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Storage Stability and Biochemical Changes of the Common Carp Slices Treated With Carotenoid Extracted from Crayfish Waste

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ABSTRACT

The physicochemical, microbiological, and sensory qualities of common carp fish slices treated with carotenoids extracted from crayfish waste and stored at 5 ± 1 °C for 12 days were evaluated in this study. The results showed that quality indicators—such as pH, peroxide value, anisidine value, total oxidation (TOTOX), total volatile basic nitrogen (TVB-N), and bacterial count—increased over time, while sensory scores declined. The control samples showed the lowest quality, whereas the carotenoid-treated samples maintained higher quality due to the antioxidant effects of the carotenoids, which helped reduce lipid oxidation, protein degradation, and microbial growth. In conclusion, fish slices treated with 0.2% and 0.3% carotenoids maintained acceptable quality for up to 10 days, compared to only 6 days for the control samples. This study suggests that crayfish waste can be effectively utilized to extract bioactive compounds that enhance the physicochemical and sensory properties of fish slices during refrigerated storage.

INTRODUCTION

Fish are nutritious, affordable source of high-quality protein, making it an essential part of the human diet **(Olalekan, 2019)**. It is low in saturated fats and rich in omega-3 fatty acids, especially DHA and EPA, which offer anti-inflammatory benefits and help protect against cardiovascular diseases (**Siscovick *et al.*, 2017; Vilavert *et al.*, 2017)**. However, fish is a highly perishable product due to its high moisture content, rich nutrients, neutral pH, and autolytic enzymes, making it prone to microbial spoilage. Even under refrigeration, enzymatic and chemical reactions cause rancidity, texture changes, and discoloration, leading to quality loss during post-harvest handling, processing, and storage **(Tesfay & Teferi, 2017; Speranza *et al.*, 2021)**.

Synthetic preservatives have long been used in the food industry for their antibacterial effects and spoilage prevention. However, health concerns such as allergies,

toxicity, and links to serious illnesses have led to increased consumer demand for natural alternatives **(Alsaiqali *et al.*, 2016; Hassoun & Karoui, 2017; Raeisi, *et al.*, 2019)**. In response to these concerns, researchers have increasingly focused on finding safe, effective, and natural preservatives to controlling in the microbial and oxidative spoilage in fish products **(Velasco, & Williams, 2011; Viji, *et al.*, 2017)**. Crayfish (*Procambarus clarkii*) by-products are rich in proteins, chitin, and carotenoids with strong antioxidant and antimicrobial properties. These compounds can delay lipid oxidation, inhibit microbial growth, and improve the shelf life of fish products **(Guillou *et al.*, 1995)**.

Carotenoids extracted from shrimp processing waste showed strong antioxidant activity. Both the crude extract and the astaxanthin-rich fractions demonstrated radical scavenging, reducing, and metal-chelating activities, comparable to those of α-tocopherol and TBHQ. They also exhibited higher singlet oxygen and nitric oxide scavenging activities than α-tocopherol **(Sachindra *et al.*, 2005a&b; Sachindra *et al.*, 2006; Sachindra & Bhaskar, 2008)**. This study aimed to evaluate the physicochemical, microbiological, and sensory quality of common carp slices treated with carotenoids extracted from crayfish waste and stored at 5 ± 1°C for 12 days.

MATERIALS AND METHODS

Materials

All waste samples of the red swamp crayfish (*Procambarus clarkii*) were obtained from the Toshka Masr Company in Cairo, Egypt. About 7kg of these wastes were transported using ice boxes to Fish Technology and Processing Laboratory, El-Kanater El-Khiria, National Institute of Oceanography and Fisheries (NIOF). Approximately 15kg common carp (*Cyprinus carpio*) were purchased from Elserw fish farm belonging NIOF and transported in icebox to the laboratory. The average weight and length of fish samples recorded 1.500-2.600kg and 43.25-53.3cm, respectively. Refined corn oil and white polyethylene pages were obtained from the local market. Nutrient agar medium was purchased from Sigma Company for chemicals.

