Effect of Transglutaminase Enzyme and some Natural Antioxidants on the Quality of Ready to Eat Catfish Fingers during Frozen Storage

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Abstract: To increase the utilization of catfish, ready to eat fish fingers were prepared and evaluated during frozen storage at -18˚C for 5 months. The effect of transglutaminase enzyme, date seed powder, citric acid and ascorbic acid, added individually or in combination, on physical, chemical, microbiological and sensory properties were investigated. The obtained results indicated that pH, TVB-N, TMA, FFA, PV and TBA were decreased significantly (P≤0.05) in the treated fish finger samples compared to the control. Also, the applied natural antioxidants retarded the microbial growth (aerobic plate count, *pseudomonas* and *Enterobacteriaceae* counts) during storage period as the treated samples gave better results compared to the control sample. Also, sensory evaluations (color, odor, taste, texture and overall acceptability) indicated that such natural treatments improved sensory scores of the prepared fingers during frozen storage in comparison with the control sample.

Keywords**:** Ready to eat fish fingers, transglutaminase enzyme**,** date seed powder, citric acid, ascorbic acid

INTRODUCTION

The consumption and popularity of fish and seafood have increased during recent years (Bochi *et al.,* 2008) due to the increase in consumption rate by the increasing world population and awareness on nutritional value of fishery products (Emborg *et al.,* 2001). Fish and fishery products contain proteins of high quality, and high content of unsaturated fatty acids, especially the n-3 PUFAs (Venugopal and Shahidi, 1995). An adequate consumption of n-3 PUFAs can help to protect humans against many adverse health effects, including mortality due to coronary heart disease (CHD), and cancer (Colombo *et al*., 2004; Iso *et al.,* 2006). These products are important in the prevention of cardiovascular and inflammatory diseases and may have a promising effect on the prevention of cognitive decline and dementia in older people (Simopoulos, 1999; Sinn *et al.,* 2012).

Catfish *(Clarias gariepinus*) is a highly nutritious that contain high amounts of unsaturated fatty acids, vitamins, proteins, and minerals (Nelson, 2006). Catfish is referred to as a fatty fish when compared to other fish species and it is also classified as dark muscle fish with strong muddy odor, hence all these characteristics have slightly hindered its wide utilization (Tadpitchayangkoon and Yongsawatdigul, 2009). Therefore, it is important to increase the palatability and economic value for such fish species. Recently, changes in life style and nutritional awareness resulted in increasing consumption of ready-to-eat foods (Cakli *et al*., 2005). Fish fingers, as battered and breaded products, are stored and distributed in the frozen state.

Oxidation of lipids occurred during raw material storage, processing, heating and distribution. Storage is one of the basic processes that cause rancidity in food products. Consequently, it may affect flavour, texture, taste, and shelf-life of fish products and their nutritional quality (Banerjee, 2006; Babovic *et al.,* 2010; Karakaya *et al.,* 2011). Antioxidant addition is one of the effective ways to prolong shelf-life and preserve the quality of food (Serdaroğlu and Felekoglu, 2005). Thus, the demand for novel natural antioxidants has rocketed due to health effects and to avoid possible adverse side effects of synthetic antioxidants as reported by Benjakul *et al.* (2005) and Sarkardei and Howel (2008).

Transglutaminase enzyme (MTGase) is able to catalyze the cross linking of many proteins such as whey proteins, soy proteins, wheat proteins, beef myosin, casein and actomyosin, leading to affect their texture (Motoki and Seguro, 1998). It also affects changes in solubility, emulsifying capacity, foaming and gelation properties of proteins. Moreover it enhances the firmness, elasticity, viscosity, and water binding capacity of many foods (Giosafatto *et al*., 2012).

Date seeds or pits, a waste product, are generally resulted from date factories producing pitted dates, syrup, juice, and jams (Rahman *et al*., 2007). Date seeds are a good source of total phenolics which is considered as a cheap source of natural antioxidants. Therefore, date seed powder can be used to produce functional foods (Al-Farsi *et al.,* 2007; Ammar and Habiba, 2010).

Ascorbic acid (AA), citric acid (CA) and their salts are used in the food industry as chelators and acidulants (Kim *et al*., 2006). They revealed a partial inhibition of primary and secondary lipid oxidation during storage at freezing temperature of catfish fillets (Pourashouri *et al*., 2009), and a combination of both acids resulted in a much better lipid oxidation retardation in frozen horse mackerel than the individual acids (Aubourg *et al*., 2004).

The objective of the present study was to determine the effects of transglutaminase, date seed powder, citric and ascorbic acids on chemical, microbiological and sensory quality of ready to eat fish fingers using catfish fillet.

MATERIALS AND METHODS

Catfish (*Clarias gariepinus*), weighting between 1 and 2 kg each, were obtained directly from the fish market (Ismailia, Egypt) in November, 2013.

