Effect of chitosan and hydroxypropyl starch complex polysaccharide on the physico-chemical properties of Tilapia fish (Oreochromis mossambicus) gel

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Abstract
The compound polysaccharide composed of chitosan and hydroxypropyl starch was aimed in this study to be used as a novel treatment to improve the quality of Tilapia fish surimi. Surimi gel properties, retention of oil, rate of water loss, colour changes, changes in protein content and gas composition were analysed. Compared with the control group, with the increase of hydroxypropyl starch content, the rate of water loss in all the groups (chitosan and hydroxypropyl starch) was improved, and 1.5% chitosan-treated samples showed significantly lower water losses. The protein content tended to increase at the beginning and then decrease. The whiteness index of surimi in both groups was significantly improved. The addition of 1.0% hydroxypropyl starch showed the highest oil retention in all treated groups (1.0% chitosan treated sample showed oil retention of 0.77 mL/g, while 1.5% chitosan treated samples showed maximum oil retention of 0.7 mL/g). Gas chromatography-mass spectroscopy (GC-MS) analysis detected two identical volatile organic compounds (VOCs) in both the control and compound polysaccharide groups. These results indicate that using complex polysaccharides can significantly improve the quality of fish surimi and, therefore, can be used as a practical basis for industries to improve freshwater fish surimi’s gel properties.

1. Introduction
Tilapia fish originated in Africa and is the main aquaculture fish in China (Yuan et al., 2017). The cultivation of Tilapia fish is developing rapidly in China’s fish culture industry, and its growth rate is increasing. Tilapia is considered one of the most advantageous fish species in China as well as in the world (Fitzsimmons et al., 2011). The fish are mainly cultured in ponds, most of which are situated in Guangxi, Guangdong, Hainan, and other subtropical climate areas in China due to their growing nature. The lifespan of this fish is similar to that of crucian carp (El-Sayed, 2019); it has the characteristics of rapid growth, strong reproductive capacity, and a wide range of food habits. Moreover, this type of fish possesses improved resistance to various diseases (Lowe et al., 2012). Tilapia fish flesh is compact and delicious, with few bones (Costa et al., 2019). It possesses a high content of protein, nutrients, and a wide variety of polyunsaturated fatty acids, which meet people’s demand for protein (Webster and Lim, 2006). Therefore, Tilapia is being treated “alternative protein source” in Japan. Hence, the choice of Tilapia fish to produce surimi and surimi products can meet the various nutritional needs of consumers (Oliveira et al., 2017).

China has a large population and a large demand for meat products. Surimi products are one of the popular items. Surimi is a new type of aquatic meat product that started in the last 50 years of the 20th century (Park, Nozaki, Suzuki et al., 2013). Surimi product is made from fresh or frozen fish through a series of procedures such as rinsing, filtering, formulation, chopping, mixing, and moulding (Park, Graves, Draves et al., 2013). However, in China, sea fish is generally used as a high-quality raw material for surimi production. For the development and utilisation of freshwater fish surimi products, researches need to be carried out to establish production and processing models. Therefore, it can effectively improve the resource utilisation of Tilapia fish-based products, which is of a certain value to improve the country’s foreign trade and economic
development. It is undeniable that when making surimi products, compared with marine fish, the gel structure of freshwater fish is often worse (Rohani et al., 1995; Zhou et al., 2008). Gel characteristics which are an important quality of surimi, degrade easily during processing. Therefore, improving the gel properties of the fish surimi is very critical and important, which puts some challenges for the processors. In general, a single polysaccharide has been added to surimi products to improve the gel properties of surimi. Several researchers used two or more combinations of polysaccharides to improve the gel characteristics of surimi products (Alipour et al., 2018; Gao et al., 2019).