Technological methods

Preparation of crayfish by products and extraction

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

Preparation of carotenoid concentrations

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

Preparation of fish slices samples

Fish were prepared for processing under hygienic conditions. All fish were rewashed, beheaded, eviscerated, skinned, and filleted. The fish slices were then washed and drained. Irregular slices (averaging 6.5 cm in length and 1.5 cm in thickness) were divided into five equal batches. The first batch, with no added carotenoid, was directly molded into slices and used as the control. Batches two, three, four, and five were immersed in carotenoid solutions at concentrations of 0.1%, 0.2%, 0.3%, and 0.4% (v/v), respectively, for 50 minutes, then air-dried for 5 minutes. All samples were then packed in polyethylene bags and stored at 5 ± 1 °C for 12 days.

Analytical methods

Moisture, protein, lipid, and ash contents of raw common carp fish slices were analyzed. Acid, peroxide, and anisidine values were determined **(AOAC, 2007)**, while carbohydrate content was calculated by difference. pH was measured according to the method of **Aitken *et al.* (1962)**. The total oxidation value was calculated using the method of **Rossell (1983)**, combining peroxide and anisidine values. TVBN and TMAN were measured using the semi-micro distillation method **(AMC, 1979)**. Total bacterial count (TBC) was assessed **(Oxoid, 2006)** using nutrient agar. Sensory evaluation (only odor and overall acceptability) was conducted by 10 panelists from the Fish Technology and Processing Laboratory, using a 9-point hedonic scale, modified from **Teeny and Miyaauchi (1972)**. All results were statistically analyzed and expressed as mean ± standard deviation (SD) (P<0.05) using Minitab software (version 14). Fish samples were taken randomly at intervals for analyzing every two days.

RESULTS AND DISCUSSION

The proximate composition (w/w) of fresh common fish meat was analyzed, as shown in Table (1). The values of moisture, protein, fat, ash, and carbohydrate contents of carp flesh were 72.80, 13.54, 12.25, 1.07, and 0.34%, respectively. Generally, the proximate composition of fresh fish in this study aligns with the findings of **Sudirman *et al.* (2018)** and **Abdulrahman *et al.* (2019)**. Variations in proximate composition is attributed to several factors, particularly differences in habitat, size, sex, and sexual condition of the fish.

The physicochemical quality indicators of fresh common carp fish fillets were evaluated and are presented in Table (1). The pH, acid value, peroxide value, anisidine value, total oxidation, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), and free amino nitrogen of the fish fillets were 6.44, 0.30 mg KOH/g, 0.76 m.eq/kg, 0.05, 1.57, 6.39 mg/100g, 1.26 mg/100g, and 0.84%, respectively. Similar values were reported by **Sönmez *et al.* (2020)**, who found the pH of common carp meat to be 6.49. These results are also in agreement with those observed by **Abbas *et al.* (2021)**. The quality indicators, including TVB-N and TMA-N values, indicated that raw carp fish in this study had high freshness. The TVB-N and TMA-N values of fresh fish were similar to those reported by **Mahmoud (2016)**, **Morshdy *et al.* (2018)** and **Kuzgun (2019)**. The total bacterial count of fresh common carp fish flesh was 2.98 log $_{10}$ cfu/g, which falls within the acceptable limits for fresh fish as set by **ICMSF (1986)**.

Table 1. Chemical composition, physicochemical and microbiological counts of common carp fish flesh

Parameter	**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

* Carbohydrate calculate by difference.

pH value

The effect of different concentrations of carotenoid incorporated into common carp fish slices on changes in pH values during refrigerated storage is presented in Fig. (1). It is evident that the carotenoid concentrations reduced the pH values of the fish slices at the initial storage time. The 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, and compared to 6.14 for the control samples. This decrease in pH can be

attributed to the basic nature of carotenoids and their concentration-dependent effect. Over the storage period, the pH level gradually increased for all treatments, as shown in Fig. (1). Data showed that, the highest pH values were obtained for control (without carotenoids) and treated with 0.4% carotenoid at every storage period recorded 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, fish slices treated with carotenoids showed greater stability in pH changes during the storage period. The obtained results were consistent with those reported in other studies **(Ibrahim, 2017)**, which attributed the increase in pH values to proteolysis caused by microbial activity, leading to the formation and accumulation of basic compounds such as ammonia.

