Effect of carotenoids on keeping quality of refrigerated common carp fish slices

تأثير الكاروتينويدات على جودة الحفظ لشرائح اسماك المبروك العادى المبردة

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Abstract

This study aimed to evaluate the physicochemical, microbiological and sensory quality of soaked common carp fish slices in carotenoids extracted from crayfish wastes during storage at 5±1°C for 12 days. The obtained results indicated that the physicochemical and microbiological parameters of PH, peroxide, anisidine, total oxidation, TVB-N values and total bacterial count of all samples increased with increasing the storage time. In contrast, the scores of sensory properties of odor and over all acceptability were decreased during storage period as a result of spoilage advancement. The control samples were exhibited the highest deterioration rate during cold storage. In the meantime, the carotenoids were retarded the spoilage as the result of their antioxidant effects, to be reduce lipid oxidation and protein degradation by the reduction of microbial growth in treated fish slices. Therefore, the sensory quality of soaked fish slices treatments positively

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affected by soaking in carotenoid concentrations, to present the best scores during storage compared to the control sample. The carotenoid concentrations particularly 0.2 and 0.3% were extended the quality of soaked common carp fish slices for 10 days, higher than of 6 days for control samples.

Key words: common carp fish, carotenoid extracts, soaking, physicochemical, microbiological, sensory evaluation.

INTRODUCTION

Fish serves as a good source of dietary protein which is very inexpensive in relation to other animal protein foods and it is an excellent component of human diet **(Olalekan, 2019)**. Fresh fish and their products are an important source of animal protein, also it often are cheap, easy to get, well nutritive and still in available stage in comparison with other animal protein sources, so the demand of fish and fish products are increased to compensate the shortage in the other animal protein resources. Fish has high protein content and low saturated fat content, which is considered as highly valuable food. In particular, fish is the primary dietary source of omega-3 polyunsaturated fatty acid (PUFA), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), both of which are well-known for the anti-inflammatory action and protective effects on cardiovascular disease **(Vilavert *et al*., 2017 and Siscovick *et al*., 2017).**

However, post-harvest handling, processing, and storage of fish lead to food losses and waste. The concept of fish and fish products spoilage closely contributed with that, the fresh fish is a highly perishable product due to its high-water activity, nutrient availability, nearly neutral-pH (factors that influence microbial growth) and the presence of autolytic enzymes;

hence, it is susceptible to post-harvest losses. Under normal refrigerated storage conditions, its shelf-life is limited by the development of enzymatic (caused by endogenous or microbial enzymes) and chemical reactions are an obviously fish spoilage factor that responsible for oxidative rancidity, rancid flavor, texture changes and non- enzymatic oxidation discoloration **(Speranza *et al*., 2021 and Tesfay & Teferi, 2017).**

For many years, synthetic preservatives have been used in the food industry because of their anti-bacterial properties. Synthetic additives can reduce food spoilage however they have been accused for some allergies, intoxications, cancer and other serious diseases. Thus, many consumers are desired to consume heal their products containing natural preservatives and additives instead of synthetic ones. For these reasons, one or more adequate preservation methods are required in order to maintain the safety and quality and extend the shelf life of such products, which gives a great attention to fish preservatives, especially natural preservatives، a great interest as alternatives to chemical preservatives. **(Raeisi *et al*., 2019; Hassoun and Karoui, 2017 and Alsaiqali *et al*., 2016).**

Hence, in the past decade, researchers have been focusing on the exploration of safe, effective and acceptable natural preservatives for controlling the microbial and chemical mechanisms responsible for spoilage in fish **(Viji *et al*., 2017 and Velasco & Williams, 2011)**.

Crayfish (*Procambarus clarkia*) by product is a rich source of many voluble products, such as protein, chitin, and pigments (e.g. astaxanthin) (**No *et al.*, 1989 and Guillou *et al.*, 1995).** Currently crayfish by products are used mainly as a supplement in animal feed, or not used at all, but deposited on the land,

constituting an important focus of environment pollution. However, because of its high protein content **(Knorr, 1982),** this by-product could be a good source of protein if appropriate processing was developed.