Chitosan, also known as deacetylated chitin, is not only a natural but also the only basic amino polysaccharide (Periayah et al., 2016). Since chitosan is a biological preservative of animal origin, it only plays a role in the preservation of fruits and vegetables (Duan et al., 2019). It is also a thickener and preservative that can be added to aquatic food (Shirvan et al., 2019). Moreover, chitosan has a wide range of sources, a simple production process, easy film-forming, is safe and natural, as well as biodegradable (Srinivasa and Tharanathan, 2007). It has friendly preservatives and bacteriostatic effects on medicine, cosmetics, and food and also has a high ability to adsorb protein (Morin-Crini et al., 2019). Therefore, chitosan can enhance gel properties in surimi products. According to Wu and Mao (2009), adding chitosan to a fish cake made of grass carp can fully inhibit the increase of the total number of microorganisms, pH value, and TVB-N value of the fish cake under specific packaging conditions. There are not many pieces of research on hydroxypropyl starch used in surimi products of freshwater fish to study its gel properties and quality. The developing market of Tilapia fish is growing, and the effect of adding auxiliary starch alone is limited. However, there are few studies on the mixed addition of two different auxiliary materials. Therefore, under the background of previous studies, this paper aims to combine chitosan and hydroxypropyl starch in different amounts to form composite polysaccharides for Tilapia fish surimi. The changes in protein, water loss, colour (whiteness), oil retention, and gel properties such as elasticity, viscosity, recovery, and hardness were studied to evaluate the gel quality and quality of the fish surimi. The purpose of this study was to use complex polysaccharides as a novel treatment to improve the gel structure and make the basis for freshwater surimi product development.

2. Materials and methods
2.1 Materials
Tilapia fish of 2 kg were collected from the local Yulin RT-Mart supermarket in China. From the same supermarket, Da Xiang glutinous rice was collected. Food-grade water-soluble chitosan was supplied by Guangdong Huasheng Food Co., Ltd, Guangdong, China. In addition, food-grade 99% hydroxypropyl starch was supplied by Henan Hengrui STarch Technology Co., Ltd, Henan, China. Salt from Guangxi Salt Group Co., Ltd, Guanxi, China, was used in the experiment.

Folin phenol reagent A, Folin phenol reagent B, and Bovine serum albumin standard solution were collected from Sigma Aldrich. N-hexane was supplied by Guangdong Guanhua Technology Co., Ltd, Shanghai, China.

2.2 Methods
The raw materials were processed, as shown by Gao et al. (2021), with minor modifications. Fishes were beheaded, and tail, viscera, and scales were removed. Then the fish samples were washed with cold tap water until complete removal of blood and other impurities. After that, the fish skins were removed as well as bones and spines, were removed.

Fish sample (50 g) was soaked and cleaned with refrigerated 4°C ice water (fish: ice = 1:5) for 15 mins, stirred for 30 s from time to time in the process of soaking; Fish samples were rinsed three times, and 2% fish quality salt was added in the last rinse. Coarse filtration was carried out with a three-layer filter cloth. The fish were then cut into small pieces and minced for 3-5 mins with a meat grinder (SD-LL07, Foshan Shunde Sandi Electric Appliance Manufacturing Co., Ltd, Foshan, China). With the minced fish, two kinds of compound polysaccharides, 2% salt and 20% ice water, were mixed for 30 s. During the processing, the temperature was kept between 4 and 10°C.
A total of two groups of polysaccharides were prepared, one of which consists of 1.0% chitosan with 1.0%, 1.5%, 2.0%, 2.5% and 3.0% hydroxypropyl starch and the other consists of 1.5% chitosan with 1.0%, 1.5%, 2.0%, 2.5%, and 3.0% hydroxypropyl starch. The amount of polysaccharides was all based on the weight of surimi, and the surimi products without adding compound Polysaccharides were compared and evaluated.

2.3 Gel formation

A traditional two-stage heating method was adopted; firstly, heating in the water bath at 45°C constant temperature for 30 mins to initiate gelatinisation and heating for 20 mins in a constant temperature water bath at 90°C. The gel was then cooled to room temperature, cooled at 4°C then, placed in the refrigerator and kept for 20-24 hrs.