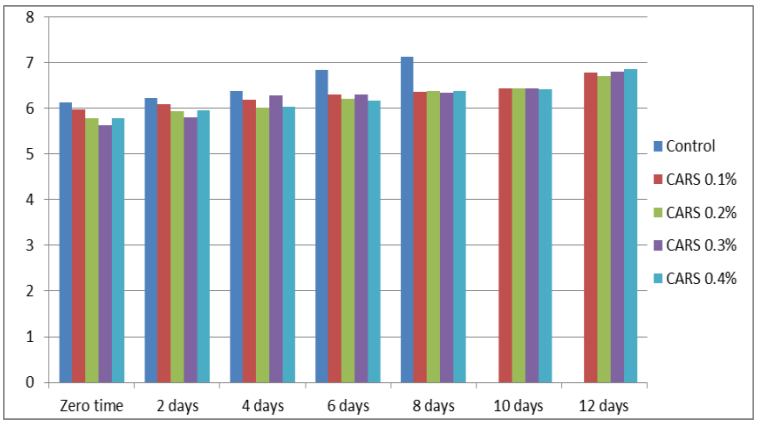

Fig. 1. Change in PH value of common carp fish fillets treated with different levels of carotenoid during storage at 5±1° C for 12 days

Peroxide value

The peroxide value of common carp fish slices samples treated with carotenoids is presented in Fig. (2). It is evident that the peroxide value of all the common carp fish slices increased as affected by cold storage conditions. These values showed a significant increase ($P \leq 0.05$) across all treatments throughout the storage period. Fish slices treated with carotenoids exhibited the highest peroxide values, with significant ($P \leq 0.05$) differences between treatments. The peroxide values were 3.92, 2.41, 2.95, and 2.22 m.eq./kg for the 0.1, 0.2, 0.3, and 0.4% carotenoid concentrations, respectively, after 8 days of cold storage. In comparison, the control sample had a peroxide value of 6.73 m.eq./kg, which did not exceed the standard limit (10 m.eq./kg).

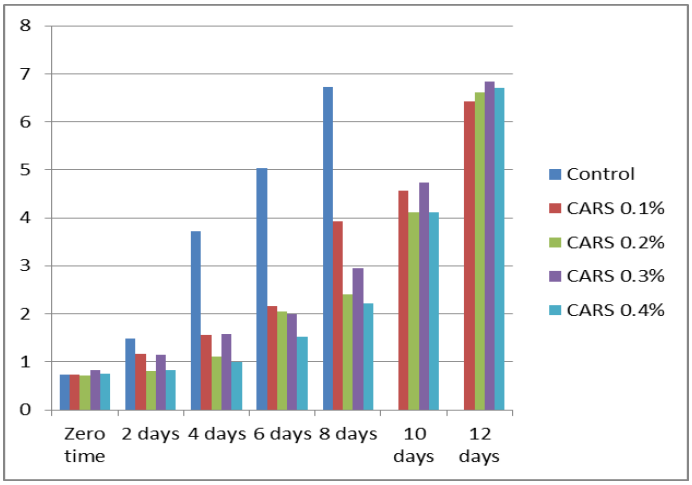

Fig. 2. Changes in peroxide value of common carp fish fillets treated with different levels of carotenoid during storage at 5±1° C for 12 days

It could be noticed that the peroxide values of all 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. Fig. (2) shows 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). **Zhu *et al.* (2016)** reported that, the highly unsaturated fatty acids commonly found in seafood are particularly sensitive to oxidative change during storage. These results are in 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 Fig. (3) indicate clearly that, the anisidine value of 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 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. Also, the data 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 compared 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 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.

