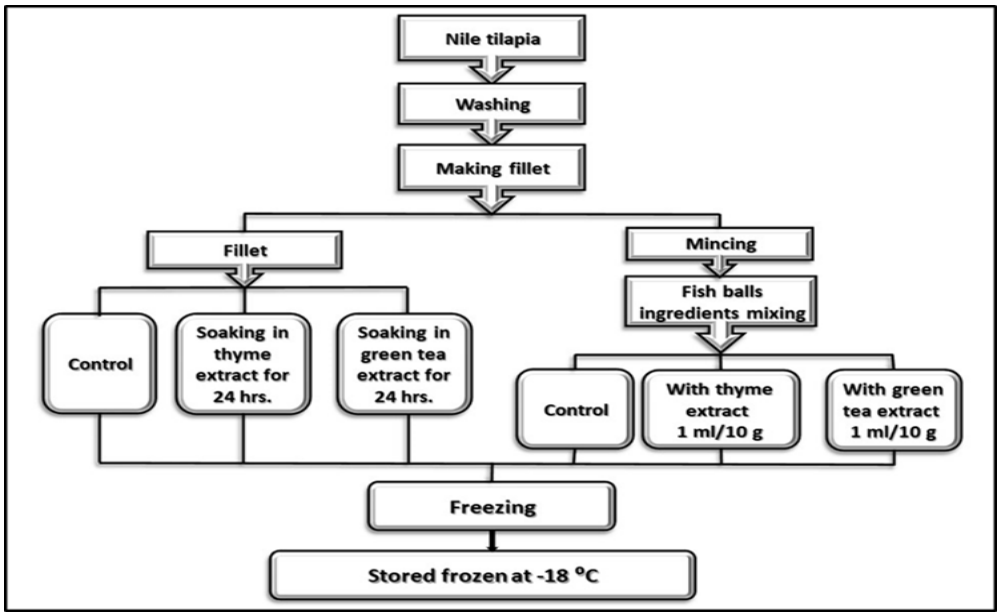

Fig. 1. Flow diagram of production of Nile tilapia (*Oreochromis niloticus*) products stored for 10 months under frozen condition

Results and Discussion

Fish is considered as one of the cheapest sources of animal protein, important nutrients, and minerals required to nourish and build the human body all over the world and Egypt. One of the most common types of fish is Nile tilapia (*Oreochromis niloticus*), as Egypt ranked third in the world in producing Nile

tilapia, and exported $8637000 of Nile tilapia within a year. The original habitat of the Nile tilapia is in large parts of Africa.

Table (2), illustrated the chemical composition, pH, and total acidity (lactic acid) of Nile tilapia (*Oreochromis niloticus*) fillet and fish balls products treated and untreated with two natural antimicrobial extracts (thyme and green tea) at the beginning of storage time (zero time). The data revealed that there were slight differences in the moisture content between the samples at zero time of storage. There were increase in the ash content, total acidity for the fish balls samples compared with the fillet samples. While for the fillet samples the crude protein content, and the ether extract values were higher than the fish balls samples. The data also showed that the pH values for the fish balls samples were lower than the fillet samples.

Table 2. Effect of natural antimicrobial extracts on the Chemical composition, pH and total acidity (lactic acid) of Nile tilapia products

Parameters	C-F	GT-F	TH-F	C-FB	GT-FB	TH-FB
Moisture content (%)	76.0±03	78.0±01	77.0±02	76.0±02	78.0±07	77.0±01
Crude protein (%/)*	74.0±07	73.5±05	74.0±03	43.75±05	43.75±01	43.75±03
Ether extract (%/)	8.5±02	8.5±03	8.6±02	8.2±07	8.2±02	8.1±03
Ash (%/)	0.98±01	0.98±05	0.97±05	0.99±03	1.09±05	1.08±07
pH	6.22±07	6.15±02	6.15±07	5.71±05	5.67±07	5.67±05
Total acidity (mg lactic %)	0.67±03	0.67±05	0.67±01	1.1±02	1.1±03	1.1±01

C-F= control fillet. GT-F= green tea extract treated fillet. TH-F= thyme extract treated fillet.