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Date seed powder was prepared from a common local date fruits (Samany variety). Wheat and corn flours, sugar, salt, cumin, onion, garlic powder, pepper and thyme were purchased from local supermarket, while transglutaminase enzyme was purchased from Ajinomoto (Tokyo, Japan). Citric and ascorbic acids and other chemicals were of food grades.

Date seed powder preparation

Date fruits (Samany variety) were purchased from local market and then pitted and pits were dried in an oven at 70ºC overnight, milled by a grinder (Moulinex, LM2421, France) to pass through 1 mm sieve, packed and stored at 4ºC until used in the experiments as an additive (Ammar and Habiba, 2010).

Preparation of fish fingers

About 40 kg of catfish was beheaded, gutted, washed and filleted. The fillets were minced using a meat mincer through a plate with 3 mm diameter holes. The control mince included 93.5% catfish mince, 1.5% salt, 1% sugar, 3% wheat flour, 0.243% each of cumin, onion, garlic powder, pepper and 0.020% thyme according to Tokur *et al*. (2006). All ingredients were mixed and homogenized by a kitchen blender. The mix was divided into six equal parts to prepare the experimental treatments. Each part was transferred to a commercial mixer, where they were mixed with tested additives except for one part, which served as the control (T1). Transglutaminase enzyme was added at a concentration of 0.5%, (T2). Date seed powder was used at a level of 1% (T3). A combination of citric acid and ascorbic acid 0.5% each, (T4). Also, a combination of the enzyme (0.5%) and date seed powder (1%), (T5); and a combination of the enzyme (0.5%) and the mixture of citric and ascorbic acids (0.5% + 0.5%) were used, (T6). Then, all fish finger treatments were manually shaped. Only treatment samples containing transglutaminase enzyme were incubated at 40 ºC for 30 min after forming. Fish fingers were well battered using a mixture of wheat flour, corn flour and cold water at 30, 10 and 60%, respectively. Then, it was covered with traditional bread crumb and finally the prepared fish finger samples were flash fried for half a minute at 180 ºC in a fryer containing sunflower oil according to Cakli *et al*. (2005) and Tokur *et al*. (2006). Then, the samples were drained and allowed to be cooled. The fried samples were packaged in a foam plate, wrapped with cling film, and stored in a freezer at - 18 ºC for five months.

Proximate composition

Moisture content of samples was determined using oven at 105°C until constant weight, while, ash was measured at 550°C (AOAC, 2000). Micro-kjeldahl method was used to determine sample crude protein, and a factor of 6.25 was applied (AOAC, 2000). For the determination of crude fat, the method of Bligh and Dyer (1959) was used, by subjecting the sample to extraction with a mixture of chloroform and methanol (1:2 v:v). Total carbohydrates were determined by subtracting the sum of % moisture (M), fat (F), % crude protein (CP) and % ash content (A) from 100. %Total carbohydrates = 100 – (M + F + CP + A).

Physical and chemical parameters

Texture of prepared fish fingers was determined using Y2 laboratory penetrometer and the results were expressed as kg/cm^2. The pH values were determined in the homogeneous mixtures of fish and distilled water (1:9, w:v), using standardized pH meter (Jenway 3010; UK). Total volatile basic-nitrogen (TVB-N) was measured by steam-distillation of the TCA-fish extract using the modified method of Malle and Tao (1987). Trimethylamine (TMA) content was determined using Malle and Poumeyrol (1989) method. Peroxide value (PV) and free fatty acid (FFA) contents were determined in the lipid extract by the Egan *et al*. (1997) method. Thiobarbituric acid reactive substances (TBARS) value as mg malonaldehyde/kg was determined using a spectrophotometric method (Tarladgis *et al*., 1960).

Antioxidant activity of date seed powder

The antioxidant activity of date seed powder sample was determined by DPPH method (Lee *et al.*, 2003) with some modifications. The stock reagent solution (1×10^{-3} M) was prepared by dissolving 22mg of DPPH in 50 mL of methanol and stored at -20 °C until use. The working solution (6×10^{-5} M) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8±0.02 at 515 nm, measured using a spectrophotometer (6505 UV/Vis, Jenway Ltd., Felsted, Dunmow, UK). Extracts each of 0.1 ml were mixed with 3.9 ml of DPPH solution for 30 s and left to react for 30 min. Then, the absorbance (A) at 515 nm was recorded. A control was also done using the extraction solvent.

Scavenging activity (%) = $[(A_{control}-A_{sample})/A_{control}] \times 100$

Microbiological analysis

A sample (10g) was taken and aseptically transferred in 90 ml of sterile 0.1% peptone water to prepare the 10^{-1} dilution, from which other decimal dilutions were prepared (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). Total plate count was determined using pour plate method on a Plate Count Agar as a medium. Plates were incubated at 35°C for 24-48 h according to (Harrigan and McCance, 1976). *Pseudomonas* counts were performed using *Pseudomonas* Isolation Agar medium supplemented with glycerol (Difco, 2009) and incubated at 25°C for 48 h. For total *Enterobacteriaceae* count, violet red bile glucose (VRBG) agar was used as a medium. Plates were incubated at 35°C for 48 h (Difco, 2009). All counts were expressed as log CFU/g.