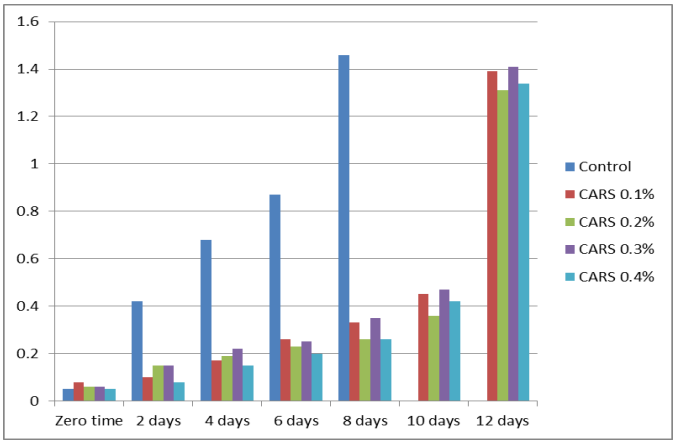

Fig. 3. Changes in anisidine value of common carp fish fillets treated with different levels of carotenoid during storage at 5±1° C for 12 days

The obtained data showed a strong correlation with the primary products of lipid oxidation, as determined by the peroxide value. This suggests that the incorporation of carotenoids extracted from crayfish waste into fish slices effectively inhibited the autoxidation of unsaturated double bonds, as well as enzymatic oxidation leading to the formation of transient intermediate compounds (such as peroxides and hydroperoxides) and their decomposed products, primarily 2-alkenals.

Total oxidation

The total oxidation value (totox) was periodically calculated during the storage of fish slices as an indicator of the formation of primary and secondary oxidative compounds. The data presented in Fig. (4) clearly show that the total oxidation values of the common carp fish slices were significantly affected ($P \leq 0.05$) by both the carotenoid treatments and the storage period. At the initial storage period (5 ± 1°C), the total oxidation value of the control sample was 1.53, while the values for samples treated with 0.1%, 0.2%, 0.3%, and 0.4% carotenoid were 1.56, 1.48, 1.70, and 1.55, respectively. After 8 days of cold storage, the totox values increased to 8.17, 5.08, 6.25, and 4.70 for the treated samples, compared to control sample (14.92). These results show a continuous increase in the totox values of all common carp fish samples as the storage period was extended under cold temperature conditions, with varying rates depending on the carotenoid concentrations used. The lowest totox values at the end of storage period were observed in samples treated with 0.1% and 0.2% carotenoid concentrations, which had values of 14.25 and 14.55, respectively. Samples treated with 0.4% and 0.3% carotenoid concentrations had slightly higher totox values of 14.76 and 15.09, respectively.

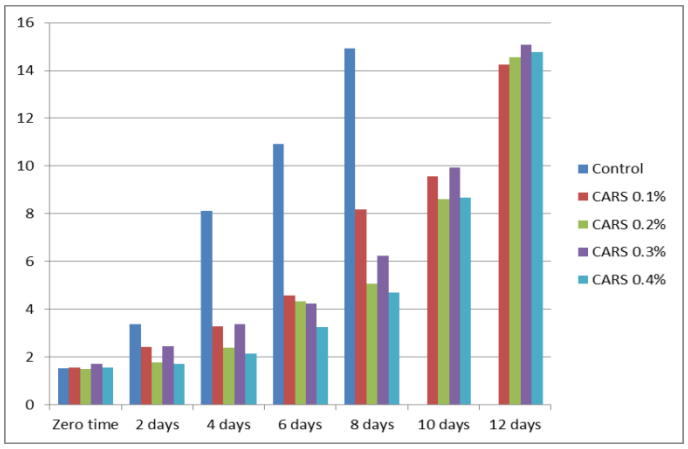

Fig. 4. Changes in total oxidation of common carp fish fillets treated with different levels of carotenoid during storage at 5±1° C for 12 days

