Effect of Chitosan Nanoparticles as Active Coating on Chemical Quality and Oil Uptake of Fish Fingers

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Abstract
The effect of different concentrations of chitosan and chitosan nanoparticles as active coating compared to commercial edible coating on chemical quality attributes of fish fingers during frozen storage at -18°C was investigated. Results illustrated that, uncoated fish fingers (T1) and that coated with commercial edible coating (T2) had significantly higher total volatile nitrogen (TVN), trimethylamine (TMAN), thiobarbituric acid (TBA) in comparison with fish fingers coated with either chitosan or chitosan nanoparticles. Moreover, T1 and T2 had a shelf life of 5 months, while, chitosan treatments had longer shelf life up to 6 months according to trimethylamine (TMAN) value which recorded by Egyptian standard. Also, data showed that, chitosan nanoparticles as active coating introduce the most effective improvement for quality attributes of fish fingers during frozen storage at -18°C. The influence of edible coating in reducing oil uptake during frying of fish fingers was investigated.

Key words: Chitosan, Nanoparticles, Chemical quality, Fish fingers, shelf life, Thixotropic effect, oil uptake

Introduction
In recent years, the increase of civilization or socio economic factors like the increasing numbers of working women of the population have led to direct consumer’s preference to ready-to-eat foods. These foods (cakes, crackers, burgers, fish fingers, marinated products, etc.) made from fish or other seafood are the products which are mostly preferred by consumers around the world and various studies on production and quality stability of these ready-to-eat foods have been done (Cakli et al. 2005). Edible films and coatings offer extra advantages such as edibility, biocompatibility, esthetic appearance, barrier to gasses properties, non-toxicity, non-polluting and its low cost. In addition, biofilms and coatings, by themselves or acting as carriers of foods additives (i.e.: antioxidants, antimicrobials), have been particularly considered in food preservation due to their ability to extend the shelf life. In this field chitosan and chitosan nanoparticles can used effectively (Entsar, et al. 2012)

Fish fingers produced from minced fish flesh as a battered and breaded product, are commonly stored and marketed in the frozen state.
However, fish and fishery products can undergo undesirable changes during frozen storage and deterioration may limit the storage time. These undesirable changes result from protein denaturation (Fijuwara et al. 1998) and Benjakul, et al. 2005) and lipid oxidation (Sarma et al., 2000 and Richards & Hultin 2002).

Time dependent flow behavior can be investigated as function of time throughout tests where both the degree of shear load and the measuring temperature are preset as constant values. Foods such as suspensions, emulsions and foams are time dependent fluids and show thixotropy and rheopexy behavior. Thixotropic behavior means the reduction in structural strength during the shear load phase and the more or less rapid, but complete structural regeneration during the subsequent rest phase (Mezger, 2002).

Frying is the cause of much fat absorption into food. There has been much activity to control fat uptake in food processing, based upon pre and post frying treatments, modifications of the frying method and edible barrier techniques.

Deep-Frying is one of the most widespread methods of food processing. Cereals and pulses are being extremely used for the manufacture of fried foods all over the world. Due to the consumer awareness, the trend has shifted to low fat fried foods. There are various approaches to reduce fat content of fried foods. (Asmita and Uday, 2013)

Fat absorption or moisture loss in foods can cause serious problems that can adversely affect the sensory and nutritive value of food. It can also critically affect product shelf-life. However, edible coatings may be used to reduce the fat absorption and moisture loss during deep frying. Physical properties like adhesion degree and cooking could cause an increase in the food volume, which can increase the mass of the product.

To obtain these properties, suitable coating materials, coating mix, and frying time are required. Also, appropriate coating materials can improve the sensory properties like colour, odour and taste (Chalupa &Sanderson, 1993; Nettler, 2006 and Nççeker & Küçüköner, 2007).