Sensory quality

Sensory evaluation was performed as described by Tokur *et al*. (2006). Thawed samples were fried in sunflower oil at 180 ºC for 2.5 min and then, introduced to the panelists for their assessment. Sensory attributes were evaluated according to their color, odour, taste, texture and overall acceptability on a 1-10 point hedonic scale. The panelists carried out the tests were staff members of Food Technology Department, Suez Canal University and semi-trained panelists.

Statistical analysis

The obtained data were subjected to Analysis of variance (ANOVA) using SPSS software (version 16.0

for Windows, SPSS Inc., Chicago). Duncan's multiple range tests were used to locate significance between treatment means at $P \leq 0.05$.

RESULTS AND DISCUSSION

Chemical composition, microbiological and chemical quality parameters of catfish mince and raw fish fingers:

The chemical compositions of catfish fillet mince and raw fish fingers are presented in Table 1. The protein and fat contents did not change significantly. Meanwhile, the carbohydrate, moisture and ash contents of fish fingers changed significantly ($P \leq 0.05$) because of the presence of coating materials such as flour, starch and bread crumb.

The pH, TVB-N, TMA, FFA, Peroxide value and TBARS values of catfish minced fillet and raw fish fingers are presented in Table 1. The obtained data showed moderate increase in these parameters in fish fingers due to processing of the mince into fish fingers. The pH value of the raw fish fingers was slightly higher (6.78) than that of catfish minced fillet (6.42). On the other hand, significantly higher values in the FFA, TVB-N and TBARS parameters were observed in the fingers. Similarly, TMA and PV values had the same trend and the increase was not significant.

Table (1) also shows the microbial count of catfish mince and raw fish fingers. Aerobic plate count decreased significantly from 5.96 to 4.52 log cfu/g during production process. This may be attributed to the antimicrobial properties of food additives such as garlic (Talab, 2014). However, *Pseudomonas* and total *Enterobacteriaceae* bacterial counts in the prepared fingers were lower but not significant. These results are in agreement with the findings of Elyasi *et al*. (2010), who prepared fish fingers from common carp and found a decrease of all microbiological counts after the production process.

Table (1): Chemical composition, chemical and microbiological quality of catfish mince and raw fish fingers*

Analysis	Parameters	Catfish mince	Fish fingers
Chemical composition (%)	Moisture	76.13[a]	72.34[b]
	Ash	1.78[b]	2.98[a]
	Crude protein	19.58[a]	19.32[a]
	Crude fat	1.63[a]	1.37[a]
	Carbohydrates	0.88[b]	3.99[a]
Chemical quality parameters	pH	6.42[a]	6.78[a]
	TVB-N (mg/100g)	9.24[b]	10.50[a]
	TMA (mg/100g)	8.54[a]	8.96[a]
	FFA (g oleic/kg^{-1} lipids)	0.178[b]	0.279[a]
	PV (meq O_2 /kg)	2.52[a]	2.64[a]
	TBARS (mg MDA/kg)	0.160[b]	0.234[a]
Microbiological quality (log CFU/g)	APC	5.96[a]	4.52[b]
	Pseudomonas	2.98[a]	2.87[a]
	Enterobacteriaceae	1.84[a]	1.74[a]

*Means within the same column having different superscript letters are significantly different at $P \leq 0.05$.

Quality parameters of fish finger samples:

Texture:

As shown in Table (2), samples containing transglutaminase (MTGase) showed higher values of texture i.e. its firmness was increased. There was a significant increase in texture values from 1.34 for control to 3.33 kg/cm^2 for MTGase. The increase in the texture values may be attributed to inducing covalent crosslinking of proteins. Ramirez-Suárez *et al*. (2001) stated that transglutaminase increases cross-linking of myosin heavy chains during setting, thus creating a denser bond network between proteins. The results of Vácha *et al*. (2006) and Muguruma *et al.* (2003) confirmed a strong improvement of texture (firmness) after the addition of transglutaminase. It could be noticed that, DSP had lower texture value (1.24 kg/cm^2) compared to control (1.34 kg/cm^2) due to the high fiber content (51%) in the added DSP. Sánchez-Alonso *et al.* (2006) found that addition the wheat dietary fiber to Alaska pollock surimi resulted in a decrease in firmness.

