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Effect of Transglutaminase Enzyme, Chitosan and Rosemary Extract on Some Quality Characteristics of Ready to Eat Fish Fingers Made from Catfish (*Clarias gariepinus*) during Frozen Storage

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Abstract

To increase the utilization of catfish; which is not preferred by consumers as compared to other fish species, ready to eat fish fingers were prepared and evaluated during frozen storage (-18°C) for 5 months. The effect of some additives such as transglutaminase, chitosan and rosemary on physical, chemical, microbiological and sensory properties, were carried out. The obtained data indicated that pH, total volatile basic nitrogen (TVB-N), trimethylamine (TMA), free fatty acids, peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were decreased significantly ($P \leq 0.05$) in the treated fish finger samples compared to the control sample. Natural additives applied retarded microbial aerobic plate count (APC), Pseudomonas and total Enterobacteriaceae) growth during storage period and treated samples gave best results, as compared to the control sample. Sensory evaluations indicated that treatments improved scores of the prepared fingers during frozen storage as compared to the control sample. Moreover, combination of added materials was better than individual addition on improving the physical, chemical, microbiological and sensory properties of catfish fingers.

Keywords: *Quality Parameters; Ready to Eat Fish Products; Transglutaminase Enzyme; Chitosan; Rosemary*

Introduction

Catfish (*Clarias gariepinus*) is a highly nutritious that contain high amounts of unsaturated fatty acids, vitamins, proteins, and minerals [1]. 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 [2]. 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 such as fish fingers, cutlets, patties, burgers, sausages and fish balls [3]. Ready to eat food can be described as the status of food being ready for immediate consumption at the point of scale, it could be raw or cooked, and can be consumed without further treatment [4].

Fish and fish products can undergo unwanted changes during frozen storage which result in deterioration that limit their shelf life. These undesirable changes are a result of protein denaturation [5], and lipid oxidation [6,7]. Lipid oxidation may affect flavour, texture, taste, and shelf-life of fish products and their nutritional quality [8-10]. It can negatively affect protein functionality [11] and may cause discoloration and poor appearance.

A direct correlation between lipid oxidation and sensory changes of ready-to eat fish products during frozen storage was reported by Boran and Kose [12]. Lipid oxidation should be inhibited to minimize quality changes and to insure sensory and nutritional values during processing and storage of fish products. Antioxidants are compounds capable of scavenging free radicals which delay, retard or prevent auto-oxidation. Antioxidants have also a wide range of biological, nutritional and health benefits, as oxidative stress is an important factor

in cell damage and the development of certain cancers and neurodegenerative diseases [13]. 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.* [5]. Sarkardei and Howel [14], and Candan and Bagdath [15].

Transglutaminase enzyme (MTGase) is able to catalyze the crosslinking of many proteins such as whey proteins, soy proteins, wheat proteins, beef myosin, casein and actomyosin, leading to affect their texture [16]. Interestingly, bonds formed by transglutaminase showed a high resistance to proteolysis [17]. 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 [18].

Chitosan [β-(1, 4)-2-amino-2-deoxy-D-glucopyranose] is an abundant natural polymer in nature next to cellulose [19]. It is mostly applied as a food additive or preservative, and as a component of packaging material, not only to retard microbial growth in food, but also to improve the quality and shelf-life of food products [20]. Due to its antimicrobial activity [21] antioxidant activity in muscle foods [22], antitumor [23] binding agent [24,25] clarifying agent in apple juice [26], texturizing effect [27], a cryoprotective effect [28], and hypocholesterolaemia functions [29], chitosan and its oligomers have received considerable attention and comprehensively reviewed by Moradi., *et al.* (2010).

Rosemary (Rosmarinus officinalis L.), is a popular herb belonging to the Lamiaceae family with high antioxidant activity. Rosemary extracts have known to exhibit a high antioxidant activity and are widely applied in the food industry. The antioxidative properties of rosemary are mainly related to their content of phenolic compounds, which quench free radicals by hydrogen donation [30]. Rosemary phenolics may be up to four times equal to butylated hydroxy anisole and as effective as butylated hydroxytoluene as antioxidants [31-33]. It has been used to retard lipid oxidation in chicken burger [34].

The objective of the present study was to determine the effects of transglutaminase enzyme, chitosan and rosemary on chemical, microbiological and sensory quality of ready to eat fish fingers made from catfish fillet during frozen storage.

Materials and Methods

Catfish (*Clarias gariepinus*), weighing between 1 and 2 kg each, were obtained directly from the fish market (Ismailia, Egypt) in August 2014. Chitosan was obtained from Sigma-Aldrich Chemical Co. Rosemary leaves were purchased from local markets. 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). Other chemicals used were of food grades.

Rosemary preparation and extraction

Dried leaves were ground into powder using a grinder (Moulinex, LM2421, France). Twenty-five grams of the dried powder was transferred into a beaker and 400 mL of methanol was added. The mixture was covered and shaken by a mechanical shaker (Julabo D-7633 Labortechmik, GMBIT, Jeelback, Germany) for 24 h at room temperature. The extract was filtered using Double Rings Filter Paper No. 102. The filtrate was collected, and the residue was re-extracted twice. The extracts were pooled. Then, the solvent in the extract was removed at 45 ºC using rotary evaporator (Steroglass, Sreike 300, Perugia, Italy) under reduced pressure. The residues were freeze-dried (CPERON, FDU-7006, Korea), and stored at 4 ºC until further uses [35].