Bouchon (2009) described the essential features of oil absorption during deep fat frying. Frying is essentially a dehydration/absorption process. When the food is immersed in hot oil the high temperature of the frying oil causes the evaporation of water at the surface of the food. As water from the external layers escapes and moves into the surrounding oil a dehydrated crust is formed, the temperature of which then increases above the boiling point of water. The loss of water from the external crust layer leaves empty spaces into which oil can migrate. It has been shown however that during frying the vigorous escape of water vapour generates a barrier to oil migrating into the porous crust, limiting oil uptake during most of the immersion period.

The purpose of this work was to study the effect of different concentrations of chitosan and chitosan nanoparticles as coating material compared with commercial coating on chemical quality attributes and shelf-life of raw fish fingers during frozen storage and to use the abovementioned coating materials to reduce fat absorption during frying process.

Material and Methods

Materials

Fish Sample

Carp fish varying from 500 to 900 gm in weight, were purchased from the private sector shop in the local market at Giza, Egypt. Fish were transferred to the laboratory in an ice box within 30 min.
Other Ingredients

Food grade sodium tripolyphosphate (99.5% purity) was obtained from El-Gomhoria for chemicals Co., Egypt. Salt, sugar, wheat and corn flour, cumin, onion, garlic powder, black pepper, thyme, egg and skimmed milk were purchased from local market at Giza, Egypt.

Methods

Extraction of Chitosan

Chitosan was extracted from marine shrimp shells. The exoskeleton of the shrimp were crushed and treated in the usual way with HCl, NaOH 1-2 M then with 40% NaOH to extract the chitosan (Abdou et al. 2008). The degree of deacetylation (DDA%) of chitosan determined by potentiometric titration (Domard & Rinaudo, 1983), and the molecular weight was calculated using the value of intrinsic viscosity (Ravindra et al. 1998) measured by an Ubbelohde viscometer. The value of (DDA%) and molecular weight of chitosan were 85% and 3.98 × 104 gm/mol respectively.

Preparation of Chitosan Nanoparticles

Nanoparticles were produced based on ionic gelation of tripolyphosphate (TPP) and chitosan as described elsewhere (Calvo et al. 1997). Nanoparticles were spontaneously obtained upon the addition of 2%, 2.8% and 4% solutions of TPP aqueous basic solution to 2%, 2.8% and 4% of the chitosan acidic solution respectively (the ratio of TTP to chitosan was 1:1) under magnetic stirring at room temperature.

Scanning Electron Microscopy

The surface morphology of chitosan nanoparticles was investigated using Transmission Electron Microscope (TEM) polymer sample was suspended in acetone for 20 min. then, a drop of the suspension was placed on a grid and letting the solvent evaporate prior to imaging.

Thixotropic Effect of Edible Coating Solutions

Viscosity of chitosan and chitosan nanoparticles were measured at different time of shearing using Brookfield Engineering labs DV-III Ultra Rheometer. The samples were placed in a small sample adapter and a constant temperature water bath was used to maintain the desired temperature. The viscometer was operated at shear rate 9.3 s⁻¹ and different time of shearing 20-200 sec. Viscosity data were obtained directly from the instrument, the SC4-21 spindle was selected for the measurement.

Preparation of Fish Fingers

Fish were washed with chilled water (4°C), beheaded, gutted, washed again with chilled water, and then filleted. The fillets were minced with meat mincer using a 4.5 mm diameter holes plate. Carp fish fingers were prepared by the following recipe according to Long et al (1983) and US Department of Agriculture (USDA, 2001): 93.5% fish mince, 1.5 % salt, 1.0 % sugar, 3.0% wheat flour, 0.243% cumin, 0.243% onion, 0.243% garlic powder, 0.243% black pepper and 0.02 % thyme. Minced fish meat and other ingredients were mixed for 3 min by using laboratory mixer (Hobart Kneading machine, Italy).