Carotenoids extracted from shrimp processing discards were evaluated for antioxidant activity. Crude extract and fractions rich in astaxanthin showed strong antioxidant activity as indicated by radical scavenging, reducing activity and metal chelating activity, comparable to that of the known antioxidants a -tocopherol and TBHQ. Singlet oxygen quenching activity of crude extract and its fractions was higher than that of a -tocopherol. Nitric oxide scavenging activity was also higher than a-tocopherol **(Sachindra *et al.*, 2005a&b; Sachindra *et al.*, 2006 and Sachindra & Bhaskar, 2008**). The antioxidant activity of carotenoid extracted from crayfish wastes at concentrations 0.5 and 1%, the results obtained showed that the scavenging radicals activity proved 3.29 and 3.76, respectively, while that antioxisdant activity values at the same concentration were 11.32 and 7.21 **(Ibrahim, 2017).**

Also studied that the addition of 0.5% carotenoid to crayfish burger stored at -18C° for three months, the obtained results showed that reduced the rates of loss of moisture, protein and fat. Physicochemical quality parameters such as PH, cooking loss TVB- N, TMA-N and FAN of crayfish burger showed that the addition of 0.5% carotenoid extracted from crayfish wastes led to maintain product quality and reduce the rate increase of above parameters. Also results showed that the adding 0.5% carotenoid improved degree of stability against oxidation and rancidity of lipid extracted from the samples during frozen storage. The results obtained cleared that decreased rate of

increase logarithm numbers of total bacterial counts. The addition of carotenoid to crayfish burger improved sensory characteristics of cooked samples by grilling method.

This study aimed to evaluate the physicochemical, microbiological and sensory quality of soaked common carp fish slice in carotenoids extracted from crayfish wastes during storge at ٥±1°C for 12 days.

MATERIALS AND METHODS

Materials

All samples of fresh water crayfish waste red swamp (*Procambarus clarkii*) were obtained from the toshka masr company in Cairo, Egypt, during, 2023. Crayfish wastes weighing 7 kg were transported using ice boxes to Fish Technology and Processing Laboratory, National Institute of Oceanography and Fisheries, El-kanater El-khiria, Ministry of Scientific Research.

All samples of common carp (*Cyprinus carpio*) were purchased from eserw fish farm belong national institute of oceanography and fisheries, Egypt, and transported in icebox to fish processing and technology laboratory, fish research station, El-Kanater El-Khiria, National Institute of Oceanography and Fisheries, Egypt. About 15 kg from fish with average weight from 1.500 to 2.600 kg, length from 43.25-53.3 cm for common carp fish and nutrient agar medium were obtained from Sigma Company for chemicals.

Refined corn oil was obtained from the local market; produced in January, 2022 by Arma Food Industries Co., 10th of Ramadan, Egypt. White polyethylene pages were obtained from the local market.

Methods

Preparation of crayfish wastes and extraction

Crayfish wastes were washed, dried at 45°C overnight, grinded, then sieved to obtain coarse powder at particle size 40 meshes and stored in dry place until extraction of carotenoids. The optimized method of carotenoid extraction reported by **Sachindra *et al*. (2006)** was used.

Preparation of carotenoid concentrations

Four carotenoid concentration solutions were prepared by mixing 1, 2, 3 and 4 ml of crude carotenoid with 1000 ml of corn oil for each and stirred at a controlled temperature of 27 ºC until the mixture became clear to obtain 0.1, 0.2, 0.3 and 0.4% (v/v) carotenoid solutions. All solutions were cooled until prior to surface application onto fish slices.

Preparation of fish slices samples

Fish prepared for processing under hygienic condition, all fish were rewashed, beheaded, eviscerated, skin removed and filleted. Fish slices were washed and drained. Fresh fish meat subjected to chemical and physical, microbiological analysis to assess freshness and quality of used fish.

Irregular slices (average 6.5cm length and 1.5cm sickness) were divided into five equal batches. The first batch without added carotenoid was directly molded into slices and used as a control. Batches number two, three, four and five of common carp fish slices were immersed in the solutions (0.1, 0.2, 0.3 and 0.4% v/v carotenoid solutions) for 50 mints and then air-dried for 5 mints. They were then packed in polyethylene bags and stored at 5 ±1 ºC for 12days. Fish samples were taken randomly at intervals for analyzing every two days.