C-FB= control fish balls. GT-FB= green tea extract treated fish balls. TH-FB= thyme extract treated fish balls. *N X 6.25

Figure (2) represents the effect of antimicrobial extracts treatment on the % moisture content values of tilapia fillet and fish balls stored for 10 months under frozen condition. The data showed that all the studied samples suffered from reduction in their moisture content along with the storage period. At the beginning of storage time the moisture contents for the different treatments were between 76, and 78 % for the control sample and the sample treated by the extracts of thyme and green tea. The difference between the two values comes from the treatments of fish fillet and fish balls by the aqueous solutions of thyme and green tea. By the end of storage period the moisture content reduced to the values between 70%, and 73 %. This could be due to the effect of thyme and green tea extracts as antimicrobial on keeping the moisture in the treated samples compared to the control ones. This comes in agreement with what was found by (Gupta *et al*., 2019).

Water holding capacity values of control and treated fillet and fish balls of tilapia fish (*Oreochromis niloticus*), stored for 10 months under frozen condition is shown in Fig. (3). The results showed a reduction in the (WHC) values for all samples. The rate of reduction was much higher in the fillet samples (from 26% - to about 17%) compared to the fish balls products (from 28% - about 22%) after 10 months of frozen storage. This could be due to the effect or the power of the other ingredients of the fish balls on catching the moisture in the matrix.

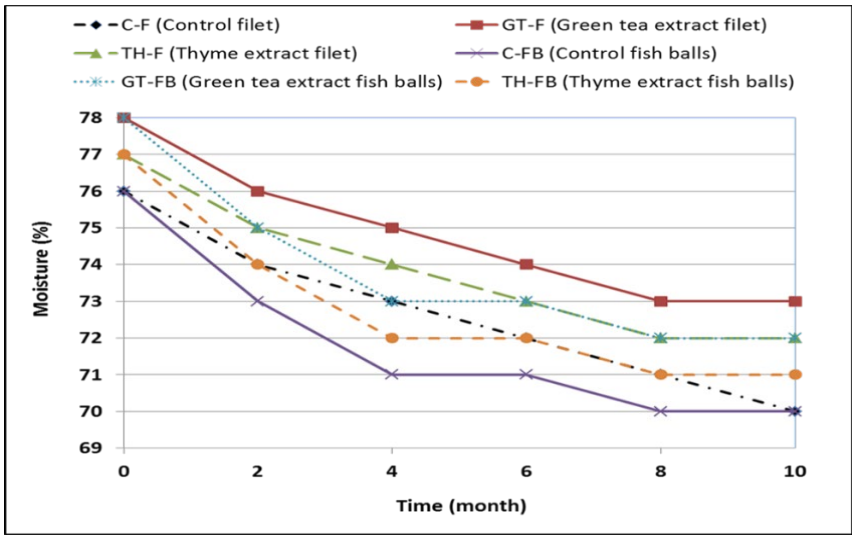

Fig. 2. The effect of antimicrobial extracts (thyme and green tea extracts) on the % moisture content values of tilapia fillet and fish balls stored for 10 months under frozen condition

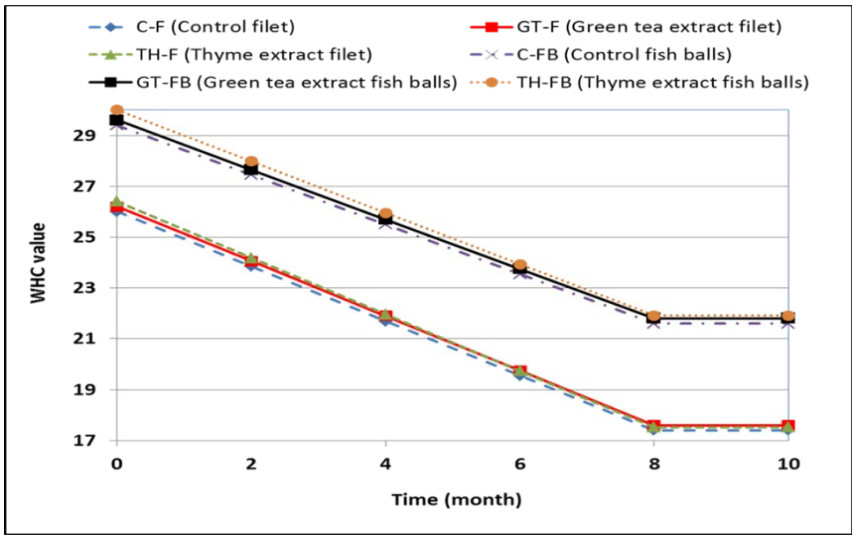

Fig. 3. The effect of antimicrobial extracts (thyme and green tea extracts) on the water holding capacity (WHC) values of tilapia fillet and fish balls stored for 10 months under frozen condition