Preparation of fish fingers

About 60 kg of catfish was beheaded, gutted, washed and filleted. The fillets were minced using a meat mincer (SAP Meat Mincer TC22. Italy) 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 [36]. All ingredients were mixed and homogenized by a kitchen blender. The mix was divided into seven 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). Chitosan was added at 1%, (T2), the prepared rosemary powder was added at a concentration of 0.05%, (T3). Moreover, combinations of chitosan 1% and rosemary 0.05% (T4); enzyme 0.5% and chitosan 1% (T5); enzyme 0.5% and rosemary 0.05% (T6); and enzyme 0.5%, rosemary 0.05% and chitosan 1% (T7) were tested. 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.,* [3] and Tokur., *et al.* [36]. 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 [37]. 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 [38] 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 [39]. Trimethylamine (TMA) content was determined using Malle and Poumeyrol [40] method. Peroxide value (PV) and free fatty acid (FFA) contents were determined in the lipid extract by the Egan., *et al.* [41] method. Thiobarbituric acid reactive substances (TBARS) value as mg malonaldehyde/ kg was determined using a spectrophotometric method [42].

Antioxidant activity of rosemary Extract

The antioxidant activity of rosemary extract was determined by DPPH method [43] 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 (%) = [(Acontrol-Asample) / Acontrol] × 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 (TPC) was determined using pour plate method on a Plate Count Agar medium. Plates were incubated at 35°C for 24 - 48 h according to [44]. Pseudomonas counts were performed using Pseudomonas Isolation Agar medium supplemented with glycerol [45] 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 [45]. All counts were expressed as log CFU/g.

Sensory quality

Sensory evaluation was performed as described by Tokur., *et al.* [36]. Thawed samples were fried in sunflower oil at 180ºC for 2.5 min and then, introduced to ten trained panelists for samples 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

Proximate composition, chemical and microbiological quality parameters of catfish mince and raw fish fingers.

The proximate composition of catfish fillet mince and raw fish fingers is 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.

Table 1: *Proximate composition, chemical and microbiological quality of catfish mince and raw fish fingers*.*

Analysis	Parameters	Catfish mince	Fish fingers
Proximate composition (%)	Moisture	76.48[a]	72.52[b]
	Ash	1.22[b]	2.87[a]
	Crude protein	19.88[a]	19.30[a]
	Crude fat	1.63[a]	1.29[a]
	Carbohydrates	0.79[b]	4.02[a]
Chemical quality parameters	pH	6.50[a]	6.75[a]
	TVB-N (mg/100g)	9.24[b]	10.08[a]
	TMA (mg/100g)	5.12[b]	5.24[a]
	FFA (g oleic/kg^{-1} lipids)	0.19[b]	0.20[a]
	PV (meq O_2 /kg)	1.60[a]	1.33[b]
	TBARS (mg MDA/kg)	0.22[b]	0.40[a]
Microbiological quality (log CFU/g)	Aerobic plate count (APC)	6.34[a]	5.90[a]
	Pseudomonas	2.59[a]	2.44[b]
	Enterobacteriaceae	1.88[a]	1.75[b]

**Means within the same row having different superscripts are significantly different at P ≤ 0.05.*

The pH, TVB-N, TMA, FFA, PV and TBARS values of catfish minced fillet and raw fish fingers are presented in Table 1. The pH value of the raw fish fingers was slightly higher (6.75) than of catfish minced fillet (6.50). The TVB-N and TMA values were significantly increased, by processing the fillet, from 9.24 to 10.08 mg/100g and from 5.12 to 5.24 mg/100g, respectively. Also, the obtained data indicated that, the FFA and TBARS values were significantly (P ≤ 0.05) increased from 0.19 to 0.20 g oleic fatty acid kg-1 lipids and from 0.22 to 0.40 mg MDA/kg, respectively. While the PV value was significantly (P ≤ 0.05) decreased from 1.60 to 1.33 meq O^2 /kg.

Table 1 also shows the microbial count of catfish mince and raw fish fingers. Aerobic plate count of fish fingers decreased during the production process from 6.34 to 5.90 log cfu/g during production process. This may be attributed to the antimicrobial properties of food additives such as garlic [46]. Pseudomonas and total Enterobacteriaceae bacteria counts were significantly (P ≤ 0.05) decreased from 2.59 to 2.44 log cfu/g and from 1.88 to 1.75 log cfu/g, respectively. This can be attributed to the bactericidal effects of one or more of the added ingredients. These results are in accordance with the findings of Elyasi., *et al.* [47], who produced fish fingers from mince and surimi of common carp (Cyprinus carpio L.) and found a decrease of all microbiological counts after the production process.