The obtained mixture was spread in thin layer (1.5 cm) in stainless steel trays and formed to fingers using a kitchen knife (9.0 × 2.0 cm) then stored in freezer at -18°C for 24 hr. the frozen fish fingers were divided to eight different batches. As seen in (Table 1) every batch was immersed or dipped into the corresponding edible coating for about 2 min. All fish fingers treatments were packaged in a foam plates, wrapped with polyethylene film and stored at -18 °C for six months. Samples were taken for analysis every month periodically.
**Table 1. Fish fingers coated with different edible coating**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Coating composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Without coating</td>
</tr>
<tr>
<td>T2</td>
<td>Commercial edible coating*</td>
</tr>
<tr>
<td>T3</td>
<td>2% chitosan solution</td>
</tr>
<tr>
<td>T4</td>
<td>2.8% chitosan solution</td>
</tr>
<tr>
<td>T5</td>
<td>4% chitosan solution</td>
</tr>
<tr>
<td>T6</td>
<td>Chitosan nanoparticles solution (2% chitosan+2% TPP)</td>
</tr>
<tr>
<td>T7</td>
<td>Chitosan nanoparticles solution(2.8% chitosan+2.8% TPP)</td>
</tr>
<tr>
<td>T8</td>
<td>Chitosan nanoparticles solution(4% chitosan+4% TPP)</td>
</tr>
</tbody>
</table>

*Commercial edible coating was prepared by mixing the mixture which consist (94% corn flour and 2% egg yolk and 2% skimmed milk, 1.8 % salt and 0.2 % cumin) with water by 1:3 (w:w), respectively to obtain coating mixture.

**Chemical Analysis**

**Determination of pH**

pH value was estimated according to Goulas and Kontominas (2005) as follows. Ten gram of raw fish fingers sample was homogenized in 100 ml of distilled water and the mixture was filtered. The pH of filtrate was measured using a pH meter (Jenway, 3510, UK) at ambient temperature.

**Determination of Total Volatile Basic Nitrogen (TVB-N)**

Total Volatile Base Nitrogen (TVB-N) value was estimated by the semi-micro distillation procedure (AMC, 1979 and Kirk & Sawyer, 1991). The bases are steam distilled into standard acid and back-titration with standard alkali.

**Determination of Trimethylamine Nitrogen (TMAN)**

Trimethylamine nitrogen (TMAN) was determined using the above mentioned TVBN method after appropriate modification: formaldehyde was used to block the primary and secondary amines (AMC, 1979 and Malle & Tao, 1987).

**Determination of 2-Thiobarbituric Acid (TBA)**

Thiobarbituric acid (TBA) value of fish fingers samples was determined colorimetrically by using the method published by (Kirk and Sawyer 1991).

**Preparation of Fish Finger before Frying**

The prepared fish fingers were dipped in different coating medium for 2 min., the samples were removed and then blotted with filter paper to remove surface moisture, then coated with an equal amount of bread crumbs. Sunflower oil was used as a frying medium, a mini fryer with 1 L capacity was used for frying operation. The samples were immersed in the hot oil (140 ± 5°C) and fried for 4 min. Fried samples were removed from the unit and the excess surface oil absorbed with filter paper. Samples were then allowed to cool to room temperature for 5 min before oil content analysis was done.

The oil and moisture contents were determined using Soxhlet extraction method and oven drying method at 105°C until constant weight respectively according to the guidelines proposed by AOAC (1995).
Analysis of the Coating Material Performance
Yield parameters were determined by measuring the mass of the raw fish fingers (X), the mass of the coated fish fingers prior to frying (Y) and the mass of the coated fish fingers after frying (Z). Calculations of the yield parameters were as follows (Hutchison, et al. 1990)

\[
\text{Adhesion degree} = \frac{Y - X}{Y} \times 100
\]

(1)

\[
\text{Yield} = \frac{Z}{X} \times 100
\]

(2)

\[
\text{Frying loss} = \frac{Y - Z}{Y} \times 100
\]

(3)

Statistical Analysis
Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for difference between means (significance was defined at (p < 0.05) as reported by (Snedecor and Cochran 1994).

Results and Discussion
Scanning Electron Microscopy
Three different concentrations with the same ratio (1:1) of chitosan/TPP are used. Transmittance electron microscope was used for the determination of the particle size and the morphological structure of the prepared polymer matrix. It was found that chitosan/TPP (T8) has average particle size of 10 nm. (Fig. 1) shows the scanning electron microscopy of chitosan nanoparticles.

