Seafood safety assessed using eye lens lavage and 3-hydroxykynurenine glucoside

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Abstract

The globalization of the food trade has brought previously unattainable foods to new places. One such food is fish, shippable even to landlocked places. The long journeys, however, can bring into question the freshness of the fish. Past studies have made note of the potential for examining the eyes to assess freshness. Based on these studies, we hypothesized that by examining various parts of the eyes, a potential method of quality assurance could be devised. First, we determined that the Golden Pompano would work best for our proof of principle experimentation. The lens of the fish eyes were lavaged before or after stored for a week in a refrigerator and a freezer. The lavage samples were examined using a spectrophotometer to assess the presence of UV-absorbing proteins, such as 3-hydroxykynurenine glucoside. Fish stored at +4°C showed the most similarity with a sample from a fish purchased directly from the market The overall absorbance profile from 230-380 nm. However, samples stored in the freezer showed expected absorbance at 260 nm and 365 nm, expected ranges for 3-hydroxykynurenine glucoside. The use of fish eyes to assess freshness shows promise.

Introduction

The Quality Index Method (QIM) is a means of assessing the quality and freshness of a whole fish product (Bernadi, et al., 2013). It is designed to be a fast and accurate way of measuring the quality of fish. The scoring is species specific. Trained evaluators examine physical characteristics of a given species, such as eyes, gills and scales, ranking them on a scale from 0 to 3, with 0 being the ideal. Fisheye anatomy is very similar to that of human eyes. It contains a lens, which is held in place by suspensory ligaments. Retractor muscles give the lens the ability to focus, by contracting or relaxing. At the back of the eye lies the retina, the membrane through which nerve impulses are set to be translated into images (Lamb, et al., 2007).

The problem with the QIM is that assessment of fish freshness, particularly in regards to fisheyes, requires qualitative scoring by trained expert.

We sought to streamline the QIM by developing a quantitative method to assess fisheye freshness.

Assay Development ("Proof of Concept")

Assay development started with two types of fish that have a high rate of consumption and are readily available at seafood markets, the Tilapia (*Oreochromis spp.)* and Golden Pompano (*Trachinotus auratus*).

Initial observations of the fish **(Figure 1)** consistently showed that the eye of the Tilapia was not optimal for assay development because the eye tended to be macerated. Focus was placed on the Golden Pompano, whose eye was bright, bulging and easier to manipulate. To determine the eye part that was most amenable for assay development, eyes were dissected into 3 sections; cornea, lens and retina. The cornea and retina required extensive fine dissection to isolate. The lens' density and mass made it easy to isolate.

The lens exterior is composed of a lens capsule made of collagen deposited by the apposing lens epithelial cells. The lens interior is composed of lens fibers composed of transparent cells that make and release crystallins and UV-absorbing proteins, such as 3-hydroxykynurenine glucoside (3-OHKG). Previous studies have shown that 3OHKG, in particular, denatures as lens' age (Hood et. al, 1999).

We hypothesized that as fish quality deteriorates ('ages'), fish eye lens proteins, like 3OHKG, would denature and degrade. So, 3OHKG denaturation could be used as an indicator of fresh freshness.

Based on trial and error, we developed a technique whereby we lavaged the interior of lens (see materials and methods) and collected the proteins (LL only) or we stored the whole lens and subsequently lavaged the lens (WL-LL). Samples were stored for 7 days (one week) at +4°C (refrigerator) or -20°C (freezer). We assessed protein stability using spectrophotometry by quantifying sample absorbance at 280 nm. The optical absorbance of tryptophan, tyrosine and cysteine amino acids is common at this wavelength and indicates presence of protein.
Bovine serum albumin (BSA) at 2 mg/mL was used as a control protein.

Results and Conclusions Assay Development

Abs_{290} were similar between LL and WL-LL samples (see table) indicating that lens lavage alone or whole lens with subsequent lavage could be used to collect fish protein. Samples stored at either +4°C or -20°C were similar to control protein levels (Abs_{290} is 0.275) indicating that sample (whether LL or WL-LL) can be stored for up to 7 days at either +4°C or -20°C .

Temperature (ºC)	Lens Lavage stored [whole eye discarded] (LL only)	Whole Lens stored [Lens Lavage after storage] (WL-LL)
+4	0.347	0.344
-20	0.168	0.173

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Introduction

When preparing seafood, such as fish, for human consumption, it should be stored properly to assure freshness. When the right storage techniques are not followed, seafood can become contaminated or spoiled. This makes the seafood unsafe for human consumption and can lead to illness. Previous reports have determined fish freshness using fish eye image energy changes (Wang, et al., 2013) and the refractive index of fish eye fluid (Yapar & Yetim, 1999). However these techniques require specialized instrumentation and technical expertise.

The purpose of this study was to develop a quick and easy way to determine fish freshness using fish eyes. We hypothesized that the length of time a fish has been dead and the conditions it is stored affect the degradation of lens proteins in the eye. A major lens protein is the UV absorbing 3-hydroxykynurenine glucosides (3-OHKG) (Garner, et al., 1999). In this regard, fish freshness is associated with lens protein, particularly 3-OHKG, stability.

Fish eyes were collected from Golden Pompano fish. After extracting the eye from the fish, the lens was isolated. Both whole lens and lens protein lavage were stored in a refrigerator (+4°C) or a freezer (-20°C) for one week. These samples along with lens lavage from direct fish purchase were tested using a spectrophotometer to assess UV light absorption.