Analytical methods

Moisture, protein, lipids and ash contents of raw slices of common carp fish. Acid, peroxide and anisidine values were estimated using the method described by were determined according to **AOAC (2007)**, while carbohydrates were calculated by difference. The pH value was estimated according to the method mentored by **Aitken *et al*. (1962).** Total oxidation value was calculated using the anisidine value conjugation with the peroxide value according to **Rossell (1983).** The method recommended by the **AMC (1979)** for the determination of TVBN and TMAN is based on a semi-micro distillation procedure. The Total bacterial count (TBC) was determined by using nutrient agar medium as described by **Oxoid (2006)**. The various samples were evaluated every two days during 12 days of common carp fish slices, the organoleptic properties of fish slices were evaluated by 10 panelists from Fish Technology and Processing Laboratory, National Institute of Oceanography and Fisheries, El-kanater El-khiria, Ministry of Scientific Research. A 9-point hedonic scale was employed in this sensory analysis by the modified method of **Teeny and Miyaauchi (1972).**

Statistical analysis

Three replicates of each trial were performed for each parameter using ANOVA and the means were separated by **Duncan (1955)** at a probability level of $P<0.05$ (**SAS, 2000**).

RESULTS AND DISCUSSION

The proximate composition (W/W) of fresh fish meat was investigated as shown **Table (1)**. The moisture, protein, fat, ash and carbohydrate contents were 72.80, 13.54 12.25, 1.07 and 0.34% of common carp fish flesh, respectively. In general, proximate composition of fresh fish in this study was in

agreement with mentioned by **(Akinwumi *et al.*, 2011; Khidhir, 2011; Akpambang, 2015; Elsayed *et al.*, 2016; Yu *et al*., 2017; Sudirman *et al*., 2018 and Abdulrahman *et al*., 2019)**. The differences in proximate composition can be caused by many factors, especially differences in habitat, size, sex and sexual condition of fish.

The similar change in chemical composition due to processing effects, such the increment in moisture and dry matter decrement due to the soaking of fish fillets in extra diluted aqueous solution could be caused water absorption into fish tissue, and outing water soluble substances such residual entire blood, pigments, enzymes, minerals and some water-soluble proteins, also surface fat loss through floating in water phase and removed afterward, such observations were also noticed by **Das *et al*. (2015); El-Shennawy *et al*. (2017) and Moawad *et al*. (2017)**. This may be explained by the fact that during the process of washing some amount of water is held by the meat as reflected by the increase in moisture percent of washed meat compared to unwashed meat. The hydrophobic residues of *(myofibrillar)* proteins may be responsible for retention of water (**Khanipour *et al*., 2014; Moawad *et al*., 2017; Yu *et al*., 2017; Bandre *et al*., 2018 and Golgolipour *et al*., 2019)**.

Table (1): Chemical composition, Physicochemical and microbiological counts of common carp fish flesh.

Parameters	Common carp fish flesh
Moisture %	72.80±1.12
Protein %	13.54±1.21
Fat %	12.25±1.19
Ash %	1.07±0.13

Carbohydrate %*	0.34±0.08
Ph	6.44±0.03
Acid value (mg KOH/g**)**	0.30±0.08
Peroxide value(meq/kg)	0.76±0.10
Anisidine value	0.05±0.01
Total oxidation	1.57±0.18
TVB–N (mg / 100gm)	6.39±0.21
TMA-N(mg / 100gm)	1.26±0.10
Free amino nitrogen (%)	0.84±0.04
Total bacterial count (log $_{10}$ cfu/g)	2.98±0.14

Means of triplicate ± Standard Deviation on wet weight basis.

The physicochemical quality indicators of fresh fish fillet were evaluated as shown in **Table (1)**.The pH, acid value, peroxide value, anisidine value, total oxidation, total volatile basis nitrogen, trimethyl amine and free amino nitrogen of common carp fish flesh were 6.44, 0.30 mg KOH/g, 0.76 (m.eq/kg), 0.05, 1.57, 6.39 mg /100gm, 1.26 mg /100gm and 0.84%**.** The similar values were obtained by **El-Lahamy *et al*. (2019)** found that pH value common carp meat was 6.49 **(Sönmez *et al*., 2020),** also, these results are in agreement with that observed by **(Khidhir, 2011 and Abbas *et al*., 2021).** Concerning quality criteria as well as TVB-N and TMA-N values of fish fillets in current study indicated to high freshness fish material. The TVB-N and TMA-N values of fresh fish similar with results found by **(Badee *et al*., 2013; Mahmoud, 2016; Morshdy *et al*., 2018 and Kuzgun, 2019).** Therefore, the total bacterial count of common carp fish flesh was 2.98 log $_{10}$ cfu/g. The microbial counts of fresh fish were in acceptable limits of fresh fish according to **ICMSF, (1986)**.

pH value

Effect of incorporated different concentration of carotenoid on the changes of pH values of common carp fish slices samples during refrigerated storage are presented in **Table (2).**