Quality parameters of fish finger samples

Texture

The texture of fish is a main feature used to appreciate the freshness quality [48]. As shown in table 2, the control had the lowest mean texture value (2.27 kg/cm^2) and the chitosan combination with MTGase treatment had the highest texture value (3.70 kg/cm^2), due to the effect of MTGase and chitosan on the texture. The data showed that MTGase has the ability to improve the texture of fish finger samples. Ramirez-Suárez., *et al.* [49] 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.* [50] and Muguruma., *et al*. [51] confirmed a strong improvement of texture (firmness) after the addition of transglutaminase. It could be noticed that, chitosan treatment had a higher texture value (3.05 kg/cm^2) as compared to control sample. Amiza and Kang [25] stated that addition of chitosan (1.5%) enhanced the WHC value of catfish surimi products.

As statistical analysis indicated, frozen storage revealed a significant effect on texture changes during storage period (table 3). This is may be attributed to use the antioxidants that retarded lipid oxidation, hence resulted in improving the texture values. A linear increase in firmness with frozen storage was reported by Badii and Howell [52] and Ozbay., *et al*. [53]. Makri [54] related the development of hardness in raw stored frozen fillets to water holding capacity, denaturation and changes of myofibrillar protein.

Table 2: *Effect of MT Gase 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	2.27^{e}	7.07^{a}	19.00^{a}	6.97^{a}	2.24^{a}	2.49^{a}	2.04^{a}
T2	3.05^{c}	6.86^{b}	13.21^{b}	5.57^{b}	0.65^{c}	0.76^{c}	0.76^{d}
T3	2.57^{d}	6.89^{b}	12.25^{c}	5.00^{cd}	1.08^{b}	1.02^{b}	1.05^{b}
T4	3.04^{c}	6.61^{c}	10.91^{e}	4.74^{de}	0.32^{d}	0.49^{d}	0.54^{e}
T5	3.70^{a}	6.85^{b}	12.17^{cd}	5.21^{c}	0.66^{c}	0.75^{c}	0.86^{cd}
T6	3.49^{b}	6.90^{b}	12.41^{bc}	5.10^{c}	1.03^{b}	1.09^{b}	0.96^{bc}
T7	3.51^{b}	6.60^{c}	11.34^{de}	4.71^{e}	0.35^{d}	0.51^{d}	0.56^{e}

T1 Control, T2 Chitosan 1%, T3 Rosemary 500 ppm, T4 Combination of chitosan 1% and rosemary 500 ppm, T5 Combination of Chitosan 1% + Enzyme 0.5%, T6 Combination of Rosemary 500 ppm + Enzyme 0.5% and T7 Combination of Chitosan 1%+ Rosemary 500 ppm + Enzyme 0.5%.

Means within the same column having different superscript letters are significantly different at P ≤ 0.05 for treatments.

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	1.84^{f}	6.62^{e}	11.07^{e}	3.86^{e}	0.35^{e}	0.43^{e}	0.61^{e}
1	2.36^{e}	6.67^{de}	11.81^{de}	4.47^{d}	0.57^{d}	0.64^{d}	0.71^{de}
2	2.98^{d}	6.73^{d}	12.35^{d}	5.09^{c}	0.82^{c}	0.99^{c}	0.83^{cd}
3	3.37^{c}	6.82^{c}	13.43^{c}	5.92^{b}	1.06^{b}	1.18^{b}	0.97^{c}
4	3.79^{b}	6.97^{b}	14.29^{b}	6.15^{b}	1.22^{b}	1.35^{ab}	1.24^{b}
5	4.19^{a}	7.13^{a}	15.28^{a}	6.47^{a}	1.40^{a}	1.50^{a}	1.34^{a}

Means within the same column having different superscript letters are significantly different at P ≤ 0.05.

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 ≤ 0.05) in pH values between fish finger treatments along storage periods. It was noticed that the pH value of the control samples had significantly (P ≤ 0.05) the highest pH value as compared to other treatments. It could be noticed that the samples containing a combination of chitosan and rosemary (T4 and T7) had the lowest pH value as compared to other samples (Table 2). This is may be due to the antimicrobial effects of chitosan, rosemary and inhibitory activity on the endogenous proteases which may have delayed the increase in pH. Mohan., [55] found that chitosan coated sardines was significantly (P≤0.05) lower in pH value than the untreated ones.

There was comparatively slow increase in pH value of fish finger samples during freezing storage period (Table 3). Similar results have been observed by Rathod and Pagarkar [56] for Pangasius fish cutlets, Pawar., *et al.* [57] for Catla catla and by Coban [58] for fish fingers (Sarda sarda). Fan., *et al.* [59] explained the increase in pH to the increase in volatile bases produced; ammonia and trimethylamine generated by endogenous or microbial enzymes. Dhanapal., *et al.* [60] associated this increase in pH to the breakage of hydrogen bond.

Total volatile basic-nitrogen

Total volatile basic nitrogen (TVB-N) is a term that includes measurement of basic compounds as trimethylamine, dimethylamine, ammonia and others associated with seafood spoilage processes. TVB-N indicated the extent of the breakdown of proteins into non-protein N-compounds due to bacterial and enzymatic actions [61]. The control sample had generally higher TVB-N mean value (19 mg/100g) than the treated samples (Table 2). Sample which containing a combination of chitosan and rosemary (T4) had the lowest TVB-N value (10.91 mg/100g) as compared to other treatments and control. Lower value in samples treated with rosemary extract was obtained as reported in other studies [62,63]. A reduction (48.33%) in TVB-N formation in grass carp surimi treated with chitosan (1%) was recorded

at the end of 15-day storage period [64]. The treatments and storage time showed statistically ($P \leq 0.05$) effects on TVB-N values of fish fingers. Similar findings were reported by Izci [65] for fish fingers.