Table (2): Effect of MTGase and some natural antioxidants on quality parameters of catfish fingers

Treatments	Texture (kg/cm^2)	pH	TVB-N (mg/100g)	TMA (mg/100g)	FFA (g oleic/kg^{-1} lipids)	PV (meq O_2 /kg)	TBARS (mg MDA/kg)
T1	1.34^{c}	7.05^{a}	24.48^{a}	11.14^{a}	2.58^{a}	3.19^{a}	1.33^{a}
T2	3.33^{a}	6.97^{b}	18.92^{b}	8.03^{b}	1.77^{b}	1.78^{b}	0.75^{b}
T3	1.24^{c}	6.72^{c}	15.77^{d}	7.38^{c}	0.79^{d}	1.18^{c}	0.37^{c}
T4	1.38^{c}	6.70^{c}	18.11^{bc}	8.12^{b}	1.26^{c}	0.48^{d}	0.33^{c}
T5	2.85^{b}	6.72^{c}	18.13^{bc}	7.33^{c}	0.87^{d}	0.99^{c}	0.38^{c}
T6	3.48^{a}	6.69^{c}	17.48^{c}	8.03^{b}	1.31^{c}	0.53^{d}	0.33^{c}

T1 Control, T2 Enzyme 0.5%, T3 Date seed powder (DSP) 1%, T4 Combination of citric acid 0.5% and ascorbic acid 0.5%, T5 Combination of date seed powder 1% and enzyme 0.5% and T6 Combination of citric acid 0.5%, ascorbic acid 0.5% and enzyme 0.5%.
Means within the same column having different superscript letters are significantly different at $P \leq 0.05$.

As statistical analysis indicated, frozen storage revealed a significant effect on texture changes during storage period (Table 3). Badii and Howell (2002) reported that increased length of storage time at -10°C hardened the fillets. Dorado-Rodelo *et al.* (2007) found changes in shear forces for frozen fillets stored at -20°C for 120 days. Makri (2009) related the development of hardness in raw stored frozen fillets to water holding capacity, denaturation and changes of myofibrillar proteins.

Table (3): Effect of frozen storage (-18 ˚C for 5 months) on the quality parameters of fish fingers

Parameters / Storage (month)	Texture (kg/cm^2)	pH	TVB-N (mg/100g)	TMA (mg/100g)	FFA (g oleic/kg^{-1} lipids)	PV (meq O_2 /kg)	TBARS (mg MDA/kg)
Zero time	2.01^{d}	6.62^{e}	16.36^{e}	5.39^{e}	0.55^{e}	0.56^{d}	0.25^{c}
1	2.06^{d}	6.67^{de}	17.31^{de}	6.77^{d}	0.73^{e}	0.95^{c}	0.34^{c}
2	2.14^{cd}	6.73^{d}	18.25^{cd}	8.17^{c}	1.21^{d}	1.20^{bc}	0.41^{c}
3	2.28^{bc}	6.82^{c}	19.18^{bc}	9.30^{b}	1.43^{c}	1.38^{b}	0.65^{b}
4	2.38^{b}	6.96^{b}	19.55^{b}	10.23^{a}	1.94^{b}	1.89^{a}	0.86^{ab}
5	2.75^{a}	7.05^{a}	22.24^{a}	10.18^{a}	2.74^{a}	2.16^{a}	1.00^{a}

Means within the same column having different superscript letters are significantly different at $P \leq 0.05$.

Chemical quality changes:

pH value

Changes of pH value in the treated fish finger samples during 5 months of frozen storage at (-18°C) are shown in Table (2) and Table (3). The data revealed that there were significant differences ($p \leq 0.05$) in pH values between fish finger treatments along storage periods. It was noticed that the pH value of the control was higher (7.05) than the other samples. For treatments (Table 2), fish fingers containing MTGase had the highest pH value (6.97) and fish finger containing citric acid 0.5%, ascorbic acid 0.5% and enzyme 0.5% showed the lowest value (6.69). Rostamzad *et al*. (2011) found that pH value of treated samples with citric and ascorbic acids were lower than that of control sample in fish fillets during six months of frozen storage.

The treatments and storage time exhibited a significant effect ($P \leq 0.05$) on the pH values of fish fingers. There was comparatively slow increase in pH vales of fish finger samples during freezing storage period (Table 3). Similar results have been observed by Rathod and Pagarkar (2013) for *Pangasius* fish cutlets and by Coban (2013) for fish fingers (*Sarda sarda*). Jay (1996) explained the increase in pH to the accumulation of metabolites caused by bacterial action in meat. Gill (1983) added that bacteria, on exhaustion of glucose, utilize amino acids (released during protein breakdown) and as a result ammonia accumulates which increases pH value.

Total volatile basic-nitrogen

Total volatile basic-nitrogen (TVB-N) is a commonly used chemical method to determine spoilage of fish. The treatments and frozen storage time had a significant effect ($P \leq 0.05$) on lowering the TVB-N values of fish fingers. The control sample had generally higher TVB-N mean value (24.48 mg/100g) than the treated samples (Table 2). Fish fingers containing MTGase had the highest mean TVB-N value (18.92 mg/100g) and Fish finger containing DSP had the lowest value (15.77 mg/100g) among treatments. European Union Commission (1995) reported the legal limits set for these indexes to be 35 mg/100 g for TVB-N. Changes in the TVB-N values of fish finger samples during frozen storage are given in Table (3). The values for the prepared fingers did not exceed such limit throughout storage period. Pandey and Kulkarni (2007) reported a significant increase in the TVBN value of fish fingers during frozen storage (6 months). The increasing of TVB-N value during storage is attributed by Chomnawang *et al*. (2007) to bacterial spoilage and the activity of endogenous enzymes.