Table (2): Changes in pH value of common carp fish slices treated with different levels of carotenoid during storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	**With carotenoid 0.1%**	**With carotenoid 0.2%**	**With carotenoid 0.3%**	**With carotenoid 0.4%**
0	6.14±0.13	5.98±0.15	5.78±0.13	5.64±0.16	5.79±0.11
2	6.23±0.17	6.09±0.15	5.93±0.13	5.81±0.12	5.96±0.09
4	6.38±0.14	6.19±0.13	6.02±0.09	6.28±0.17	6.04±0.10
6	6.84±0.12	6.31±0.18	6.21±0.12	6.31±0.07	6.16±0.13
8	7.13±0.17	6.37±0.12	6.39±0.08	6.35±0.15	6.39±0.14
10	R	6.44±0.18	6.44±0.13	6.43±0.15	6.42±0.13
12	R	6.78±0.12	6.71±0.09	6.80±0.17	6.86±0.11

Means of triplicate ± Standard Deviation on wet weight basis.

It could be noticed that, carotenoid concentration decreased the pH values of common carp fish slices samples at zero time of storage since initial pH values were 5.98, 5.78, 5.64 and 5.79

for common carp fish slices treated with 0.1%, 0.2%, 0.3% and 0.4% extracted carotenoid, respectively, compared to 6.14 for control samples. This decrease in pH could be attributed to the basic nature of carotenoid and its concentrations dependent.

The pH level increased gradually during storage for all formulas **(Table 2).** Data showed that, the highest pH values were obtained for common carp fish slices samples control (without preservatives) and treated with 0.4% carotenoid at every storage period reaching 7.13 and 6.86 after 8 and 12 days, respectively. However, the pH of samples treated with 0.1, 0.2 and 0.3% carotenoid registered the second order having respective values of 6.78, 6.71 and 6.80. On the other hand, common carp fish slices samples treated with carotenoid extracted from crayfish wastes were more stable for the changing in the pH during storage period. The obtained results were similar to those reported in other studies (**Fernandez – Kim, 2004 and Lopez- Caballero *et al.*, 2005)** which attributed the increases in pH values to proteolysis, derived from microbial action, resulting in formation and accumulation of basic compounds such as ammonia.

Peroxide value

The peroxide value of common carp fish slices samples as affected by carotenoid solutions and cold storage was determined, and the obtained results are presented in **Table (3).** It could be easily noted that, peroxide value of all common carp fish slices samples increased as a result of cold storage effect. The values were significantly increased ($p \leq 0.05$) among all treatments during the whole storage period.

Common carp fish slices samples treated with carotenoid extracted from crayfish wastes exhibited the highest peroxide

values with significant ($p \leq 0.05$) differences between them, being 3.92, 2.41, 2.95 and 2.22 m.eq. /kg at concentration of 0.1, 0.2, 0.3 and 0.4 % both of them, respectively, at 8 days of cold storage period, compared to that obtained by control samples 6.73 m.eq./kg which non exceeded the value of standard.

Table (3): Changes in peroxide value of common carp fish slices treated with different levels of carotenoid during storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	With carotenoid 0.1%	With carotenoid 0.2%	With carotenoid 0.3%	With carotenoid 0.4%
0	0.74^{Ea}	0.74^{Fa}	0.71^{Ga}	0.82^{Fab}	0.75^{Fa}
2	1.48^{Dab}	1.16^{De}	0.81^{Fb}	1.15^{De}	0.82^{Fb}
4	3.72^{Ca}	1.56^{Cd}	1.10^{Bc}	1.57^{Cd}	0.99^{Gab}
6	5.03^{Ba}	2.16^{Ed}	2.05^{Dab}	2.00^{Eab}	1.52^{Ce}
8	6.73^{Aa}	3.92^{dcd}	2.41^{Fbc}	2.95^{Dab}	2.22^{Edb}
10	R	4.56^{Bc}	4.12^{Dbc}	4.74^{Dbe}	4.12^{Dbc}
12	R	6.43^{Aa}	6.62^{Ba}	6.84^{Bc}	6.71^{Ac}

Different superscript letters mean significant differences between different treatments and concentrations ($P \leq 0.5$).