Changes in the TVB-N values of fish finger samples during frozen storage are given in Table 3. A gradual increase ($P \leq 0.05$) during storage for all samples was observed and the rate of increase was higher in the control samples. This increase may be due to ammonia and other volatile amines in the fish muscles by enzymatic action [66]. Ozogul., *et al.* [67] justified the increased TVB-N content of certain fish products to the endogenous enzymes and growth of spoilage bacteria. Significant increases in TVBN during frozen storage were reported in tilapia cutlets [68] and in grass carp fish fingers [69].

Trimethylamine (TMA)

Trimethylamine nitrogen (TMA) is used as an index to assess the quality and shelf life of seafood products [70]. TMA values were statistically lower ($P \leq 0.05$) in all treated samples compared to the control (Table 2). Treatment containing chitosan with rosemary and MTGase had the lowest TMA mean (4.71 mg/100g) as compared to other treatments and control sample (6.97 mg/100g). This result may be attributed to the antimicrobial effect of chitosan and/or rosemary. Moreover, a slower increase in TMA was obtained in treated fish finger samples in contrast to a faster increase in TMA of the control. A level of 5-10 mg of TMA/100 g of flesh is set as a rejection limit for fish product [71].

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 is a result of enzymatic decomposition of lipid during storage [36]. Glycerides, glycolipids, and phospholipids are hydrolysed by lipases to free fatty acids, which may be oxidized to give aldehydes and ketones [72]. Control sample had, generally, higher FFA value than the treated samples throughout the storage time (Table 2). FFA values of prepared fingers decreased significantly ($P \leq 0.05$) by the addition of chitosan and/or rosemary which exhibited antibacterial and antioxidant effects. Samples containing a combination of chitosan and rosemary (T4 and T7) had the lowest FFA values (0.32 and 0.35 g oleic fatty acid kg-1 lipids, respectively, suggesting a synergistic effect of chitosan and rosemary. Georgantelis., *et al.* [73]. found that chitosan (1%) in combination with rosemary or alpha-tocopherol was more effective than rosemary or alpha-tocopherol alone on retarding lipid oxidation.

Changes in the FFA of fish finger samples during frozen storage are given in Table 3. The FFA values significantly ($P \leq 0.05$) increased with increased storage time in all treatments. Brake and Fennema [74] (1999) found that the FFA contents of mackerel (Scomber scombrus) mince in frozen storage (-10 ºC) increased with the duration of storage and the degree of mincing of the meat. Similar results were reported by Tokur., *et al.* [75] (2004) and Pandey and Kulkarni [69].

Peroxide value (PV)

Peroxide value (PV) is widely used to measure the primary oxidation products (peroxides) in oils and fats [76]. Peroxides are unstable compounds, and they break down to aldehydes, ketones and alcohols that causing off-flavour in food products [77]. Changes in the PV of fish finger samples during frozen storage are given in Table 2, 3. All treatments significantly reduced the PV values throughout storage as compared to the control sample. The lowest PV value (0.49 meq O_2/kg lipid) occurred in the sample containing chitosan and rosemary, while the highest PV value (2.49 meq O_2/kg lipid) occurred with the control sample. The best effect ($P \leq 0.05$) was obtained by the combination of rosemary and chitosan. This is may be due to the high antioxidant activity of the rosemary extract (48.28%) as determined in a laboratory assessment. Kamil., *et al.* [78] (2002) found a decrease (61%) in the peroxide value of herring (Clupea harengus) samples treated with 200 mg/kg chitosan compared to the controls. Also, when two oxidation inhibitors are used, significant synergic effect is achieved when one of them breaks lipid peroxide chain reaction and another one destroyed the peroxides [79].

The analysis of variance for the PV data indicated that the PV values were significantly ($P \leq 0.05$) affected by both the storage time and the treatments. Generally, increased frozen storage time resulted in an increase in the hydroperoxide formation. A similar increase in the PV content was reported by Tokur., *et al.* [75] for frozen stored fish burgers produced from tilapia

The thiobarbituric acid reactive substances (TBARS)

TBARS values were significantly (P ≤ 0.05) lower in all treated samples than that of the control (Table 2). The lowest TBARS mean value (0.54 mg MDA/kg) was observed in the sample containing chitosan and rosemary (P ≤ 0.05), the data (not shown) of antioxidant activity of rosemary extract would endorse the obtained results of rosemary treatment. These results are in good agreement with those of Georgantelis., *et al*., [80] and Weist and Karel [81]. The authors explained the antioxidant mechanism of chitosan in that the primary amino groups of chitosan would form stable compounds with volatile aldehydes generated from breakdown of fat. They added that, strong effect of synergism is exhibited when two or three antioxidants with different mechanism of action are jointly added. Erdmann., *et al.* [82] reported that the compounds responsible for antioxidant activity are phenolic diterpenes which can delay or inhibit lipid oxidation.