Fig. 1. Scanning electron microscopy of chitosan nanoparticles (T8)
Thixotropic Effect of Chitosan and Chitosan Nanoparticles

Time dependency of the chitosan and chitosan nanoparticles was evaluated by determining the change in apparent viscosity under constant shear rate of 9.3 s\(^{-1}\) for 180 s (Figs. 2 and 3).

All samples were found to have thixotropic behavior since viscosity of chitosan and chitosan nanoparticles decreased with increased mixing time. Chitosan solution has higher viscosity than chitosan nanoparticles. The lower apparent viscosity of chitosan nanoparticles than chitosan solution may be due to TPP-cross linked chitosan molecules turned into more dense particles whose hydrodynamic volumes were smaller than pure chitosan chains. (Li, 2012)
Chemical Evaluation

pH value

pH changes can be used as a spoilage indicator in fishery products. Changes in pH values of different fish fingers treatments during frozen storage at -18°C are presented in Table (2). The initial pH values of all fish fingers treatments ranged from 6.29 to 6.64. The pH of uncoated fish fingers treatment (T1) slightly or not significantly higher than fish fingers coated with commercial edible coating (T2) but significantly (p<0.05) higher than other coated fish fingers treatments which coated with different concentrations of chitosan (T3, T4 and T5) and chitosan nanoparticles (T6, T7 and T8).

Moreover, fish fingers treatments which coated with different concentrations of chitosan nanoparticles had slightly higher pH value when compared with that coated with chitosan, this may be due to the basic characteristics of sodium tripolyphosphate which used in preparation of chitosan nanoparticles with different concentrations. Also, the reduction in pH values for all fish fingers treatments coated with chitosan or chitosan nanoparticles may be caused by the acidic coatings formed on the surface of fish fingers. The lowest reduction in pH values was recorded for T8 (chitosan nanoparticles which prepared with chitosan/sodium tripolyphosphate by 1:1).

On the other hand the higher reduction in pH values was recorded for T5 (6.29) which prepared with the highest concentration of chitosan immediately after processing. Generally, the pH value of uncoated fish fingers treatment (T1) was higher than coated ones during frozen storage.

It is obvious that, with advancement of frozen storage time, the pH values of chitosan treatments (T3, T4 and T4) and chitosan nanoparticles treatments (T6, T9 and T8) were decreased. This reduction in pH value of the fish fingers was probably resulted from the protein breakdown and the release of phosphoric and lactic acids occurred during freezing and thawing processes (Singh and Balange, 2005 and Duan et al., 2010). Moreover, the pH values of uncoated fish fingers (T1) and that coated with commercial coating (T2) were also decreased with increasing frozen storage time up to 4 months and then slightly increased at the fifth and sixth month of storage.

The increase of pH value was postulated to be due to an increase in volatile bases produced e.g. ammonia and trimethylamine by either endogenous or microbial enzymes (Manat et al., 2005 and Fan et al., 2009). The highest decrement of pH value was recorded for T5 which dropped from 6.29 at zero time to 5.49 at the end of frozen storage. On the contrary, the lowest decrement of pH value was recorded for T8 which dropped from 6.53 at zero time to 6.09 at the end of frozen storage.
Table 2. Changes in pH values of different raw fish fingers treatments as affected by coating type during frozen storage at -18°C up to six month.

<table>
<thead>
<tr>
<th>Storage period (months)</th>
<th>Raw fish fingers coated treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>zero time</td>
<td>6.64a</td>
</tr>
<tr>
<td>1</td>
<td>6.50a</td>
</tr>
<tr>
<td>2</td>
<td>6.46a</td>
</tr>
<tr>
<td>3</td>
<td>6.32a</td>
</tr>
<tr>
<td>4</td>
<td>6.27a</td>
</tr>
<tr>
<td>5</td>
<td>6.37a</td>
</tr>
<tr>
<td>6</td>
<td>6.41a</td>
</tr>
</tbody>
</table>

Where: Mean values in the same row with the same letter are not significantly different at 0.05 level.