It could be noticed that the peroxide values of all common carp fish slices samples treated with the different carotenoid concentration were significantly ($p \leq 0.05$) lower at all storage periods than those of control sample. From the same table, it could be found that the best anti oxidative effect ($p \leq 0.05$) was obtained by both of the all samples treated with the carotenoid at the end of storage period (12 days)**,** (**Rodrigues *et al*., 2016 and Zhu *et al*., 2016)** also reported that, the highly unsaturated fatty acids commonly found in seafood are particularly

sensitive to oxidative change during storage. These results are agreement with those given by **Ibrahim, (2017).**

Anisidine value

The anisidine value was evaluated periodically during cold storage of common carp fish slices samples as an indication of secondary oxidative compounds formation. Data presented in **Table (4)** indicate clearly that, the anisidine value of common carp fish slices samples was affected significantly ($p \leq 0.05$) by carotenoid, also affected by storage period. The anisidine values were 0.08, 0.06, 0.06 and 0.05 for common carp fish slices sample treated with concentrations 0.1, 0.2, 0.3 and 0.4 % extract carotenoid, respectively compared to 0.05 for control sample.

Data from **Table (4)** revealed that, the anisidine values were gradually and markedly stepped up by advancing storage period. They increased to 0.33, 0.26, 0.35 and 0.26 in the above treated common carp fish slices samples, respectively in comparison to 1.46 for control sample at 8days of storage period. The lowest anisidine value at the end of storage period (12 days) were recorded to common carp fish slices samples treated with 0.2% carotenoid, 1.31 followed by 0.4, 0.1, and 0.3% carotenoid concentration which were 1.34, 1.39 and 1.41%, respectively.

Table (4): Changes in anisidine value of common carp fish slices treated with different levels of carotenoid during storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	With carotenoid 0.1%	With carotenoid 0.2%	With carotenoid 0.3%	With carotenoid 0.4%

0	0.05^{Gab}	0.08^{Fab}	0.06^{Gab}	0.06^{Gab}	0.05^{Gab}
2	0.42^{Fab}	0.10^{Gde}	0.15^{Gbc}	0.15^{Gbc}	0.08^{Fab}
4	0.68^{Eab}	0.17^{Fab}	0.19^{Gbd}	0.22^{Efb}	0.15^{Gbc}
6	0.87^{Dae}	0.26^{Ebb}	0.23^{Efb}	0.25^{Efb}	0.20^{Gfc}
8	1.46^{Cab}	0.33^{Dbb}	0.26^{Ebb}	0.35^{Dbc}	0.26^{Ebb}
10	R	0.45^{Fab}	0.36^{Dbc}	0.47^{Fbc}	0.42^{Fab}
12	R	1.39^{Cbb}	1.31^{Cbe}	1.41^{Cbb}	1.34^{Cbe}

Different superscript letters mean significant differences between different treatments and concentrations ($P \leq 0.05$).

The obtained data was in good relationship with those of primary products of lipid oxidation determined as peroxide value. It means that the incorporation of carotenoid extracted from crayfish wastes to common carp fish slices samples inhibited the autoxidation of unsaturated double bonds as well as enzymatically oxidation to transitory intermediate compounds (peroxides and hydroperoxides) and their decomposed products, principally 2- alkenal.

Total oxidation

The total oxidation value was calculated periodically during storage of common carp fish slices samples as an indication of primary and secondary oxidative compounds formation. The data presented in **Table (5)** indicate clearly that, the totox values of common carp fish slices samples were affected significantly ($p \leq 0.05$) by treatments with carotenoid concentrations, also affected by storage period. The levels of totox value of common carp fish slices samples (control) were 1.53 compared to samples treated with 0.1, 0.2, 0.3 and 0.4% carotenoid were 1.56, 1.48, 1.70 and 1.55 at the initial storage period at 5±1°C, against, 8.17, 5.08, 6.25 and 4.70 at the end period (8 days) of cold storage, respectively compared to the control sample 14.92

Table (5): Changes in total oxidation of common carp fish slices treated with different levels of carotenoid during storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	With carotenoid 0.1%	With carotenoid 0.2%	With carotenoid 0.3%	With carotenoid 0.4%
0	1. 53^{aa}	1.56^{aa}	1.48^{ab}	1.70^{ac}	1.55^{aa}
2	3.38^{bd}	2.42^{bb}	1.77^{ac}	2.45^{bb}	1.72^{ac}
4	8.12^{ec}	3.29^{cc}	2.39^{bb}	3.36^{bc}	2.13^{bc}
6	10.93^{fb}	4.58^{db}	4.33^{dd}	4.25^{dc}	3.24^{cb}
8	14.92^{gg}	8.17^{eb}	5.08^{ee}	6.25^{cb}	4.70^{dd}
10	R	9.57^{fe}	8.60^{ed}	9.95^{fe}	8.66^{ed}
12	R	14.25^{gc}	14.55^{gd}	15.09^{ge}	14.76^{gd}

Different superscript letters mean significant differences between different treatments and concentrations ($P \leq 0.05$).