Storage time of the prepared catfish fingers had a significant (P ≤ 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 ([75] for fish burgers; Ninan *et al*., [68] for fish cutlet; Georgantelis *et al*., [80]. (2007a) for minced meat; Sánchez- Escalante., *et al.* [83]. (2001) for beef patties and Mielnik *et al.*, [84] (2003) for Turkey meat).

Microbiological changes of fish fingers during frozen storage

Aerobic plate counts (APC)

Bacterial growth is considered to be the main cause of fish and fish product spoilage. Thus, it is recommended to use bacterial count as an index of the quality of food products [85]. In fish products, Aerobic plate counts (APC) are used as an acceptability index. Table 4 shows the APC of fish finger samples. At zero-time, APC of control was initially 4.72 log cfu/g, which increased with the storage and reached close to 5.93 log cfu/g at the end of storage period. APC mean of treated fish finger samples (T2-T7) were around 3.33-4.10 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 [86]. It is very obvious to notice that treatments containing chitosan and rosemary (T4 and T7) showed the lowest count of APC due to their antimicrobial effects. Darmadji and Izumimoto [87], Georgantelis *et al*., [80], and Mohan., *et al*. [55] have observed a significant reduction of microbial population by the addition of chitosan. Also, components such as phenolic diterpenes (e.g. α-pinene) are responsible for the antimicrobial effects of rosemary [88]. The obtained data also showed that during storage time, significant (P ≤ 0.05) increase in the APC counts of fish finger samples was observed.

Table 4: *Changes of Aerobic plate count (log cfu/g) in treated fish fingers (T2-T7) during 5 months of storage at -18°C as compared to the control sample (T1).*

Treatments	Storage period (months)						Mean
	0	1	2	3	4	5	
T1	4.72	4.91	5.30	5.81	5.89	5.93	5.43^{a}
T2	3.85	3.89	3.92	4.02	4.14	4.24	4.01^{b}
T3	3.81	3.90	3.99	4.11	4.21	4.32	4.06^{b}
T4	3.14	3.21	3.39	3.42	3.54	3.65	3.39^{c}
T5	3.86	3.89	3.99	4.12	4.18	4.29	4.06^{b}
T6	3.79	3.85	3.96	4.25	4.32	4.41	4.10^{b}
T7	3.10	3.22	3.31	3.34	3.45	3.58	3.33^{c}
Mean	3.75^{f}	3.84^{e}	3.98^{d}	4.15^{c}	4.25^{b}	4.35^{a}	

T1 Control, T2 Chitosan 1%, T3 Rosemary 500 ppm, T4 Combination of chitosan 1% and rosemary 500 ppm, T5 Combination of Chitosan 1% + Enzyme 0.5%, T6 Combination of Rosemary 500 ppm + Enzyme 0.5% and T7 Combination of Chitosan 1%+ Rosemary 500 ppm + Enzyme 0.5%.

Means within the same column having different superscript letters are significantly different at P ≤ 0.05 for treatments, while Means within the same row having different superscript letters are significantly different for time of storage.

Pseudomonas

Pseudomonas count in fish samples is of highly importance because this bacterium can be used as an indicator of food quality as spoilage organism [89,90]. Table 5 shows Pseudomonas count in treated fish finger samples during 5 months of frozen storage. At zero-time,

Pseudomonas count for control was 2.12 log cfu/g and the count reached 3.25 log cfu/g at the end of storage period. The average of Pseudomonas count of treated fish finger samples (T2-T7) ranged from 1.49 to 1.95 log cfu/g. It is interesting that the lowest Pseudomonas counts among all samples were those containing both rosemary and chitosan, indicating a synergistic effect. Again, the inhibitory effect of chitosan was significantly enhanced with rosemary extract supplementation. In agreement with the present findings, Petrou., *et al.* [91]. demonstrated a positive effect of chitosan applied singly or in combination with oregano oil, as regard Pseudomonas inhibition, in fresh refrigerated chicken breast meat. Chen., *et al.* [92]. mentioned that the growth of Pseudomonas on oysters was retarded by chitosan addition.

Table 5: *changes of Pseudomonas count (log cfu/g) in treated fish finger (T2-T7) during 5 months of storage at -18°C as compared to the control sample (T1).*

Treatments	Storage period (months)						Mean
	0	1	2	3	4	5	
T1	2.12	2.32	2.73	2.94	3.15	3.25	2.75^{a}
T2	1.55	1.99	1.73	1.92	2.04	2.24	1.91^{b}
T3	1.64	1.72	1.79	1.92	2.09	2.20	1.89^{b}
T4	1.32	1.36	1.45	1.48	1.60	1.72	1.49^{c}
T5	1.60	1.83	1.94	1.99	2.11	2.20	1.95^{b}
T6	1.70	1.79	1.82	1.95	2.13	2.25	1.94^{b}
T7	1.33	1.37	1.42	1.55	1.56	1.75	1.50^{c}
Mean	1.61^{f}	1.77^{e}	1.84^{d}	1.96^{c}	2.10^{b}	2.23^{a}	

T1 Control, T2 Chitosan 1%, T3 Rosemary 500 ppm, T4 Combination of chitosan 1% and rosemary 500 ppm, T5 Combination of Chitosan 1% + Enzyme 0.5%, T6 Combination of Rosemary 500 ppm + Enzyme 0.5% and T7 Combination of Chitosan 1%+ Rosemary 500 ppm + Enzyme 0.5%.