Total Volatile Bases Nitrogen (TVBN)

Total volatile base nitrogen (TVBN) formed in fish and fishery products could be used as an indication of decomposition which occurs by bacteria and protein breakdown during storage. Changes in TVBN values of different fish fingers treatments as affected by coating materials during frozen storage are shown in Table (2). The initial total volatile base nitrogen of different fish fingers treatments was 13.72mg/100gm, this value indicative of freshness of fish fingers treatments. Also, from the same table, it could be noticed that the total volatile base nitrogen (TVBN) increased progressively with time of frozen storage for all fish fingers treatments either with or without coating.

The increase of (TVBN) during storage may be attributed to the growth of spoilage bacteria (Connell, 1990). This increase was significantly lower (p<0.05) in the fish fingers coated with both chitosan (T3, T4 and T5) and chitosan nanoparticles (T6, T7 and T8) than in the other treatments (uncoated or coated with commercial edible coating). This could be due to the protective chitosan coating which inhibited the bacterial growth and slowed spoilage. Higher microbial counts which breakdown compounds like trimethylamine oxide (TMAO), peptides, amino acids…. etc (Gram and Huss, 1996 and Mohan et al. 2012), resulted in an increase in the basic nitrogen fraction for uncoated and coated with commercial edible coating samples compared to chitosan coated samples.

Table 3: Changes in total volatile nitrogen (mg N/100g) of different raw fish fingers treatments as affected by coating type during frozen storage at -18°C up to six months.

<table>
<thead>
<tr>
<th>Storage period (months)</th>
<th>Raw fish fingers coated treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Zero time</td>
<td>13.72a</td>
</tr>
<tr>
<td>2</td>
<td>18.12a</td>
</tr>
<tr>
<td>3</td>
<td>21.30a</td>
</tr>
<tr>
<td>4</td>
<td>25.81a</td>
</tr>
<tr>
<td>5</td>
<td>29.16a</td>
</tr>
<tr>
<td>6</td>
<td>34.97a</td>
</tr>
</tbody>
</table>

Where: Mean values in the same row with the same letter are not significantly different at 0.05 levels.
The data also showed that the increase of TVBN values during frozen storage was lower in fish fingers coated with different concentrations of nanoparticles than in fish fingers coated with different concentrations of chitosan. This reflects the impact of higher chitosan nanoparticles as antimicrobial. This may be due to the antimicrobial effect of chitosan was higher at the end of 12 day storage period.

**Trimethylamine Nitrogen (TMAN)**

Trimethylamine nitrogen formed in fish fingers is an index of spoilage. Changes in trimethylamine values are presented in (Table 4). The initial TMAN value of different fish fingers treatments was 2.13 mg N₂/100gm. This value was lower than that reported by Manhan et al., (2012) found that the initial TMAN was 6.01 mg N₂/100g but slightly higher than that reported by (Cakli et al. 2005) mentioned that (TMAN) of whiting fish fingers immediately after processing was 1.76 mg/100gm.

Statistical analysis of these data showed that there were significant differences (p<0.05) in (TMAN) value among fish fingers treatment during frozen storage period. Also, at any time of frozen storage, uncoated fish fingers treatment (T1) had significantly higher TMAN than other fish fingers treatments.

**Table. 4. Changes in trimethylamine (mg N₂/100g) of different raw fish fingers treatments as affected by coating type during frozen storage at – 18°C up to six months**

<table>
<thead>
<tr>
<th>Storage period (months)</th>
<th>Raw fish fingers coated treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>zero time</td>
<td>2.13</td>
</tr>
<tr>
<td>1</td>
<td>4.04</td>
</tr>
<tr>
<td>2</td>
<td>4.78</td>
</tr>
<tr>
<td>3</td>
<td>5.21</td>
</tr>
<tr>
<td>4</td>
<td>8.97</td>
</tr>
<tr>
<td>5</td>
<td>11.07</td>
</tr>
<tr>
<td>6</td>
<td>13.87</td>
</tr>
</tbody>
</table>