Therefore, there was a continuous increase in the totox values of all common carp fish samples with extending the storage period, under the cold temperature conditions, at different rates depending upon the preservatives used and the period of cold storage. The lowest totox values at the end of storage period were 14.25 and 14.55 for common carp fish slices treated with 0.1 and 0.2% carotenoid concentrations followed by samples treated with 0.4 and 0.3% carotenoid concentrations were 14.76 and 15.09, respectively. These results are agreement with those given by **Ibrahim, (2017).** It is also important to study the behavior of antioxidants in membrane systems. Liposomes have been used to evaluate the behavior of carotenoids in membrane model systems. Comparison of the antioxidant activity of polar carotenoids including astaxanthin and

astaxanthin- b-glucoside from marine bacteria on PC liposome indicated that they are highly active antioxidants **(Matsushita *et al.*, 2000).**

From the obtained results for oxidation measures, cleared that the using carotenoids by different concentrations were more effects on the oxidation reasons inhibition especially lipolysis enzymes and keeping quality of common carp fish slices, These results are accordance with those given by **Sachindra *et al.*, (2005a&b) and Sachindra *et al.*, (2006**) they found that the carotenoids extracted from shrimp processing wastes were evaluated for antioxidant activity. Crude extract and fractions rich in astaxanthin showed strong antioxidant activity as indicated by radical scavenging, reducing activity and metal chelating activity, comparable to that of the known antioxidants a -tocopherol and TBHQ.

Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) is mainly composed of ammonia, primary, secondary and tertiary amines, is widely used as an indicator of protein decomposition and deterioration in fish, meat and its products. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (**Kyrana *et al.*, 1997; Moawed, 1995 and Gibriel *et al.*, 2007).** According to **Connell, (1990)** a level of 35- 40 mg TVB-N/100g of fish flesh is usually regarded as spoilage. Total volatile basic nitrogen (TVB-N) content of common carp fish slices samples was determined at several time intervals during cold storage experiment (for 12 days) and the results were express in term of mg TVB-N/100g sample on wet weight basis.

Results in **Table (6)** showed that, the TVB-N content was increased during storage of different common carp fish slices samples. Results also revealed that, the control fish slices sample had the highest increase in TVB-N content which was 6.74 mg/100g sample at zero time of refrigerated storage, and continuously increased to 31.51 mg/100g after 6 days. While, the corresponding value for the common carp fish slices samples which containing carotenoid 0.1%, 0.2%, 0.3% and 0.4% extracted from crayfish wastes, had the lowest TVB-N content at the beginning of refrigerated storage 6.54, 6.44, 6.48 and 6.71 mg/ 100g, respectively. To the end of refrigerated storage period after 12 days 31.44, 31.28, 31.89 and 31.84 mg/ 100g; respectively.

Table (6): Total volatile basic nitrogen (TVB-N mg/100g) of common carp fish slices treated with different levels of carotenoid during storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	With carotenoid 0.1%	With carotenoid 0.2%	With carotenoid 0.3%	With carotenoid 0.4%
0	6.74±0.43	6.54±0.16	6.44±0.07	6.48±0.15	6.71±0.22
2	13.49±0.44	13.67±0.17	8.03±0.08	10.89±0.07	13.75±0.31
4	22.36±0.43	19.26±0.17	12.29±0.07	18.81±0.08	18.72±0.23
6	31.51±0.45	19.37±0.17	22.09±0.06	20.51±0.09	19.07±0.19
8	R	22.65±0.17	23.47±0.08	24.10±0.18	21.50±0.14
10	R	26.93±0.17	26.85±0.06	27.94±0.08	25.96±0.42
12	R	31.44±0.16	31.28±0.07	31.89±0.19	31.84±0.41

Means of triplicate ± Standard Deviation on wet weight basis.