Trimethylamine (TMA)

Trimethylamine nitrogen (TMA) is used as an index to assess the quality and shelf life of seafood products (Hebard *et al.*, 1982). TMA values were statistically lower ($P \leq 0.05$) in all treated samples compared to the control (Table 2). The treatment containing a combination of date seed powder 1% + enzyme 0.5% had the lowest TMA value (7.33 mg/100 g) followed by The DSP treatment (7.38 mg/100 g), due to the high antioxidant activity of the DSP (42.5%) as determined in a laboratory assessment. Changes of TMA in treated fish finger samples during 5 months of frozen storage at -18°C are shown in Table (3). The TMA values of all fish finger treatments were gradually increased with increasing storage period.

Free fatty acids (FFA)

FFA content is a result of enzymatic decomposition of lipid during storage (Tokur *et al.,* 2006). The FFA content in the lipid of a fish is an indication of lipid hydrolysis. The lowest FFA value (0.79 g kg^{-1} lipids) was found in the fish fingers with the addition of DSP, while the highest value in FFA values (2.58 g/kg^{-1} lipid, Table 2) was found in the control sample. This is due to the high antioxidant activity of the added DSP (42.5%). Also, addition of citric and ascorbic acids resulted in a decrease in FFA value compared to control sample. Because ascorbic and citric acids act as oxygen scavengers and metal chelators, thus causing delay in lipid oxidation (Rostamzad *et al.,* 2011).

Changes in the FFA of fish finger samples during frozen storage are given in Table 3. A gradual increase in FFA content with storage duration was observed in all samples. Confirmation of these results was indicated by the results of Pawar *et al.* (2012) for *Catla* fish cutlets, Rathod and Pagarkar (2013) for *Pangasius* fish cutlets and Ninan *et al.* (2010) for tilapia fish cutlets. Also, Tokur *et al.* (2006) reported FFA rise from the beginning of storage up to the end of storage.

Peroxide value (PV)

The peroxide value was employed for determining the formation of primary oxidation products during the storage period. The PV of MTGase treatment was 1.78 meq O_2/kg which was lower than that of the control (3.19 meq O_2/kg). Treatments containing CA+AA (T4) and CA+AA+ MTGase (T6) had the lowest PV values, which were 0.48 and 0.53 meq O_2 /kg, respectively. These results are in agreement with those recorded by (Naveena *et al.,* 2008; Taheri *et al.,* 2012) for chicken fillet and fillet due to the antioxidant effects of ascorbic acid in terms of inhibiting lipid oxidation. They also observed that PV value showed a slow increase with frozen storage time. Treatments containing DSP gave 1.18 and 0.98 meq O_2 /kg for DSP and the combination of DSP + MTGase treatments, respectively. In this concern, Sarkardei and Howel (2008) reported that the increase of peroxide value in samples treated with antioxidants was significantly lower than that of control sample.

Changes in the PV of fish finger samples during frozen storage are given in Table 3. All treatments significantly reduced the PV values throughout storage as compared to the control sample.

The thiobarbituric acid reactive substances (TBARS)

TBARS values were significantly lower ($P \leq 0.05$) in all treated samples as compared to the control sample (Table 2). Egyptian Standard (2005) asserted that TBARS value in frozen fish fingers is not to exceed 4.5 mg malonaldehyde/kg. Yanar and Fenercioğlu (1999) reported similar results (0.6 and 2.2 mg MDA/kg) in minced fish meat from carp.

For the variation in treatment's effects, a significant ($p \leq 0.05$) increase in TBARS value was observed for MTGase treatment (0.75 mg MDA/kg) as compared to other treatments. Addition of CA, AA and DSP significantly ($P \leq 0.05$) decreased TBARS values of prepared fingers, due to their antioxidant effect. This means that usage of CA, AA and DSP had a positive influence on reducing lipid oxidation and, therefore, would enhance shelf-life of fish fingers. Storage time of the prepared catfish fingers had a significant ($P \leq 0.05$) effect on TBARS values as shown in Table (3). The increase of the TBARS value during frozen storage was affirmed by many researchers (Tokur *et al.,* 2006; İzci, 2010; Boran and Köse, 2007).