The effect of adding natural antimicrobial substances on the % expressible water values of frozen stored tilapia fish products is illustrated in Fig. (4). The data showed an increase in the (% EW) values with the increasing in the storage time for all treated and untreated samples. The rate of increment was higher in the control, and fillet treated with thyme or green tea extract samples than the control, and treated with thyme or green tea extract fish balls samples. At the beginning of storage period the (% EW) values for the control and the thyme or green tea extracts treated tilapia fish fillet samples were 50 %, 50.8 %, and 52 % and reached 53.7 %, 54.5 %, and 55.4 % at the end of storage period respectively. Whereas, for the fish balls the values increased from 46.8 %, 47.0 %, and 48.3 % to 48.2 %, 49.0 %, and 50.2 % respectively. The data comes in a logic harmony with what was previously obtained from the water holding capacity (WHC) values of the same products. From the obtained data one can realized that the fish balls

ingredients could help in capturing the water in the matrix and this goes with what was found in the case of the water holding capacity.

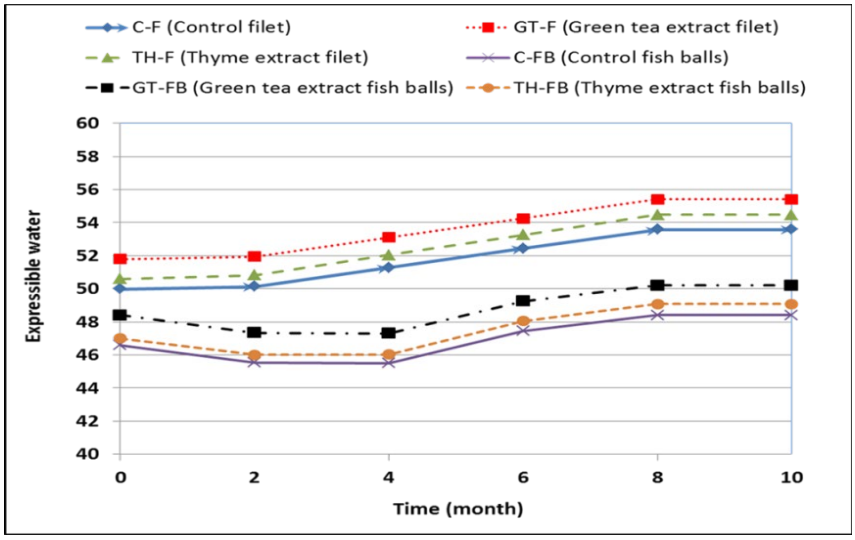

Fig. 4. The effect of antimicrobial extracts (thyme and green tea extracts) on the expressible water (EW) values of tilapia fillet and fish balls stored for 10 months under frozen condition

Figure (5) illustrates the effect of adding thyme and green tea extracts on the pH values of tilapia fish fillet and fish balls, stored for 10 months under frozen condition. The data revealed an increase for the pH values for all samples along with the storage time. The rate of increment was the highest in the tilapia fish balls. The pH values for the tilapia fish balls were lower than the values for the fillet product which means that the ingredients add to form fish balls have lowered the pH values for the final products. The data also revealed that, the increase in pH values for fish products during storage indicates spoilage likely caused by bacterial contamination as the metabolic activity of bacteria decomposes nitrogen compounds and forms basic compounds like ammonia and trimethylamine (Taskaya, *et al*., 2003).

The effect of adding natural antimicrobial on the total acidity values (expressed as mg/100g lactic acid) of tilapia fish (*Oreochromis niloticus*) fillet and fish balls, stored for 10 months under frozen condition is shown in Fig. (6). The data showed a reduction in the total acidity values for all samples along with the progressing in the storage time. The rate of reduction for the tilapia fish balls samples were greater than the fillet samples. This could be related to the other ingredients used in preparing the fish balls products. The reduction in the total acidity value could be due to the formation of TMA and ammonia as the action of body and microbial enzymes in the fish flesh (Chomnawang *et al*., 2007).