The increasing in TVB-N during refrigerated storage of common carp fish slices samples might be attributed to the

breakdown of nitrogenous substances by microbial activity. These results are in agreement with those given by **Huidobro *et al*. (2002)** and **Gibriel *et al*. (2007).** On the other hand, the final TVB-N values of treated samples exceed the upper acceptability limit after 10 days, except the control sample directed to the critical limit after 6 days of refrigerated storage. This fact was indicative of either a faster reduction of bacterial population or decrease capacity of bacteria for oxidative domination of non-protein nitrogen compounds (or both) due to the effect of carotenoid solutions in the common carp fish slices samples by **Ibrahim, (2017).**

Total bacterial count (TBC)

Data indicated that the total bacterial count (TBC) of common carp fish slices sample untreated (control), common carp fish slices treated with 0.1, 0.2, 0.3 and 0.4% carotenoid concentrations, at the initial storage time, versus 4.73, 4.88, 4.80, 4.86 and 5.03 log $_{10}$cfu/g, respectively. Thus, the TBC of common carp fish slices, as the same previous order expect control sample, was increased notably by the rate of 5.40, 5.34, 5.51 and 5.30 log $_{10}$cfu/g, at the end of refrigerated storage, as enumerated on wet weight bases.

It could be noted that all treatments at zero time related to the good hygiene conditions followed during common carp fish slices preparation. The International Commission on Microbiological Specifications for food **(ICMSF, 1986)** recommended that the flesh aerobic plate count should not exceed 10^6cfu/g on wet weight.

Table (7): Total bacterial count (TBC Log_{10}CFU/g) of common carp fish slices treated with different levels of carotenoid during storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	With carotenoid 0.1%	With carotenoid 0.2%	With carotenoid 0.3%	With carotenoid 0.4%
0	4.73±0.23	4.88±0.13	4.80±0.22	4.86±0.14	5.03±0.14
2	5.06±0.16	4.92±0.27	5.00±0.17	4.92±0.14	5.09±0.11
4	5.25±0.22	4.94±0.22	5.03±0.13	5.22±0.21	5.20±0.09
6	5.43±0.18	5.01±0.25	5.12±0.15	5.30±0.16	5.22±0.08
8	5.50±0.24	5.20±0.19	5.23±0.29	5.42±0.22	5.24±0.11
10	R	5.32±0.15	5.30±0.28	5.46±0.25	5.26±0.16
12	R	5.40±0.22	5.34±0.16	5.51±0.22	5.30±0.15

Means of triplicate ± Standard Deviation on wet weight basis.

The highest value of TBC at the end of storage period showed in common carp fish slices treated with 0.3% extracted carotenoid was 5.51 log $_{10}$cfu/g. On the other hand, the lowest value of TBC showed in common carp fish slices treated with 0.4% extracted carotenoid was 5.30 log $_{10}$cfu/g. The increase of total bacterial count may be due to the effect of the semi effective of refrigerated temperature on the vegetative cells. TBC and its elevation rate throughout storage, may controlling in preparation steps, and the storage period, moreover the slightly increase in TBC related to the high concentration was used in this study especially at the period from 8 to 12 days.

It is worth to mention that the TBC of all tested common carp fish slices were within the permissible count as recommended by **Egyptian Standard Specifications, (1988),** even after 12days of refrigerated storage. These results are in agreement with those given by **Lopez-Caballero *et al.* (2005)**

The odor

The means score of common carp fish slices odor shown in **Table (8)**. The data illustrated that scores were very good for both common carp fish slices untreated (control), common carp fish slices sample treated with 0.1, 0.2, 0.3 and 0.4% carotenoid concentrations at the initial storage period, which were 6.50, 8.33, 8.08, 8.00 and 8.00, respectively, and continued their good scores of odor for common carp fish slices samples treated with 0.1, 0.2, 0.3 and 0.4% still the fourth days of refrigerate storage period which were 6.60, 6.40, 6.50 and 6.81, respectively. While, at the same period recorded accepted scores of odor quality for common carp fish slices without preservatives (control). With regards the odor criterion, as shown in the former results, it could be observed that the judging scores for odor of all treated common carp fish slices samples were semi constant from the six days till the end of ten days of refrigerated storage, after that it tended to highly decrease up to the ten days of storage, the scores of odor for treated common carp fish slices samples were rejected at the end of storage period. Such alteration in properties of odor criterion in common carp fish slices during storage may be attributed to the dehydration of slices. Thereafter, the received scores highly decreased at the end of storage period because the odor appeared fattier. These results are supported by **Knorr (1982); Gibriel *et al*. (2007) and Ibrahim, (2017).**

Table (8): Mean values of odor for common carp fish slices during refrigerated storage at 5±1°C for 12 days.