Where: Mean values in the same row with the same letter are not significantly different at 0.05 level.
Also, from the recorded data, it could be observed that the trimethylamin nitrogen (TMAN) took the same trend of TVN (Table 2). The TMAN values of all fish fingers treatments were gradually increased with increasing storage period. Uncoated fish fingers treatment (T1) and that coated with commercial edible coating (T2) exhibited a significantly (p<0.05) higher increase reaching a (TMAN) value of 13.37 and 12.69 mg/100gm respectively at the end of frozen storage. On the other hand, fish fingers treatments which coated with chitosan nanoparticles coating especially T8 had the lowest TMAN increment being 4.56 mg/100gm at the end of frozen storage followed by T7 (5.24 mg/100gm) with significant differences (p<0.05) between them. The reduction percentages of TMAN at the end of frozen storage were 49.46, 49.96, 57.89, 60.99, 62.22 and 67.12% in T3, T4, T5, T6, T7 and T8 respectively as compared with uncoated fish fingers (T1).

Finally uncoated fish fingers (T1) and that coated with commercial edible coating (T2) were unsafe for human consumption after five months as their TMAN values reached 11.07 and 10.71 mg/100gm respectively. It must be mentioned that the Egyptian standard (2005) rejected frozen fish fingers which had more than 10 mg N₂/100gm sample (TMAN) value.

**Thiobarbituric Acid (TBA)**

The thiobarbituric acid (TBA) test is used as an index for measuring oxidative rancidity (malonaldehyde formation) which place in fisher products changes in (TBA) values of different fish fingers treatments during frozen storage are shown in (Table 5). From statistical analysis of these data, it could be observed that, there were significant differences (p<0.05) in TBA values between different fish fingers treatments along storage periods.

The initial TBA value of all fish fingers treatments was ranged between 0.64 and 0.468 mg malonaldehyde/kg with non-significant differences between them.

During frozen storage, TBA values progressively increased as the period of frozen storage increased for all fish fingers treatments. This increment could be an indicator for continuous oxidation of lipids and consequently the production of oxidation by products. Uncoated fish fingers treatment T1 and that coated with commercial edible coating T2 showed a significantly (p<0.05) higher increase reaching a TBA value of 3.714 and 3.411 mg malonaldehyde/kg respectively at the end of frozen storage (after 6 months). On the other hand, this increment was significantly lower (p<0.05) in the fish fingers coated with chitosan nanoparticles especially T8 (0.918 mg/kg at the end of frozen storage) than other fish fingers treatments. This observation was indicated that chitosan clearly inhibited lipid oxidation.

Similar results were found by (Sathivel, 2005) reported that chitosan coatings reduced the lipid oxidation in pink salmon fillets during frozen storage. In addition chitosan may reduce lipid oxidation by chelating ferrous ions present in fish proteins, thus eliminating their prooxidant actively or their conversion to ferric ion (Kamil et al. 2002). Moreover, Weist and Karel (1992) reported that the antioxidant mechanism of chitosan could be explained as the primary amino groups of chitosan would form a stable fluorosphere with volatile aldehydes such as malondialdehyde which is derived from breakdown of fats during the oxidation.

Fish fingers coated with commercial edible coating (T2) had slightly lower thiobarbituric acid value than uncoated fish fingers (T1) at any time of frozen storage except at sixth month the differences between them was significantly.
Also, thiobarbituric acid values of all fish fingers treatments at any time of frozen storage were lower than those mentioned by the Egyptian standard (2005) which reported that TBA value in frozen fish fingers is not be exceed than 4.5 mg malonaldehyde/kg sample.

Table 5. Changes in thiobarbituric acid (mg malonaldehyde/kg) of different raw fish fingers treatments as affected by coating type during frozen storage at -18°C up to six months

<table>
<thead>
<tr>
<th>Storage period (months)</th>
<th>Raw fish fingers coated treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>zero time</td>
<td>0.468&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.673&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>0.884&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.427&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>2.081&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5</td>
<td>3.107&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>3.714&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Where: Mean values in the same row with the same letter are not significantly different at 0.05 levels.