Microbiological changes of fish fingers during frozen storage at -18°C:

Aerobic plate counts (APC)

Aerobic plate counts (APC) is an indicator of shelf-life of food products (Arvanitoyannis *et al*., 2005). The microbial load of fish fingers depends on the microbial load of the raw fish meat, sanitary conditions, time and temperature of storage as well as other ingredients which are used in preparation of fish fingers. Table (4) shows the microbial content of fish fingers. The APC of control sample increased throughout 5 month at -18°C. The increase of APC may be attributed to the increase in simple nitrogenous compounds (amino acids and nucleotides) and fatty acids which were produced by hydrolysis of protein and fat by natural fish enzymes which consequently lead to suitable conditions for bacterial growth. Log APCs of other treatments (T2-T6) were around 4.03- 5.22 log cfu/g. These levels did not exceed the maximum limits (7 log APC/g) set for fresh and frozen fish given by the International Commission on Microbiological Specifications for Foods (1978).

In this study the APCs of the fish fingers were lower than the maximum limits during the storage period. It was observed that the APCs decreased just after addition of DSP, AA and CA, due to their antimicrobial effect. The obtained data also showed that during storage time, the APC counts of fish finger samples were Significantly ($P \leq 0.05$) increased.

Table (4): Changes of aerobic plate count (APC) (log cfu/g) in treated fish fingers (T2-T6) during 5 months of storage at -18°C as compared to the control sample (T1)

Treatments	Storage period (month)						Mean
	0	1	2	3	4	5	
T1	4.62	4.85	5.32	5.79	5.82	5.95	5.39^{a}
T2	4.61	4.83	4.95	5.39	5.69	5.83	5.2^{b}
T3	3.54	3.72	3.85	4.23	4.63	4.80	4.13^{d}
T4	3.52	3.82	4.11	4.25	4.52	4.83	4.19^{cd}
T5	3.53	3.69	3.76	4.15	4.27	4.78	403^{e}
T6	3.51	3.71	4.21	4.48	4.64	4.87	4.24^{c}
Mean	3.90^{f}	4.10^{e}	4.37^{d}	4.72^{c}	4.93^{b}	5.18^{a}	

T1 Control, T2 Enzyme 0.5%, T3 Date seed powder (DSP) 1%, T4 Combination of citric acid 0.5% and ascorbic acid 0.5%, T5 Combination of date seed powder 1% and enzyme 0.5% and T6 Combination of citric acid 0.5%, ascorbic acid 0.5% and enzyme 0.5%.
Means within the same column having different superscript letters are significantly different at $P \leq 0.05$ for treatments, while means within the same row having different superscript letters are significantly different for time of storage.

Pseudomonas

Pseudomonas spp count in fish samples is of highly importance because this bacterium can be used as an indicator of food quality as spoilage organism (Jeya Sekaran *et al.,* 2006 and Yagoub, 2009). Table (5) shows *Pseudomonas* count in treated fish finger samples during 5 months of frozen storage. At zero time, the *Pseudomonas* count of control was 2.21 log cfu/g and reached 3.11 log cfu/g at the end of storage. However, Log *Pseudomonas* of other treatments (T2-T6) ranged from 1.78 to 2.20 cfu/g. Enzyme treatment (T2) didn't show any significant difference In *Pseudomonas* count as compared to control. This means that the enzyme had no antimicrobial effect. Treatment combination of citric acid, ascorbic acid and enzyme had the lowest *Pseudomonas* count mean (2.06 log cfu/g) compared to other treatments and control (2.71 log cfu/g), because their antimicrobial action. *Pseudomonas* counts decreased at the second month of the storage then increased through storage time. Gram and Melchiorsen (1996) found that *Pseudomonas spp.* counts increased as the storage time increased.

Total Enterobacteriaceae

Enterobacteriaceae counts in fish finger samples are shown in Table (6). Total *Enterobacteriaceae* counts in fish finger samples at the zero time ranged from 0.53 to 0.65 log cfu/g. *Enterobacteriaceae* mean counts in fish finger samples ranged from 0.94 – 1.12 (log cfu/g). DSP treatment sample had the lowest *Enterobacteriaceae* count as compared to other treatments and control. Also, addition of citric and ascorbic acids led to a decrease in *Enterobacteriaceae* count of fish fingers. *Enterobacteriaceae* levels permitted by Center for Food Safety (2014) are $< 10^{2}$ CFU/g for ready-to-eat food; therefore, counts are within the acceptable limits for frozen fish products. For all studied samples, the *Enterobacteriaceae* counts were within the acceptable limits. Thus, the prepared fish fingers were proper from the hygienic point of view.

Table (5): Changes of *Pseudomonas* count (log cfu/g) in treated fish finger (T2-T6) during 5 months of storage at 18°C as compared to the control sample (T1)

Treatments	Storage period (month)						Mean
	0	1	2	3	4	5	
T1	2.21	2.36	2.74	2.2	2.94	3.11	2.71a
T2	2.20	2.32	2.58	2.81	2.92	3.02	2.64a
T3	1.84	1.90	2.14	2.35	2.47	2.64	2.22bc
T4	2.01	2.21	2.32	2.43	2.52	2.61	2.35b
T5	1.83	1.87	2.23	2.36	2.40	2.54	2.21c
T6	1.98	1.07	2.37	2.55	2.62	1.76	2.06d
Mean	2.01c	1.96c	2.40b	2.57a	2.65a	2.61a	

T1 Control, T2 Enzyme 0.5%, T3 Date seed powder (DSP) 1%, T4 Combination of citric acid 0.5% and ascorbic acid 0.5%, T5 Combination of date seed powder 1% and enzyme 0.5% and T6 Combination of citric acid 0.5%, ascorbic acid 0.5% and enzyme 0.5%.
Means within the same column having different superscripts are significantly different at $P \leq 0.05$ for treatments, while Means within the same row having different superscripts are significantly different for time of storage.