These results are in agreement with those reported by **Ibrahim (2017)**. It is also important to study the behavior of antioxidants in membrane systems, and liposomes have been used to evaluate the behavior of carotenoids in such models. A comparison of the antioxidant activity of polar carotenoids, including astaxanthin and astaxanthin-β-glucoside, extracted from marine bacteria on PC liposomes, demonstrated that these carotenoids are highly effective antioxidants **(Matsushita *et al.*, 2000)**. The results of oxidation measurements indicated that the use of carotenoids at different concentrations had a significant effect on inhibiting oxidation, particularly by reducing the activity of lipolytic enzymes and improving the keeping quality of common carp fish slices. These findings are consistent with the studies by **Sachindra *et al.* (2005a, b)** and **Sachindra *et al.* (2006)**, which showed that carotenoids extracted from shrimp processing wastes exhibited strong antioxidant activity, as demonstrated by radical scavenging, reducing activity, and metal chelation. The antioxidant activity of these carotenoids was comparable to that of well-known antioxidants such as α-tocopherol and TBHQ.

Total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N), which mainly consists of ammonia as well as primary, secondary, and tertiary amines, is widely used as an indicator of protein decomposition and spoilage in fish, meat, and related products. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (**Gibriel *et al.*, 2007; Moawad *et al.*, 2017)**. 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 expressed in terms of mg TVB-N/100g sample on wet weight basis. Results in Fig. (5) showed that, the TVB-N content increased during storage of different common carp fish slices samples. Results also

revealed that the control fish slice samples showed the highest increase in TVB-N content, starting at 6.74 mg/100 g at the beginning of refrigerated storage and rising continuously to 31.51 mg/100 g after 6 days. In contrast, the common carp fish slice samples treated with carotenoid concentrations of 0.1%, 0.2%, 0.3%, and 0.4% (extracted from crayfish waste) had lower initial TVB-N values of 6.54, 6.44, 6.48, and 6.71 mg/100 g, respectively. At the end of the 12-day refrigerated storage period, the TVB-N values for these treated samples were 31.44, 31.28, 31.89, and 31.84 mg/100 g, respectively.

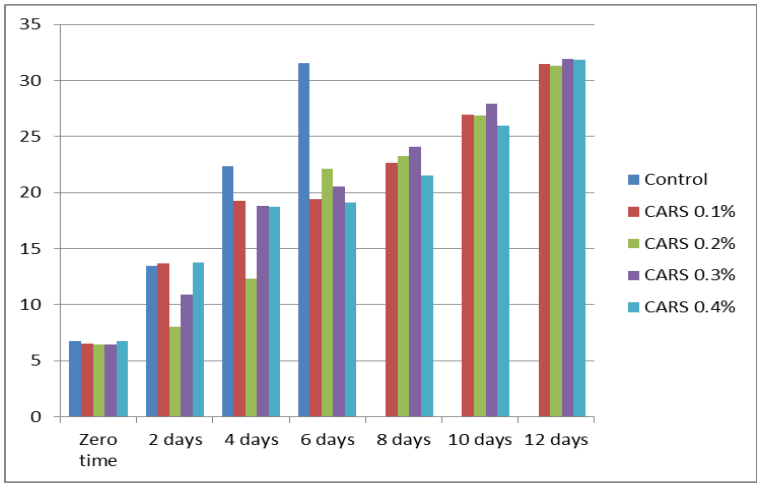

Fig. 5. Total volatile basic nitrogen (TVB-N) of common carp fish fillets treated with different levels of carotenoid during storage at 5±1° C for 12 day

The increase in total volatile base nitrogen (TVB-N) during the refrigerated storage of common carp fish slices can be attributed to the breakdown of nitrogenous compounds by microbial activity. These findings are consistent with the results reported by **Huidobro *et al.* (2002)** and **Gibriel *et al.* (2007)**. On the other hand, the final TVB-N values of the treated samples exceeded the upper acceptable limit after 10 days of storage, while the control sample reached the critical limit after 6 days of refrigerated storage. This suggests either a more rapid reduction in bacterial population or a diminished capacity of bacteria to oxidize non-protein nitrogen compounds (or both), likely due to the effect of the carotenoid treatments on the common carp fish slices, as indicated by **Ibrahim (2017)**.