Also, from the same table, it could be noticed that, TBA values in different fish fingers treatments were decreased with increasing chitosan or chitosan nanoparticles concentration. This may be attributed to highest viscosity values which probably due to the presence of a large number of ionic functional groups, which create strong polymers interactions that restrict the chain motion in high-viscosity chitosan, resulting in good oxygen barrier properties (Jeon et al. 2002).

Coating material performance
According to present study, the edible films form a protective layer on the surface of the fish fingers. This protective layer inhibits the transfer of moisture and fat between the sample and the frying medium. Adhesion degrees and yields were found to be high in coated fish fingers compared to the control samples. The highest adhesion (46%) and yield (120.59%) were recorded for fish fingers coated with 4% chitosan (T5) followed by 4% chitosan nanoparticles (T8) which were 38.10 and 115.53% respectively, as previously discussed by (Osman, 2011) who found that higher yield in Chicken nuggets was 117%. Frying loss was low in fish fingers coated with chitosan nanoparticles compared to the control. The lowest frying loss value seen in the fish fingers coated with 4% chitosan nanoparticles (13.63%) as shown in Table (6). The edible film coated samples retained more moisture in the surface layer than control sample, the moisture content was increased from 34.61% to 52.7% in coating with chitosan nanoparticles (T8).

The surface layer coated sample absorbed less oil compared to control sample. The results show that fat rate of T1 and T2 fish fingers was 16.42 and 13.9% respectively after frying and decreased in samples coated with chitosan and chitosan nanoparticles, the lowest percent of fat were found in samples coated with 4% chitosan nanoparticles (T8) This may be attributed to the fact that coating material acts as oil barrier which causes the oil to diffuse in a counter direction (from inside to outside of the food) This is in agreement with (Diaz, et al. 1999 and Salvador, et al. 2008).
Table 6. The effect of coating materials on the values of adhesion degree, yield, frying loss, moisture and fat percent

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield %</th>
<th>Frying loss %</th>
<th>Adhesion %</th>
<th>Fat %</th>
<th>Moisture %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>65.98</td>
<td>36.95</td>
<td>6.761</td>
<td>16.42</td>
<td>34.61</td>
</tr>
<tr>
<td>T2</td>
<td>72.57</td>
<td>29.15</td>
<td>25.32</td>
<td>13.9</td>
<td>40.43</td>
</tr>
<tr>
<td>T3</td>
<td>77.61</td>
<td>33.28</td>
<td>28.04</td>
<td>13.69</td>
<td>42.27</td>
</tr>
<tr>
<td>T4</td>
<td>88.44</td>
<td>30.74</td>
<td>32.23</td>
<td>12.62</td>
<td>47.00</td>
</tr>
<tr>
<td>T5</td>
<td>120.59</td>
<td>25.67</td>
<td>46.96</td>
<td>11.95</td>
<td>49.78</td>
</tr>
<tr>
<td>T6</td>
<td>91.19</td>
<td>22.25</td>
<td>23.16</td>
<td>6.88</td>
<td>46.82</td>
</tr>
<tr>
<td>T7</td>
<td>111.96</td>
<td>21.05</td>
<td>29.18</td>
<td>6.72</td>
<td>49.00</td>
</tr>
<tr>
<td>T8</td>
<td>115.53</td>
<td>13.63</td>
<td>38.10</td>
<td>4.56</td>
<td>52.70</td>
</tr>
</tbody>
</table>

T1: Fish fingers coated with bread crumbs only (control sample).
T2: Coated with commercial coating + bread crumbs
T3: Coated with 2% chitosan+ bread crumbs.
T4: Coated with 2.8 % chitosan+ bread crumbs.
T5: Coated with 4% chitosan+ bread crumbs.
T6: Coated with (2% chitosan+ 2% TPP) + bread crumbs.
T7: Coated with (2.8% chitosan+ 2.8% TPP) + bread crumbs.
T8: Coated with (4% chitosan+ 4% TPP) + bread crumbs.