Table (6): Changes of *Enterobacteriaceae* count (log cfu/g) in treated fish fingers (T2-T6) during 5 months of storage at -18°C as compared to the control sample (T1)

Treatments	Storage period (month)						Mean
	0	1	2	3	4	5	
T1	0.65	0.88	0.98	1.28	1.45	1.50	1.12^{a}
T2	0.64	0.83	0.87	0.93	1.40	1.54	1.04^{b}
T3	0.53	0.72	0.82	1.02	1.20	1.33	0.94^{c}
T4	0.57	0.76	0.98	1.21	1.30	1.35	1.03^{b}
T5	0.54	0.73	0.83	0.92	1.30	1.38	0.95^{c}
T6	0.56	0.78	0.85	0.96	1.32	1.54	1.00^{b}
Mean	0.58^{f}	0.78^{e}	0.89^{d}	1.05^{c}	1.33^{b}	1.44^{a}	

T1 Control, T2 Enzyme 0.5%, T3 Date seed powder (DSP) 1%, T4 Combination of citric acid 0.5% and ascorbic acid 0.5%, T5 Combination of date seed powder 1% and enzyme 0.5% and T6 Combination of citric acid 0.5%, ascorbic acid 0.5% and enzyme 0.5%.
Means within the same column having different superscripts are significantly different at $P \leq 0.05$ for treatments, while means within the same row having different superscripts are significantly different for time of storage.

Sensory changes of fish fingers:

The acceptability of fish and fishery products during storage depends on changes in their sensory attributes. The sensory parameters of fish finger samples prepared from catfish were evaluated in terms of color, odour, taste, texture, and overall acceptability (Figure 1). In this study, the panelists evaluated color of fish fingers meat not the external layer. As data indicated, all fish finger treatments and frozen storage had a significant effect on color changes during storage time. Scores of color decreased when the storage time increased. It is clear to notice that the MTGase sample had the highest color value as compared to the control and other samples.

It was found that treatments and storage time had a statistically significant effect ($P \leq 0.05$) on odour scores of fish fingers. Starting from the fourth month, scores of the odour for all treatments gradually decreased throughout storage period, but there was a significant difference in odour between the fourth and fifth month. Treatment containing a combination of citric acid, ascorbic acid and MTGase had the highest odour values compared to other treatments throughout storage period. At the end of storage, the control sample had the lowest odour scores (7.11) compared to treated fish finger samples. Similar results were obtained by Pourashouri *et al.* (2009) for catfish fillet treated with AA and CA.

The MTGase treatment had the highest taste scores compared to other treatments and control. Scores of taste increased during the first and second months then started to decrease at the fourth month even the end of storage.

The MTGase treatment had the highest texture scores and Treatment containing citric acid and ascorbic acid had the lowest texture scores. There was a slight decrease in texture scores throughout storage period.

At zero time, the overall acceptability score of control was 8.72 and reached a score of 8.06 at the end of storage. The MTGase treatment demonstrated the highest overall acceptability score compared to other treatments. Adding natural antioxidants in ready-to-eat fish products prevented or reduced lipid oxidation and preserved sensory attributes of such products (Yanishlieva *et al.,* 2006; Naveena *et al.,* 2008).