Means within the same column having different superscripts are significantly different at P ≤ 0.05 for treatments, while Means within the same row having different superscripts are significantly different for time of storage.

Enterobacteriaceae

Total Enterobacteriaceae counts in fish finger samples are shown in table 6. The Enterobacteriaceae count mean in fish finger samples ranged from 0.29 to 1.28 log cfu/g. A significant ($p \leq 0.05$) increase in Enterobacteriaceae counts was observed for control sample as compared to the treated samples. It is clear to notice that, treatments containing chitosan and rosemary (T4 and T7) showed the lowest count of Enterobacteriaceae due to their antimicrobial effects. Georgantelis., et al. [80] found that the chitosan and rosemary treatment had the lowest Enterobacteriaceae count in fresh pork sausages stored at 4 °C, indicating a possible synergistic effect. Enterobacteriaceae levels permitted by Center for Food Safety [93]. are < 102 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.

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 panellists evaluated colour of fish fingers meat not the external layer. As data indicated, the colour scores of the treated samples were slightly higher than the control sample over the storage period. Treatment containing chitosan and rosemary had the highest color scores as compared to other treatments and control. Also, there was a slight decrease in color scores when the storage time increased. Li., *et al.* [94]. demonstrated that colour loss in fish muscle is due to the oxidation of protein.

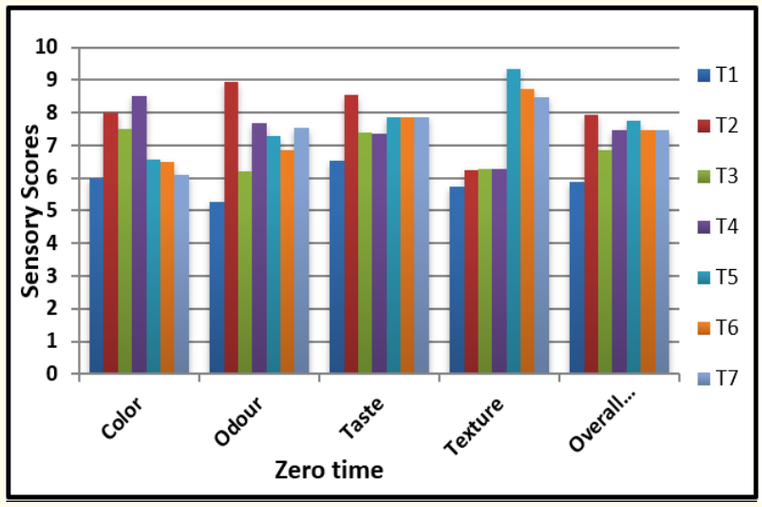
Odor is very important in sensory analysis of fish freshness. Chitosan treatment had the highest odour score while the control had the lowest odour score. Odour scores of fish finger samples were gradually decreased during the storage. Addition of antioxidants might have protected the prepared fish finger samples from rancidity development during the studied storage period.

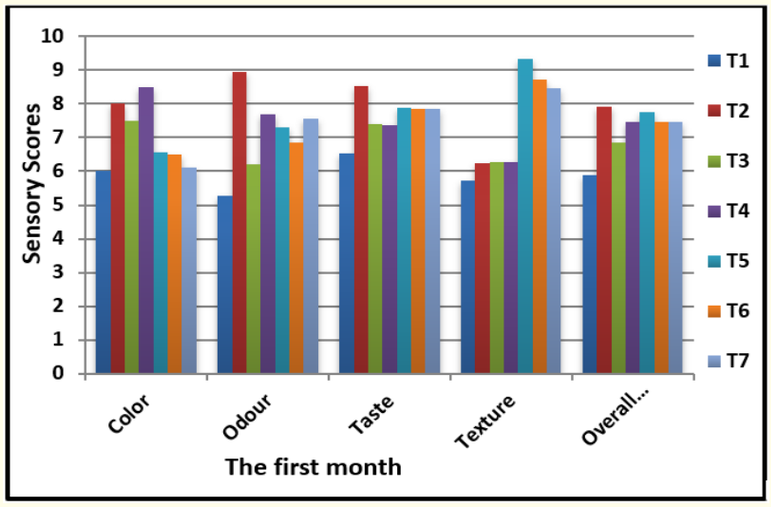
Table 6: *changes of Enterobacteriaceae count (log cfu/g) in treated fish fingers (T2-T7) during 5 months of storage at -18°C as compared to the control sample (T1).*