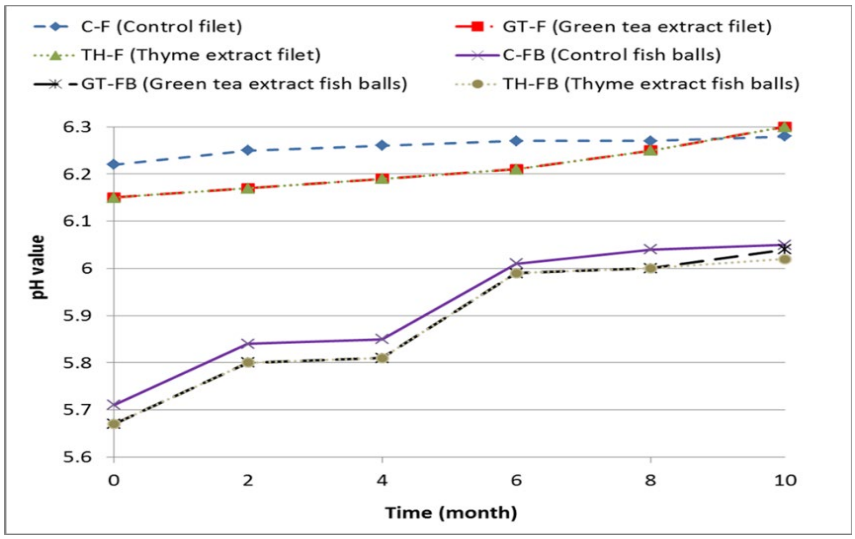

Fig. 5. The effect of antimicrobial extracts (thyme and green tea extracts) on the pH values of tilapia fillet and fish balls stored for 10 months under frozen condition

The effect of storage time under frozen condition, and the addition of natural antimicrobial on the TBA (mg malonaldehyde/kg sample) and the total volatile basic nitrogen compounds (TVB-N) values of tilapia fish (*Oreochromis niloticus*) fillet and fish balls are shown in Figs. (7&8).

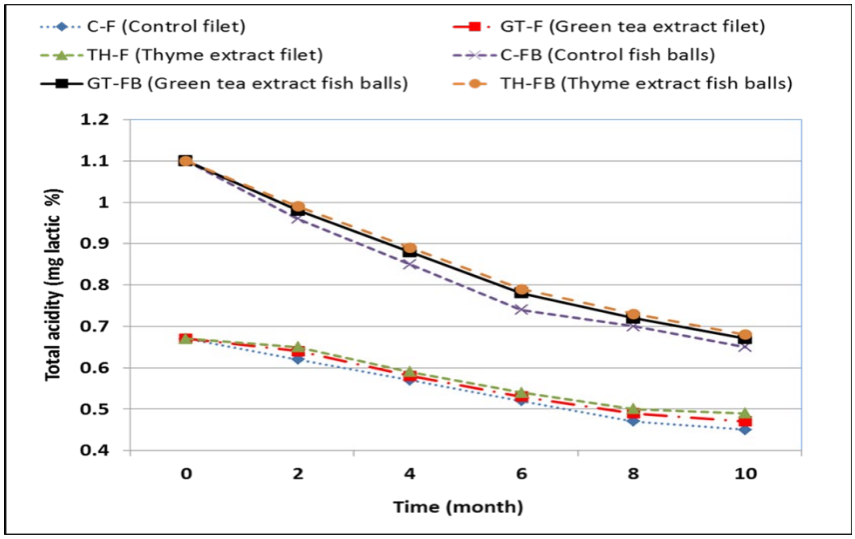

Fig. 6. The effect of antimicrobial extracts (thyme and green tea extracts) on the total acidity (lactic acid) values of tilapia fillet and fish balls stored for 10 months under frozen condition