2.4 Determination of moisture loss

Surimi sample of 3±0.03 g was weighed, wrapped with filter paper and placed into a 15 mL centrifugal tube. The samples were centrifuged at 4°C and 3000 rpm using a high-speed refrigerated centrifuge for 10 mins. After that, the surimi samples were weighted. Moisture loss was calculated using the method shown by Mao and Wu (2007).

\[
\text{Rate of water loss (\%) } = \frac{m_1 - m_2}{m_1} \times 100\%
\]

Where \( m_1 \) represents the initial weight in g, \( m_2 \) is the weight after centrifugation in g.

2.5 Measurement of colour

The surimi samples were cut into 2×2×2 cm cubes, and colour properties were determined as shown by Islam et al. (2014) using a jz-300 universal colourimeter. Each sample was determined three times, and then the average values of \( L^* \), \( a^* \), and \( b^* \) were calculated. \( L^* \) represents the brightness of the sample, 0-100 represents black to white. \( a^* \) represents the redness to the greenness of the sample; a positive value indicates redness, a negative value indicates greenness. \( b^* \) represents the yellowness to the blueness of the object; a positive value indicates yellowness while a negative value indicates blueness. The whiteness index was calculated using the following formula

\[
W = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}
\]

2.6 Determination of oil retention

The oil retention capacity of the surimi samples was measured according to the method proposed by Zhou et al. (2021) with minor modifications. Approximately 1 g surimi sample and 5 mL peanut oil were stirred in a centrifugal tube. After well stirring, the tube was placed in a centrifugal machine and centrifuged at 4°C and 3500 rpm for 15 mins. After centrifugation, the volume of free oil was measured. Each experiment was carried out thrice.

\[
\text{OR} = \frac{5 - V}{m}
\]

Where OR is the oil retention capacity in mL/g, \( V \) is the volume of free oil in mL, and \( m \) is the sample mass in g.

2.7 Determination of textural quality

The fish samples were cut into 1.5×1.5×1.5 cm small cubes. The hardness, cohesiveness, elasticity, resilience, stickiness, and chewiness of the gelative gel were measured by the TMS-PRO texture analyser (FTC company, USA). The parameters of texture measurements were cylindrical probe, test speed of 60 mm/min, initial force 0.1 N, and deformation of 25%. Each sample was measured thrice, and the average value was calculated.

2.8 Determination of protein

Protein content was determined using Folin phenol colourimetry, as shown by Xu et al. (2021). Six consecutive concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0) of bovine serum albumin standard solution were taken, and the absorbance value at the wavelength of 750 nm was noted. With the absorbance values, a standard curve was prepared.

About 100 g of surimi were weighed and then smashed with a mortar. The samples were diluted 1000 times with water. The diluted sample was then filtrated and kept for 10 mins to settle down. An aliquot of 1 mL sample was taken and mixed with 3 mL Folin phenol reagent A, at 25°C for 10 mins. After that, 0.3 mL reagent B was added and kept for 30 mins at 25°C. The absorbance was measured at 750 nm with an Ultraviolet-visible spectrophotometer UV-5200 (Shanghai Yuanxi Instrument Co., Ltd.).

2.9 Determination of volatile compounds

Volatile organic compounds were determined using the method shown by Wang et al. (2019), with little modification. A 1 g sample was taken in a 15 mL centrifugal tube, and 9 mL of n-hexane was added to it. The tube was subjected to sonication for 40 mins and kept for 10 hrs. After that, the tube was centrifuged at 6000 rpm for 5 mins. Then the aliquot was filtrated with a 0.22 \( \mu \)M organic needle filter, and 1 mL of aliquot was taken for analysis.

The gas chromatography-mass spectrometry (GC-
MS) conditions were as follows: the temperature of the sample inlet was set at 260°C, the column temperature was set at 49°C, and then the temperature was kept stable for 3 mins after that temperature was increased up to 150°C at 3°C/min, and further increased to 260°C at 6°C/min. The carrier gas used was helium, the temperature of the crate controller was kept at 270°C, and the temperature of the quadrupole was kept at 150°C. The ion source temperature of the electron ionisation system was 231°C, and 34.9 µA filament was used. The chromatogram was analysed using the NTST14.L library.