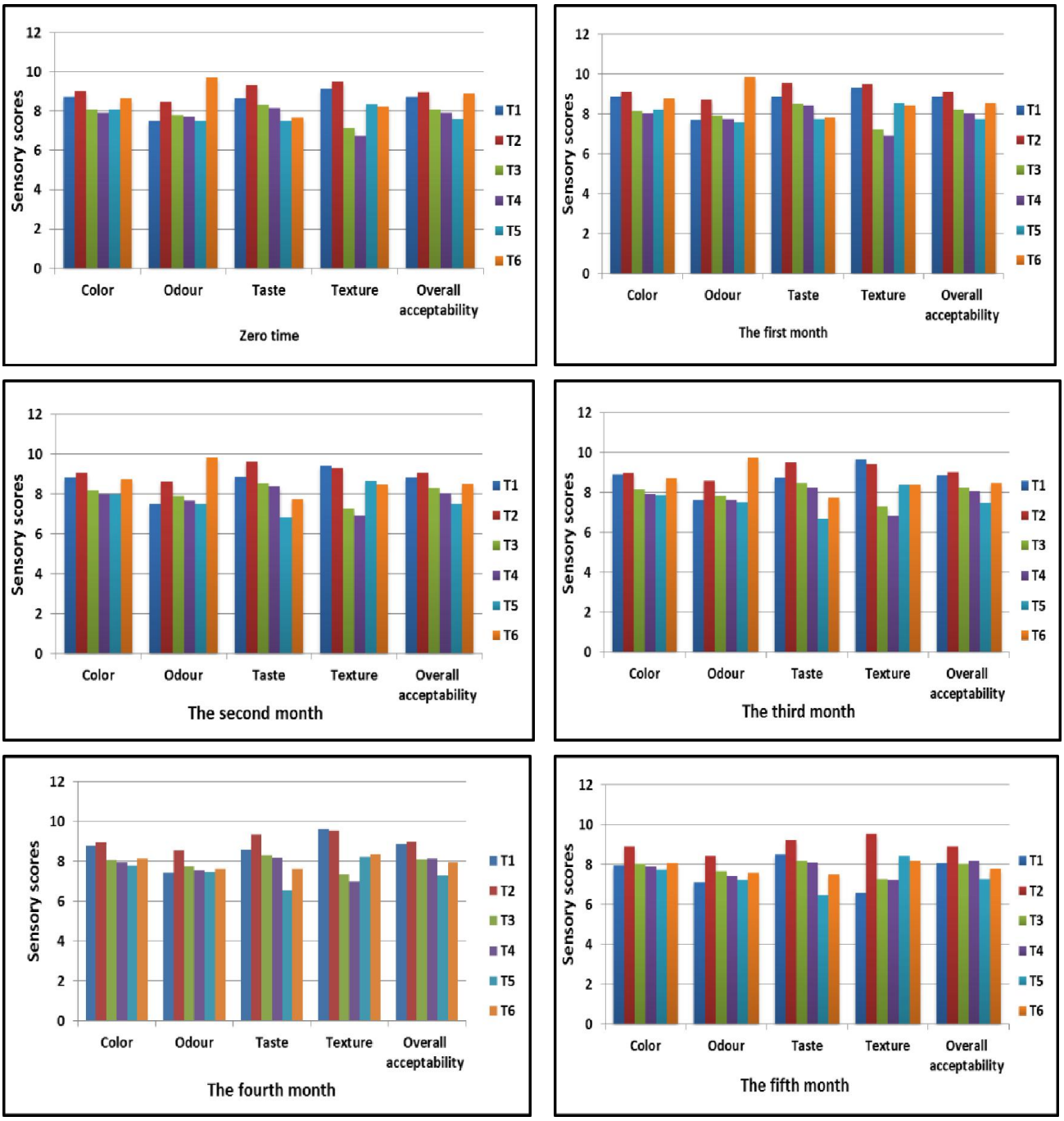

Figure (1): Changes in sensory scores of fish finger samples during the frozen storage period at -18°C.

CONCLUSION

Acceptable fish fingers can be produced from catfish by using some natural additives like date seed powder, citric acid and ascorbic acid. Also, date seed powder treatment was more effective in improving the chemical, microbiological and sensory properties of the prepared fingers than other studied treatments. Moreover, addition of MTGase enzyme, especially in combinations with other tested additives, resulted in improving in textural and sensory properties of prepared fish fingers.

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تأثير إنزيم الترانس جلوتامينيز وبعض مضادات الأكسدة الطبيعية على جودة أصابع القراميط الجاهز للأكل أثناء التخزين المجمد

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لزيادة استهلاك سمك القراميط، تم إعداد وتقييم أصابع السمك الجاهزة للأكل أثناء التخزين على - ١٨°م لمدة ٥ شهور. تم تقدير تأثير إنزيم الترانس جلوتامينيز، مسحوق نوى البلح، حامض الستريك وحامض الاسكوربيك، سواء في صورة فردية أو خليط، على الخصائص الفيزيائية، الكيميائية، الميكربيولوجية والحسية. أظهرت النتائج المتحصل عليها انخفاض قيم pH، TVB-N، TMA، FFA، PV و TBA معنويا في عينات أصابع السمك المختبرة مقارنة بالكنترول. أيضا، أدت مضادات الأكسدة الطبيعية المضافة إلى تأخير النمو الميكروبي (العدد البكتيري الكلى (*Pseudomonas* و *Enterobacteriaceae*) خلال فترة التخزين، وأعطت العينات المختبرة نتائج جيدة مقارنة بالكنترول. أيضا، أوضحت نتائج التقييم الحسي (اللون، الرائحة، الطعم، القوام والقابلية العامة) أن المعاملات حسنت قيم الخصائص الحسية لأصابع السمك المعدة أثناء التخزين المجمد مقارنة بالكنترول.

الكلمات الدالة المرشدة: أصابع السمك الجاهزة للأكل، إنزيم الترانس جلوتامينيز، مسحوق نوى البلح، حامض الستريك، حامض الاسكوربيك