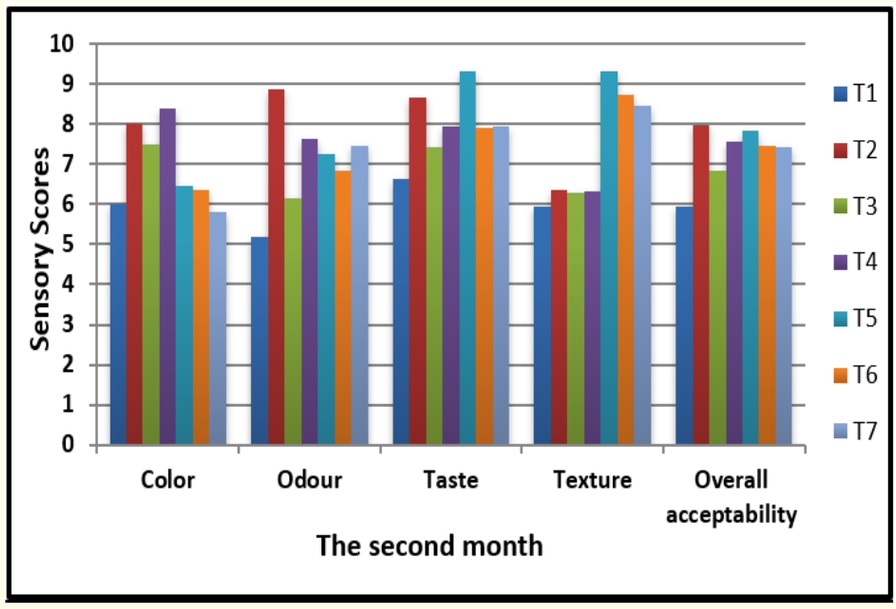
Treatments	Storage period (months)						Mean
	0	1	2	3	4	5	
T1	0.88	0.95	1.25	1.46	1.52	1.60	1.28^{a}
T2	0.35	0.38	0.43	0.45	0.52	0.53	0.44^{b}
T3	0.42	0.43	0.44	0.45	0.48	0.52	0.46^{b}
T4	0.24	0.25	0.28	0.32	0.34	0.37	0.30^{c}
T5	0.37	0.38	0.41	0.42	0.48	0.55	0.44^{b}
T6	0.40	0.42	0.44	0.50	0.52	0.54	0.47^{b}
T7	0.25	0.26	0.28	0.30	0.32	0.35	0.29^{c}
Mean	0.42^{d}	0.44^{d}	0.50^{c}	0.56^{bc}	0.60^{ab}	0.64^{a}	

T1 Control, T2 Chitosan 1%, T3 Rosemary 500 ppm, T4 Combination of chitosan 1% and rosemary 500 ppm, T5 Combination of Chitosan 1% + Enzyme 0.5%, T6 Combination of Rosemary 500 ppm + Enzyme 0.5% and T7 Combination of Chitosan 1%+ Rosemary 500 ppm + Enzyme 0.5%.

Means within the same column having different superscripts are significantly different at P ≤ 0.05 for treatments, while Means within the same row having different superscripts are significantly different for time of storage.






Sensory Scores
10
9
8
7
6
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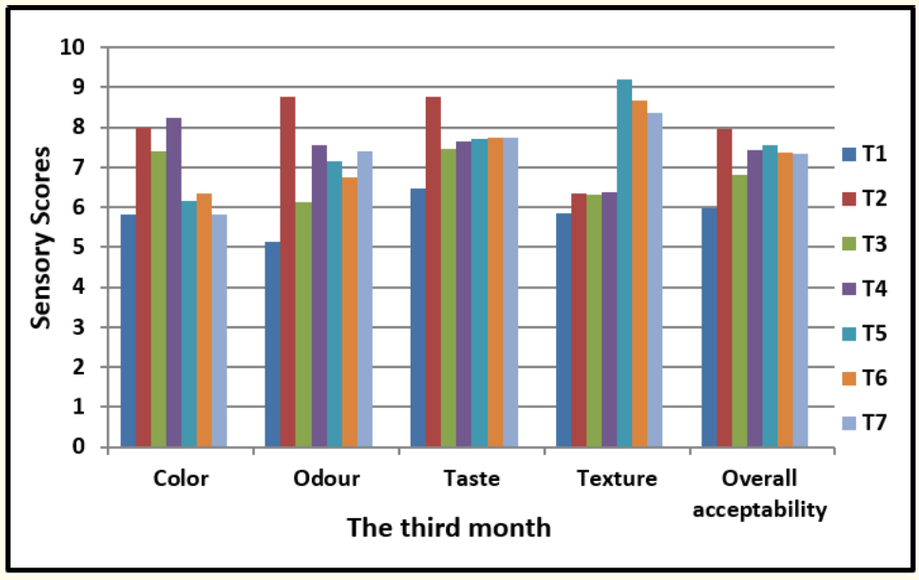
4
3
2
1
0
Color
Odour
Taste
Texture
Overall acceptability
T1
T2
T3
T4
T5
T6
T7
The second month