2.10 Statistical analysis

Analysis of variance (ANOVA) was applied to the data, and multiple comparisons were carried out using Duncan’s multiple range tests (DMRT) with SPSS software (SPSS 20.0, IBM, Chicago, IL, USA). All diagrams were drawn using Origin 8.0 software (OriginLab Corporation, Roundhouse Plaza, Suite 303 Northampton, Massachusetts, USA). All measurements were carried out in triplicate.

3. Results and discussion

3.1 Rate of water loss

The rate of water loss of surimi products prepared with different amounts of chitosan and hydroxypropyl starch has shown in Figure 1. The rate of water loss is an important property of surimi, which has an essential effect on the gel structure, tenderness of muscle as well as water holding capacity of surimi products (Sánchez-González et al., 2008). In general, surimi with high water holding capacity, internal water does not readily transfer to the outside, even with centrifugal dehydration (Park, Graves, Draves et al., 2013).

![Figure 1. Effect of different amount of compound polysaccharide on water loss rate of Tilapia surimi. Bars with different notations are significantly different (P<0.05).](image)

The addition of polysaccharides helps to improve the water holding capacity of surimi and thus retains more water molecules (Jannat-Alipour et al., 2019). The rate of water loss of the surimi samples prepared with different polysaccharide content was higher than that of the control sample. The rate of water loss of the treated samples was significantly (P<0.05) higher than 1.5% of control samples. This indicates that the polysaccharide molecules were completely combined with the water molecules of the surimi gel, which effectively retained the moisture content of the surimi, and enhanced the gel properties of the surimi products. The rate of water loss of surimi with 1.5% hydroxypropyl starch was 8.9%. This may be because too few complex polysaccharide combinations failed to fully combine with the water molecules in surimi, resulting in the extravasation of water molecules. At the same time, too many complex polysaccharide combinations may have reached the saturation state, and thus the water loss rate of surimi products did not change much. An excessive saturation state reduces the binding capability of water molecules and complex polysaccharides (Wang et al., 2018).

3.2 Changes in colour

Colour is another crucial quality parameter for surimi products. Usually, surimi products with high whiteness value are more likely to be loved and pursued by consumers (Park, 1995). To some extent, the addition of compound polysaccharides can make starch molecules fully combine with water molecules under heating, increase the expansion degree of surimi, and obtain more light penetration, to improve the whiteness value of Tilapia surimi (Chen and Xue, 2009).

The effects of different concentrations of compound polysaccharides on the whiteness of Tilapia surimi are shown in Table 1. It can be seen from the table that, the $L^*$ values of the 1.5% chitosan group and 1.0% chitosan group was significantly (P<0.05) higher than those of the control group. The $L^*$ value of 2% hydroxypropyl starch in both the 1.5% and 1.0% chitosan groups was the highest. The $L^*$ value represents the brightness of the surimi. The larger the value, the greater the brightness of the gel structure. The $a^*$ values, $b^*$ values, and whiteness of the treated groups showed a significantly increasing trend compared with the control samples (P<0.05), while the surimi colour developed toward yellow-green. However, the whiteness value did not significantly (P<0.05) change among the 1% chitosan group. The addition of hydroxypropyl starch had little effect on the colour change of surimi. However, with the addition of compound polysaccharides, the whiteness of Tilapia surimi was found to be higher than that without compound polysaccharides. Therefore, adding a certain amount of compound polysaccharide can improve the whiteness of Tilapia surimi, which is in line with the
One of the purposes of this experiment was to improve the oil retention of surimi. In each experimental group, adding compound polysaccharides on oil retention of Tilapia surimi. As a consequence, the emulsifying stability increases significantly (P<0.05). The effects of different concentrations of compound polysaccharides on the oil retention of Tilapia surimi has shown in Figure 2. It can be seen from the figure that the oil retention of surimi with 1.0% and 1.5% chitosan was significantly higher (P<0.05) compared with that of the control samples. Surimi with 1.0% and 1.5% chitosan polysaccharides significantly increased the oil retention of Tilapia surimi, which is consistent with the purpose of this study. The previous study with Silver Carp fish surimi has shown a similar trend of oil retention (Jiao et al., 2019).