The data showed an increase in the TBA and the (TVB-N) values for all samples along with the progressing of storage period. For the TBA, the rate of increasing for the tilapia fillet samples were greater than for the fish balls samples. In the same trend the rate of increasing for the control sample was the highest followed by the green tea treated sample and the thyme extract treated one. Whereas for the (TVB-N), the values were between 14.0 and 14.7 mg/100g at the beginning of storage time for the control and thyme extract or green tea extract treated samples. At the end of storage period the values raised between 20.7, and 21.7 mg/100g for the same samples. The addition antimicrobial extracts (thyme

and green tea) could reduce and retard the oxidation of tilapia fish products because such materials have been described to be an excellent source of natural antimicrobial and antioxidants, thus, increase the shelf life of foods containing fat for the presence of many different types of antioxidant components such as flavonoids, ascorbic acid, carotenoids and phenolic (Al-Juhaimi *et al*., 2015).

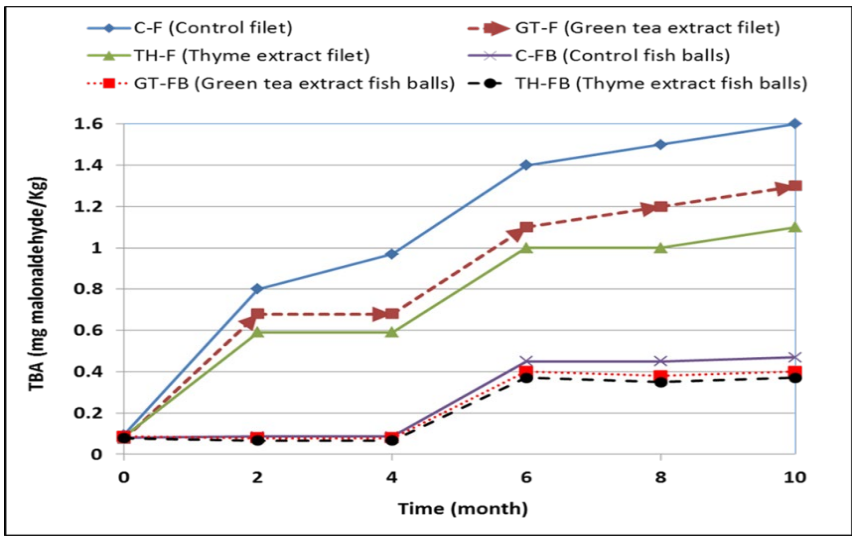

Fig. 7. The effect of antimicrobial extracts (thyme and green tea extracts) on the TBA (mg malonaldehyde/kg sample) values of tilapia fillet and fish balls stored for 10 months under frozen condition

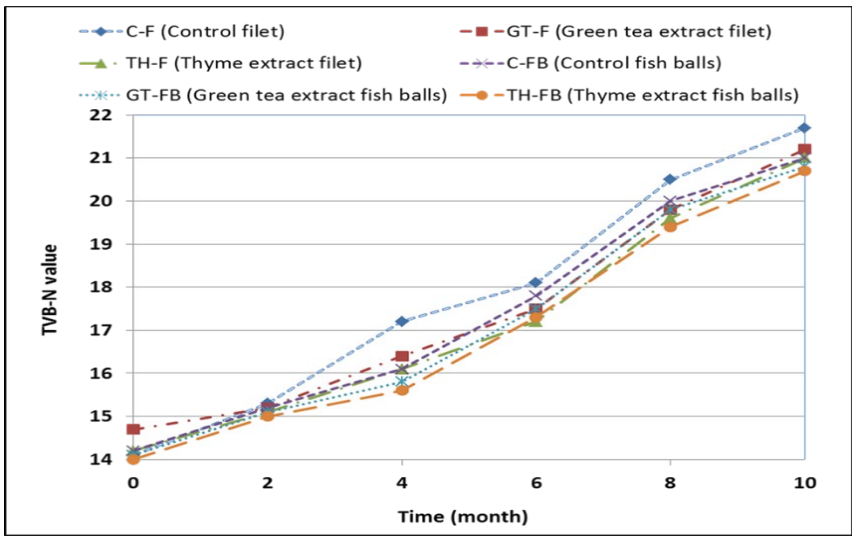

Fig. 8. The effect of antimicrobial extracts (thyme and green tea extracts) on the total volatile basic nitrogen (TVB-N) values (mg/100 g) of tilapia fillet and fish balls stored for 10 months under frozen condition