Sensory Scores
10
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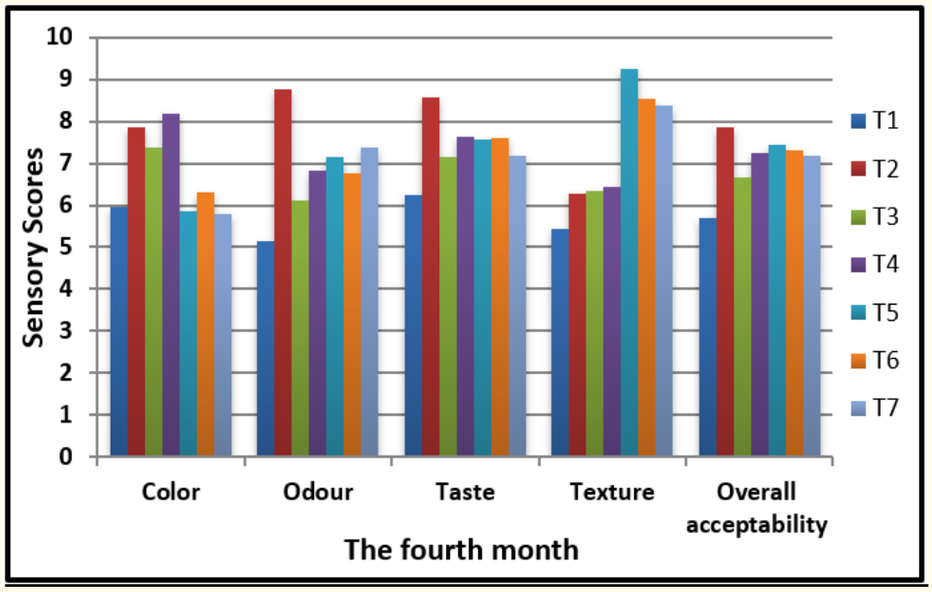
Color
Odour
Taste
Texture
Overall acceptability
T1
T2
T3
T4
T5
T6
T7
The third month


Sensory Scores
10
9
8
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6
5
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3
2
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0
Color
Odour
Taste
Texture
Overall acceptability
T1
T2
T3
T4
T5
T6
T7
The fourth month

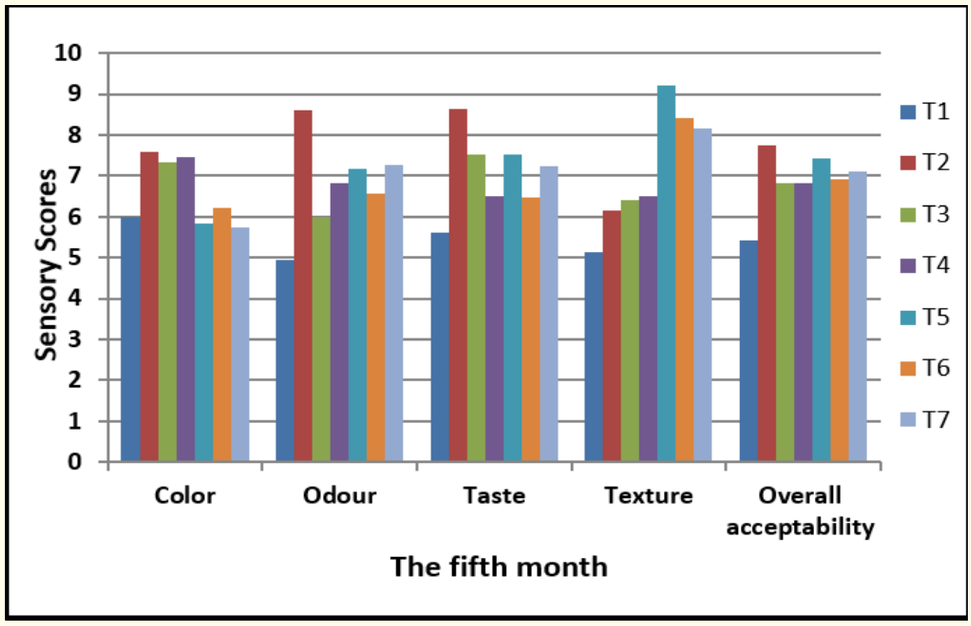

Figure 1: *Changes in sensory scores of fish finger samples during the frozen storage period at -18°C.*

Also, chitosan treatment had the highest taste score and the control had the lowest taste score. Results showed that there was a clear decrease in taste scores at the fourth and fifth months of storage.

Texture is an important attribute contributing to the acceptability of the consumer because correct texture reflects freshness and high quality [95]. Initially the best texture score was found in treatment containing chitosan and MTGase, due to the effect of chitosan and MTGase on improving texture, as Mohan., *et al.* [55]. stated that chitosan coating sardine fillets improved the textural properties significantly as compared to untreated ones. In the scope of these findings, Hajidoun and Jafarpour [96] reported that addition of 1% and 1.5% chitosan improved color, odor and texture of the surimi gel samples.

Chitosan treatment had the highest overall acceptability score as compared to other treatments as well as control. At zero time, the overall acceptability score of control was 5.91 and decreased to 5.41 at the end of storage. Chitosan treatment was 8.05 at the beginning of storage and reached to 7.74 at the end of storage. Generally, all treated fish finger samples had higher overall acceptability scores as compared to control. Starting from the third month, the overall acceptability scores showed a slight decrease. Tokur., *et al.* [75] reported few changes in the sensory scores of fish burger developed from tilapia (Oreochromis niloticus) at -18°C for 8 months storage. The sensory data of the prepared fish fingers showed that samples acceptability was decreased but still acceptable by panellists even at the end of storage period.

Conclusion

In conclusion, acceptable fish fingers can be produced from catfish by using some natural additives like chitosan and rosemary. Also, a combination of chitosan and rosemary treatment was more effective in improving the chemical, microbiological and sensory properties of the prepared fingers than other studied treatments. Moreover, addition of MT Gase enzyme, especially in combinations with other tested additives, resulted in improving textural and sensory properties of prepared fish fingers.

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