### 3.4 Gelling properties

The effect of adding different amounts of compound polysaccharides on the hardness, cohesiveness, resilience, elasticity, stickiness, and chewiness of the fish surimi has presented in Table 2. It can be seen from Table 2 that the addition of hydroxypropyl starch did not significantly (P>0.05) change cohesiveness.

In the surimi group with different amounts of chitosan, the elasticity of fish surimi first decreased and then increased (P<0.05), which indicated that the compound polysaccharide combination could effectively enhance the gel structure restoring force (Ortiz and Aguilera, 2004) of surimi in the range of a certain amount of polysaccharides. Studies have shown that the addition of 2.5% hydroxypropyl starch or other cassava starch can lead to a decrease in the hardness of myofibrillar protein starch mixed gel (Tabilo-Munizaga and Barbosa-Cánovas, 2004). The reason is that the addition of excessive starch impedes the cross-linking in the surimi products to some extent, thus making the formation of that network (Campo and Tovar, 2008).

However, the results of this experiment are contrary. This indicates that the addition of hydroxypropyl starch and chitosan did not decrease the hardness of Tilapia fish surimi and thus can effectively improve the adverse factors of fish surimi caused by hydroxypropyl starch. With the addition of compound polysaccharides, the stickiness and chewiness of surimi in the 1.0% chitosan group increased significantly (P<0.05).
Table 2. Effect of different addition of compound polysaccharides on the gel properties of Tilapia fish surimi

| Chitosan (%) | Hydroxypropyl starch (%) | Hardness (N) | Cohesiveness | Elasticity (mm) | Resilience | Stickiness (N) | Chewiness (mj) |
|--------------|--------------------------|--------------|--------------|----------------|------------|---------------|---------------|
| 1            | 1                        | 2.57±0.12bc  | 0.81±0.05a   | 3.51±0.04a     | 0.56±0.03a | 2.07±0.22c    | 7.27±0.85c    |
|              | 1.5                      | 7.13±0.42bc  | 0.66±0.06a   | 2.97±0.06c     | 0.38±0.04c | 4.72±0.70b    | 14.04±2.33b   |
|              | 2                        | 7.63±0.20b   | 0.75±0.06a   | 3.03±0.04bc    | 0.50±0.04b | 5.70±0.59b    | 17.29±2.06b   |
|              | 2.5                      | 7.24±0.21bc  | 0.76±0.06a   | 2.78±0.06d     | 0.46±0.04ed| 5.46±0.59b    | 15.16±2.01b   |
|              | 3                        | 9.69±0.05a   | 0.78±0.06a   | 3.47±0.04a     | 0.50±0.04b | 7.51±0.57a    | 26.00±2.23a   |
|              | 0                        | 6.42±0.06a   | 0.76±0.06a   | 3.14±0.05b     | 0.49±0.03b | 4.87±0.41b    | 15.27±1.52b   |
| 1.5          | 1                        | 9.19±0.17a   | 0.78±0.01a   | 2.66±0.01ab    | 0.44±0.02bc| 7.18±0.25c    | 19.09±0.73c   |
|              | 1.5                      | 3.79±0.30i   | 0.66±0.04a   | 2.74±0.09ab    | 0.31±0.01f | 2.52±0.36f    | 6.92±1.21i    |
|              | 2                        | 6.06±0.41i   | 0.66±0.08a   | 2.34±0.07ab    | 0.35±0.04ef| 4.01±0.79d    | 9.40±2.11i    |
|              | 2.5                      | 17.44±0.27a  | 0.69±0.04a   | 3.11±0.04a     | 0.40±0.03ed| 11.94±0.85a   | 37.10±3.08e   |
|              | 3                        | 12.49±0.16b  | 0.80±0.05a   | 2.88±0.01a     | 0.54±0.04a | 9.91±0.47b    | 28.50±1.49d   |
|              | 0                        | 6.42±0.06a   | 0.76±0.06a   | 3.14±0.05a     | 0.49±0.03b | 4.87±0.41b    | 15.27±1.52b   |

Values are presented as mean±SD. Values with superscripts within the same row are significantly different among different amounts of compounds polysaccharides (P<0.05).