Conclusion

Chitosan and chitosan nanoparticles solutions were found to have thixotropic behavior since apparent viscosity of chitosan and chitosan nanoparticles decreased with increased mixing time. The effect of different concentrations of chitosan and chitosan nanoparticles as active coating compared to commercials edible coating on chemical quality attributes of fish fingers during frozen storage at -18°C was investigated. The results indicate that chitosan treatments had longer shelf life up to 6 months according to trimethylamine (TMAN) value which recorded by Egyptian standard.

Also, data showed that, chitosan nanoparticles as active coating introduce the most effective improvement for quality attributes of fish fingers during frozen storage at -18°C. Coating with chitosan and chitosan nanoparticles reduced oil uptake from 16.42 to 4.56% for fish fingers coated with chitosan nanoparticles. Also coating fish fingers with chitosan nanoparticles increased moisture content from 34.61% to 52.7%.
References


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Shell fish processing industry is very common in coastal areas. While processing, only the meat is taken, the head and shells are discarded as waste. On an.. Abdou ES, Osheba AS, Sorour MA (2012) Effect of chitosan and chitosan-nanoparticles as active coating on microbiological characteristics of fish fingers. Int J Appl Sci Technol 2(7):158-169. Google Scholar. Agbodjato NA, Noumavo PA, Adjanohoun A, Agbessi L, Baba-Moussa L (2016) Synergistic effects of plant growth promoting rhizobacteria and chitosan on in vitro seeds germination, greenhouse growth, and nutrient uptake of maize (zea mays l.). Biotechnol Res Int 2016:1-11. Article Google Scholar. "Effect of Chitosan and Chitosan-Nanoparticles as Active Coating on Microbiological Characteristics of Fish Fingersâ€ International Journal of Applied Science and Technology 2(7), 158-169. [7]. Franco, Luciana de Oliveira; Stamford, Thayza Christina Montenegro; Stamford, Newton Pereira and Campos-Takaki, Galba Maria de.Â (2010) Effects of chitosan-based coating and modified atmosphere packaging (MAP). on browning and shelf life of fresh-cut lotus root (Nelumbo nucifera Gaerth). Innov Food Sci Emerg 11: 684-689. Effect of Chitosan and Chitosan-Nanoparticles as Active Coating on Microbiological Characteristics of Fish Fingers. Entsar S. Abdou Food Eng. and Packaging Dept., Food Tech. Research Institute.Â Nevertheless, frozen storage does not completely inhibit microbial and chemical reactions that lead to quality deterioration of fish (Reddy & Srikar, 1996). Moreover, fish and its products such as fish burgers, fish fingers, fish balls, frankfurters and sausages can undergo undesirable changes during frozen storage that lead to deterioration which may limit their storage time. Silver nanoparticlesâ€“chitosan composite spheres were formed in 15 minutes. The distance between solidifying liquid surface and the tip was one centimeter.Â by chemical reduction with some natural reductant (such as chitosan, dextran, sodium citrate, ascorbate and other reducing biomolecules like polypeptides, flavones, and alkaloids).59â€”61 A number of literatures have reported that silver nanoparticles incorporated with chitosan-based matrix present a broad spectrum of antimicrobial activity to inhibit both Gram-positive and Gram-negative bacteria including multidrug-resistant pathogens such as P. aeruginosa.Â Long time effect on the stability of silver nanoparticles in aqueous medium: Effect of the synthesis and storage conditions. of active ingredients, stabilization of biological molecules hydrogen bonding by parallel chains. Â 1-Chitin is a combi- like proteins, peptides or genetic material (Ghormade et al. nation of Ï± and Ï³ chitin and has parallel and antiparallel 2011). structure (Yen and Mau 2007) (Franca et al. 2008). Chitosan, Chitosan is a modified biopolymer, derived by partial obtained by deacetylation of chitin, has Ï³ 1-4 linked A unit deacetylation of chitin.Â 1996). The potential use of chitosan nanoparticles as carriers has paved way for development of wide variety of colloidal delivery vehicles (Malmiri et al.Â Even though, activated carbon can be used for adsorb- chemicals led to chitosan and chitosan nanoparticles.Â It was incor- Chitosan has a significant effect on the growth and porated into antimicrobial