Storage period (days)	Common carp fish slices				
	Control	With carotenoid 0.1%	With carotenoid 0.2%	With carotenoid 0.3%	With carotenoid 0.4%

0	6.50±0.75	8.33±0.80	8.08±1.17	8.00±1.18	8.00±1.13
2	6.40±0.34	7.25±0.88	7.25±0.65	7.75±1.31	7.75±1.02
4	5.71±0.85	6.60±1.05	6.40±0.96	7.50±1.13	6.81±1.09
6	5.29±0.88	5.85±1.06	5.85±1.05	6.50±0.83	5.71±1.02
8	R	5.75±1.16	5.75±1.18	5.42±0.95	5.57±0.97
10	R	5.28±0.82	5.18±0.99	5.00±1.12	5.00±0.88
12	R	R	R	R	R

Means of triplicate ± Standard Deviation.

The overall acceptability

Overall acceptability scores for common carp fish slices samples at zero time and during refrigerated storage periods are presented in **Table (9).** At zero time, there were the highest values of overall acceptability recorded in common carp fish slices samples with the different treated by carotenoid concentrations 0.3 followed by 0.2 and 0.3% which have 8.75, 8.33 and 8.33, respectively, while the lowest scores of overall acceptability were 7.00 and 8.17 for common carp fish slices samples untreated (control) and treated with 0.1% carotenoid concentration at the same time, respectively. Scores of overall acceptability were decreased progressively in all investigated common carp fish slices during storage as affected by refrigerated storage temperature.

Table (9): Mean values of overall acceptability for common carp fish slices during refrigerated storage at 5±1°C for 12 days.

Storage period (days)	**Common carp fish slices**				
	Control	**With carotenoid 0.1%**	**With carotenoid 0.2%**	**With carotenoid 0.3%**	**With carotenoid 0.4%**
0	7.00±0.62	8.17±1.03	8.33±1.07	8.75±0.93	8.33±0.99
2	7.00±0.80	7.58±0.83	7.00±0.97	7.75±0.78	7.75±0.98
4	6.00±1.05	7.00±1.12	7.00±0.71	7.20±1.03	7.25±1.08

6	5.75±0.97	7.00±0.94	6.75±1.18	7.00±1.05	6.86±1.11
8	R	6.00±1.32	6.25±1.16	5.87±1.20	6.00±0.89
10	R	5.57±1.14	5.71±1.14	5.55±1.19	5.57±1.14
12	R	R	R	R	R

Means of triplicate ± Standard Deviation.

Common carp fish slices treated with carotenoid concentrations showed highest loss in overall acceptability at the end of storage period which values were unaccepted. In conclusion, common carp fish slices samples treated with concentrations (0.3 and 0.4%) carotenoid concentrations were more preferred to the other common carp fish slices samples exactly on the fourth day were values 7.20 and 7.25, respectively. From the previous data the changes in sensorial properties and it's decreased of common carp fish slices may be due to the protein hydrolysis and their producing the many products such total volatile basic nitrogen and trimethylamine nitrogen amine nitrogen.

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الملخص :

هدفت هذه الدراسة الى تقييم الجودة الفيزيائية والكيميائية والميكروبيولوجية والحسية لشرائح أسماك المبروك العادى المنقوعة فى الكاروتينويدات المستخلصة من مخلفات الإستاكوزا المياه العذبة خلال التخزين عند درجة حرارة ٥±١ درجة مئوية لمدة ١٢ يوم ، أشارت النتائج المتحصل عليها إلى أن المعايير الفيزيوكيميائية والميكروبيولوجية للرقم الهيدروجينى والبيروكسيد والأنيسيدين والأكسده الكلية وقيم نيتروجين البروتين المتطايرة الكلي والعد البكتيرى الكلى لجميع العينات ذادت مع ذيادة مدة التخزين. فى المقابل ،إنخفضت درجات الخواص الحسية للرائحة والقبول العام خلال فترة التخزين نتيجة لتطور التلف. أظهرت عينات التحكم (الكنترول) أعلى معدل تدهور أثناء التخزين البارد. فى هذه الأثناء ، كانت الكاروتينويدات تؤخر التلف نتيجة لتأثيراتها المضادة للأكسدة ، للحد من أكسدة الدهون وتدهور البروتين عن طريق خفض النمو الميكروبى فى شرائح الأسماك المعالجة. ولذلك فإن الجودة الحسية لمعاملات شرائح السمك المنقوعة تأثرت إجابياً بالنقع فى تركيز الكاروتينويدات ، لتقديم أفضل الدرجات بمقارنة بعينة التحكم (الكنترول). تم ذيادة تركيز الكاروتينويدات وخاصةً ٠.٢ و ٠.٣ % فى جودة شرائح أسماك المبروك العادى المنقوعة لمدة ١٠ أيام ، أعلى من ٦أيام فى عينات التحكم (الكنترول).