Total bacterial count (TBC)

The data presented in Fig. (6) show the total bacterial count (TBC) of untreated common carp fish slices (control) and slices treated with 0.1%, 0.2%, 0.3%, and 0.4% carotenoid concentrations at the initial storage time. The initial TBC values were 4.73, 4.88, 4.80, 4.86, and 5.03 log 10 cfu/g, respectively. Over the refrigerated storage period, the TBC of the treated common carp fish slices (except for the control) increased significantly, with increases of 5.40, 5.34, 5.51, and 5.30 log 10 cfu/g at the end of

storage, based on wet weight. It is noteworthy that the TBC values at the start of storage were relatively low due to the good hygiene practices followed during the preparation of the fish slices. According to the International Commission on Microbiological Specifications for Food **(ICMSF, 1986)**, the aerobic plate count of flesh should not exceed 10^6 cfu/g on a wet weight basis. The highest TBC value at the end of the storage period was observed in the common carp fish slices treated with 0.3% carotenoid, which reached 5.51 log 10 cfu/g. Conversely, the lowest TBC value was found in the slices treated with 0.4% carotenoid, at 5.30 log 10 cfu/g. The increase in TBC may be attributed to the limited effectiveness of the refrigerated temperature in inhibiting the growth of vegetative bacterial cells.

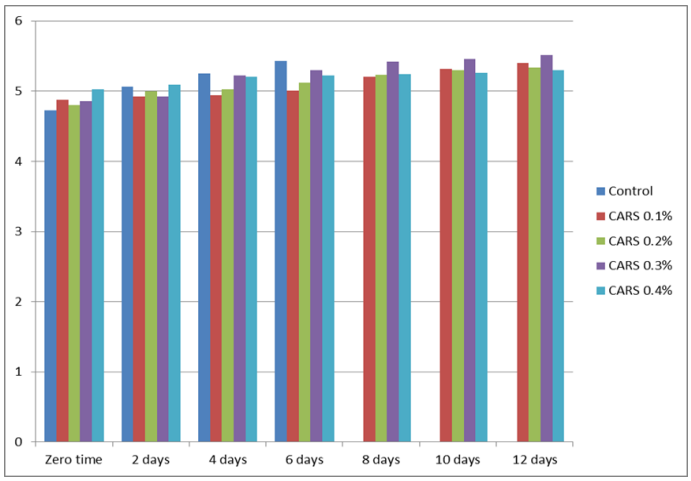

Fig. 6. Total bacterial count (TBC Log_{10}CFU/g) of common carp fish fillets treated with different levels of carotenoid during storage at 5±1°C for 12 days

The total bacterial count (TBC) and its rate of increase throughout the storage period were influenced by the preparation steps and storage conditions. Moreover, the slight increase in TBC observed, particularly between days 8 and 12, was linked to the high concentration used in this study. It is important to note that the TBC of all the tested common carp fish slices remained within the permissible limits as recommended by the **Egyptian Standard Specifications (1988)**, even after 12 days of refrigerated storage.

The odor

The mean odor scores of common carp fish slices are shown in Fig. 7. The data indicate that, at the beginning of the storage period, odor scores were very good for both the untreated (control) and carotenoid-treated samples at concentrations of 0.1%, 0.2%, 0.3%, and 0.4%, with scores of 6.50, 8.33, 8.08, 8.00, and 8.00, respectively. The treated samples maintained good odor scores up to the fourth day of refrigerated storage, with values of 6.60, 6.40, 6.50, and 6.81 for the 0.1%, 0.2%, 0.3%, and 0.4% treatments, respectively. In comparison, the control sample showed only acceptable odor quality during the same period.