The results gathered from using the spectrophotometer indicate that yes, the lens lavage method can be used to determine fisheye lens protein stability. Preliminary results show that the absorbance of protein stored at both +4°C and -20°C mimic the absorbance of freshly bought fish. Intact 3-hydroxykynurenine glucosides (3-OHKG) show a peak at 260nm and 350nm (Garner, et al., 1999), which was modeled similarly by the lens lavage proteins collected from the fisheyes.

In this study, fish freshness was defined by limiting the degradation of lens protein, and measuring that by UV ray absorbance. The preliminary results gathered at this point show promising results. Further testing is required. By conducting this research, it is now understood that lens protein of a fish can be used to assess protein stability and ultimately determine fish freshness and safety of consumption.

Figure 1

Figure 1. **Golden Pompano as model fish**. two types of fish that have a high rate of consumption and are readily available at seafood markets, the Tilapia (Oreochromis spp.) [left] and Golden Pompano (Trachinotus auratus) [right] were assessed. Tilapia consistently showed macerated eye, while Golden Pompano did not.

Figure 3

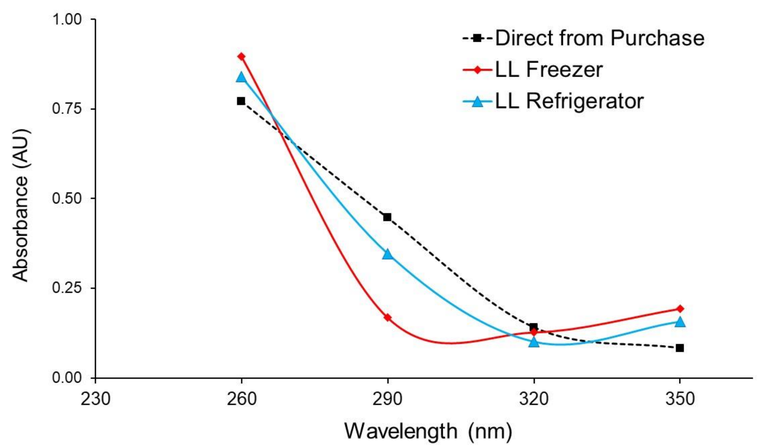

Figure 3. **Lens Lavage (LL) method effective in collecting Fisheye Lens Proteins**. Golden Pompano (*Trachinotus auratus*) subjected to lens lavage (LL) performed directly after purchase (square, black-dotted line) or after whole lens was stored for 7 days at -20°C (diamond, red-solid line) or +4°C (triangle, blue-solid line). Light absorption levels of samples taken with spectrophotometer at 30 nm intervals from 260 to 350 nm are represented. All samples show peak at 260 nm, consistence with 3-OHKG protein.

Materials & Methods

Fish (Golden Pompano (*Trachinotus auratus*)) were purchased on ice from local seafood market. Upon arrival to the lab, the lab bench and dissections instruments were sanitized using 70% ethanol. Both eyes were dissected away from the body of the Golden Pompano fish and each lens was isolated (see figure 2). The lens of each eye was subjected to a thorough PBS wash. The isolated lenses were placed in a refrigerator (+4°C) or a freezer (-20°C) for one week. After one week of storage, each specimen was placed on ice for processing.

While stabilizing the lens with tweezers by the lens capsule, an 25G needle outfitted with a 3mL syringe was inserted through the lens capsule into the lens fibers and the interior of the lens was lavaged to collect the lens protein (LL). Direct from purchase lens lavage samples were collected and assayed immediately upon arrival to the lab. For each sample, 500 µL was pipetted into a UV capable cuvette (REF #759235, LOT #602193) placed into a spectrophotometer. Bovine Serum Albumin (BSA) at 2 mg/mL was used as a control (not shown). Measurements were taken in the UV absorption range of 260-350 nm.

Figure 2

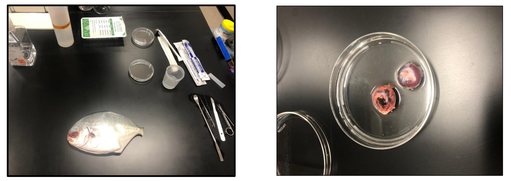

Figure 2. **Lens Lavage Method**. Golden Pompano (*Trachinotus auratus*) before dissection [left]. Golden Pompano eyes in PBS bath [right].

Conclusions

This proof of principle study indicates that there is some promise in utilizing *in situ* eye lens lavage as a way to access fish freshness (defined here as the limited degradation of lens protein).

Lens lavage samples or whole lens for subsequent lavage can be stored up to one week at +4°C (refrigerator) or -20°C (freezer) without appreciable changes from direct from purchase fish lens lavage when considering light absorption associated with protein, such as the UV-absorbing protein 3-hydroxykynurenine glucoside (3-OHKG).

This study showed that all fish are not conducive to a lens lavage procedure as evidenced by the state of Tilapia eyes and the possibility of cataracts. We are working on improving the technique to take these parameters into account.

Future Work

The lens lavage method developed here will be improved so that it can be performed *in situ* without eye dissection.

NMR will be used to confirm that the protein absorbed at 260 nm is 3-OHKG.

The method developed here will be applied to Tilapia and other fish types. Based on personal communications with fisherman (Andy LaGier), we will assess how this method is impacted by lens injuries associated with method of fish capture or cataracts developed by UV exposure.

Time frame of experiments will be extended to include times previously shown until fish spoilage (3 days in fridge and 3 months in freezer).

Acknowledgments

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