Figures (9-11) show how adding thyme or green tea extracts as natural antimicrobials affected the total bacterial count, Psychrophilic bacterial count and Coliform group (log CFU/g) in Nile tilapia (*Oreochromis niloticus*) fish fillets and fish balls that were stored frozen for 10 months. The data showed that all the treated and untreated samples have total bacterial count (log CFU/g) less than 6. There was a reduction in the total bacterial count with all treatments along with storage

time. The control samples were the highest in the total bacterial count compared with the treated ones. Samples treated by thyme extract as antimicrobial have no bacterial colonies after the zero time which shows the effective effect of thyme extract as an antimicrobial substance. No microbial growths were shown in the fish ball samples treated with green tea after the eighth month of storage. The number of psychrophilic bacteria (log CFU/g) decreased throughout all treatments and storage times. In comparison to samples treated with antimicrobial extract (thyme and green tea extract), more colonies were present in the control samples. That means the addition of thyme or green tea extract as natural antimicrobial is negatively affected the number of psychrophilic bacterial count in the samples.

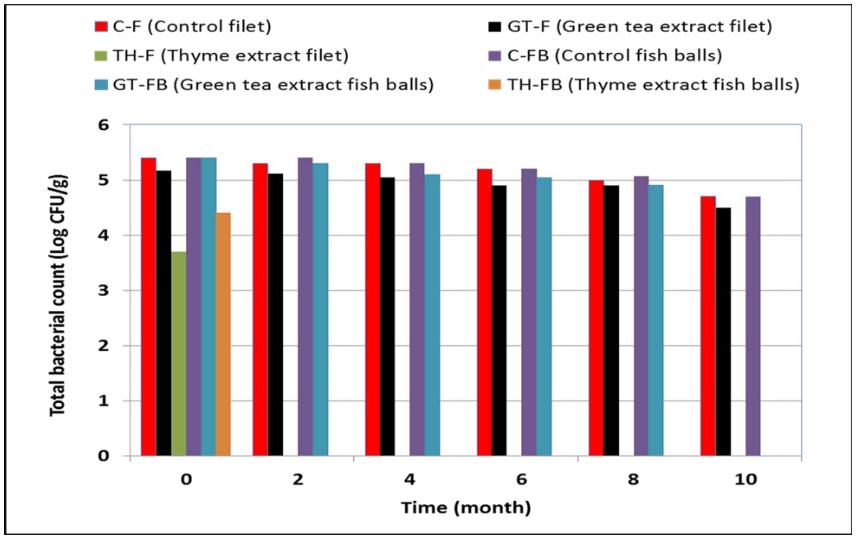

Fig. 9. The effect of antimicrobial extracts (thyme and green tea extracts) on the total bacterial count (log CFU/g) of tilapia fillet and fish balls stored for 10 months under frozen condition

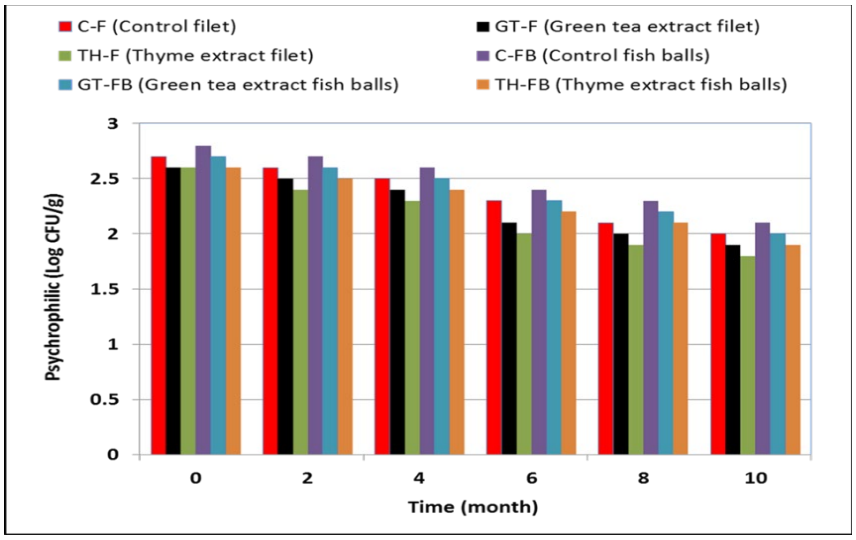

Fig. 10. The effect of antimicrobial extracts (thyme and green tea extracts) on the Psychrophilic bacterial count (log CFU/g) of tilapia fillet and fish balls stored for 10 months under frozen condition