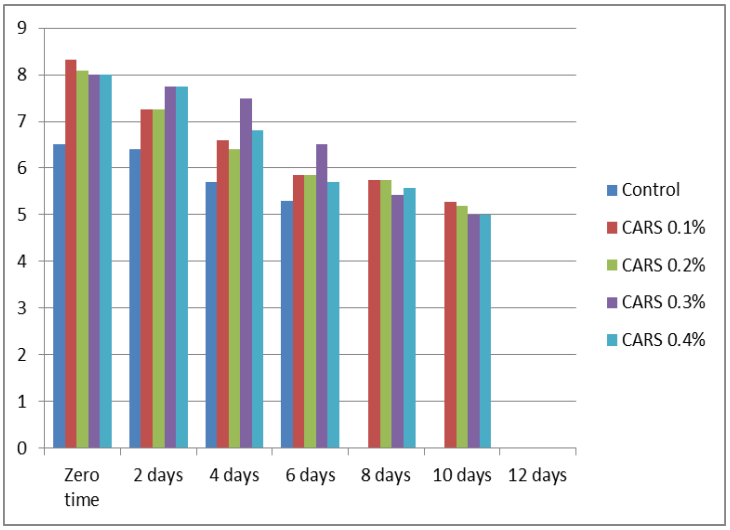

Fig. 7. Mean values of odor for common carp fish fillets treated with different levels of carotenoid during storage at 5±1°C for 12 days

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 **Gibriel *et al.* (2007)** and **Ibrahim (2017)**.

The overall acceptability

Overall acceptability scores for common carp fish slice samples at zero time and during refrigerated storage are presented in Fig. 8. At the beginning of storage, the highest overall acceptability scores were recorded in samples treated with 0.3% carotenoids (8.75), followed by 0.2% and 0.4% treatments (both 8.33). The lowest scores were observed in the control (7.00) and the sample treated with 0.1% carotenoids (8.17).

Overall acceptability scores decreased progressively in all samples during storage, influenced by refrigerated temperature and duration. By the end of the storage period, samples showed significant declines in acceptability, with some falling below acceptable levels.

However, fish slices treated with 0.3% and 0.4% carotenoid concentrations remained the most preferred, particularly on the fourth day of storage, with acceptability scores of 7.20 and 7.25, respectively.

The decline in sensory properties of the common carp fish slices over time may be attributed to protein hydrolysis and the subsequent formation of spoilage compounds such as total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N).

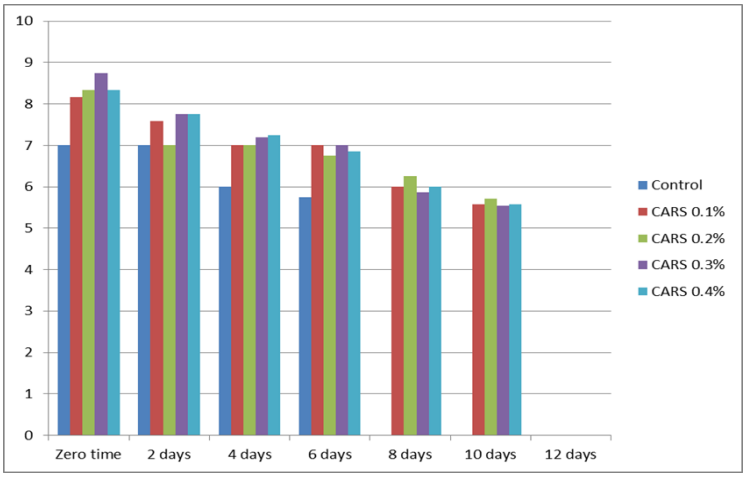

Fig. 8. Mean values of overall acceptability for common carp fish fillets treated with different levels of carotenoid during storage at 5±1°C for 12 days

CONCLUSION

This study highlights the potential of utilizing freshwater crayfish waste to extract valuable compounds such as carotenoids, which can serve as natural antioxidants and antimicrobial agents—offering a sustainable alternative to synthetic preservatives. Common carp fish slices treated with carotenoid concentrations, particularly at 0.2% and 0.3%, showed improved physicochemical and sensory properties during refrigerated storage.

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