3.5 Protein content

The Tilapia fish is rich in protein, and its protein composition includes hemolytic muscle protein, salt-soluble myogenic protein, and insoluble muscle matrix. The protein content significantly affects the gel characteristics of surimi, such as elasticity, hardness, and stickiness, even water loss and oil retention. Therefore, the protein content of surimi is also an important factor in determining the quality. The effects of different amounts of compound polysaccharides on the protein content of fish surimi have shown in Figure 3. Compared with the control sample, the protein content of the 1.0% chitosan surimi group first significantly (P<0.05) increased and then decreased significantly (P<0.05) with the increase of hydroxypropyl starch. The reason may be that the combination of low dosage of hydroxypropyl starch and chitosan reduced the interaction between protein molecules and increased the interaction between water and protein molecules, that is, the interaction between bound water and protein molecules and thus, binding force was improved (Luo et al., 2020), but the binding force was destroyed when it exceeded the range of this additional amount (Hall, 2011). In the 1.5% chitosan surimi group, the protein content of Tilapia surimi added with 2.5% hydroxypropyl starch reached the maximum, while the protein content of the rest of the surimi did not change significantly (P>0.05) compared with the control group. The reason may be that the addition of 1.5% chitosan had little effect on the protein content of surimi.

3.6 Effect on volatile organic compounds

GC-MS was used to analyse the volatile organic components of surimi products with different amounts of compound polysaccharides. The analytical results have shown in the following Figure 4 and Table 3. The table shows that only two volatile compounds were identified in both the blank group and the surimi added with compound polysaccharides, namely aromatic compound 2,4-di-tert-butyl phenol and lipid compound n-butyl octyl phthalate. Thus, it can be concluded that the addition of compound polysaccharides has little effect on the volatile compounds in Tilapia surimi. In general, different kinds of surimi products with different storage methods and different additives contribute to different levels of odour, and some odours may be difficult to accept (An et al., 2020). In the blank group, the content of lipids accounts for 25% of all substances, and aromatic compounds account for 0.95%. In contrast, in the compound polysaccharide surimi, the content of lipids is 12%, and aromatic compounds are 4%. Therefore, for Tilapia fish surimi products, carbonyl compounds play a vital role in their flavour substances. Research shows that carbonyl compounds can produce a mellow fragrance that is more consistent with the original substance, while aromatic compounds are generally more exciting and unpleasant. But if the benzene ring is added with the hydrocarbon group, the
taste will be more acceptable. The lipids in this study belong to high molecular weight compounds. Under normal circumstances, there will be a faint fruit flavour (Fu et al., 2011; Feng et al., 2017). With the addition of compound polysaccharides, the content of esters decreased, while the content of aromatic compounds increased. However, many of the compounds could not be detected due to limited resources. The compounds identified from this experiment could be a base for future experiments.

4. Conclusion

In this study, the rate of water loss increased greatly, with the addition of 1.5% Chitosan and hydroxypropyl starch (1.0%, 1.5%, 2.0%, 2.5%, 3.0%). The whiteness value and oil retention properties of surimi products were also improved. The whiteness index of surimi did not show a noticeable shifting trend in the 1.5% chitosan group, but it did improve significantly in the other group. To a certain extent, with composite polysaccharides, gel properties of surimi, such as masticatory and glue viscosity, increased, but the cohesion of surimi did not change. In terms of the comprehensive indicators, the application range of the 1.5% chitosan surimi group was better than that of the 1% chitosan group. This indicates the possibilities for the industrial production of high-quality freshwater fish surimi. Further studies need to be carried out to extensively research the optimal amount of complex polysaccharides for the best-accepted surimi by consumers.

Conflict of interest

The authors declare no conflict of interest.

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