The data showed that at the beginning of storage time (zero time), only three samples (control fillet, control fish balls, and green tea extract treated fish balls)

have coliform group bacterial count (less than 2.5 log CFU/g), then no colonies have been shown in the rest of storage period. This showed the negative combined effects of the natural antimicrobial extracts (thyme and green tea extract), and storage temperature on the surviving power of the coliform bacteria.

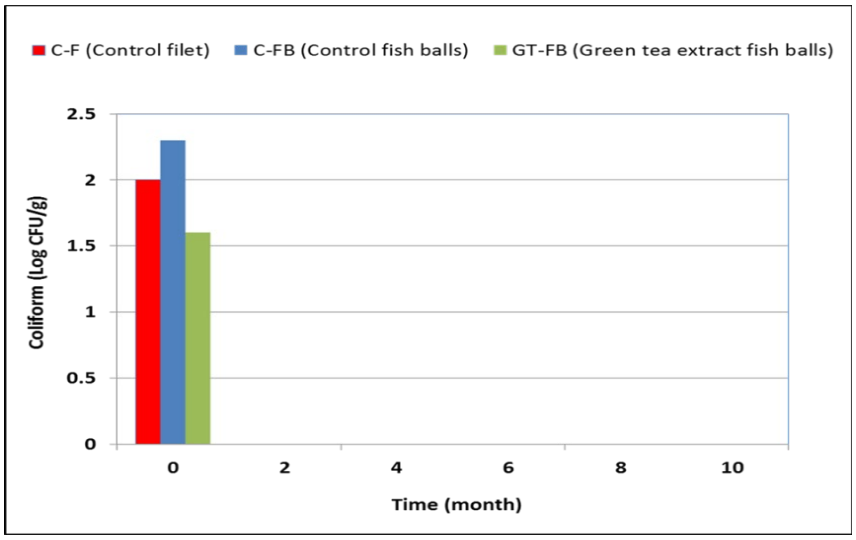

Fig. 11. The effect of antimicrobial extracts (thyme and green tea extracts) on the Coliform bacterial count (log CFU/g) of tilapia fillet and fish balls stored for 10 months under frozen condition

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تأثير بعض المستخلصات الطبيعية لمضادة للميكروبات على ثبات منتجات أسماك البلطي النيلي (*Oreochromis niloticus*) أثناء التخزين المجمد

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الملخص

تم استخدام أسماك البلطي النيلي (*Oreochromis niloticus*) في إنتاج شرائح السمك وكرات الأسماك بعد معالجتها بمستخلص الشاي الأخضر ومستخلص الزعتر كمضاد طبيعيي للميكروبات وتخزينها تحت ظروف التجميد لمدة 10 أشهر. أظهرت النتائج انخفاضًا في محتوى الرطوبة ومستويات WHC والحموضة الكلية لجميع العينات، بينما زادت قيم EW و pH و TBA و TVB-N لجميع العينات مع تمديد فترة التخزين تحت ظروف التجميد. أظهرت البيانات أن العدد الإجمالي للبكتيريا (log cfu / g) كان أقل من 6 في جميع العينات المعالجة وغير المعالجة. خلال فترة التخزين، كان هناك انخفاض في العدد الإجمالي للبكتيريا. بالمقارنة مع العينات المعالجة، كانت عينات الكونترول أعلى فى العد البكتيري الإجمالي. أظهرت النتائج أن أعداد البكتيريا لكل جرام عينة من البكتيريا المحبة للبرودة انخفضت بعد كل العلاجات وفترات التخزين. وفقًا للنتائج، فإن ثلاث عينات فقط (فيليه الكنترول، وكرات السمك الكنترول، وكرات السمك المعالجة بمستخلص الشاي الأخضر) كان التعداد البكتيري لمجموعة القولونيات في بداية وقت التخزين (الوقت صفر) اقل من 2.5 (log cfu / g) ولم يتم العثور على مستعمرات لـــ وقت التخزين المتبقي.