The Pharma Innovation Journal 2023; SP-12(8): 800-805

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 800-805

www.thepharmajournal.com
Received: 01-05-2023
Accepted: 10-06-2023

Nidhi Dhansukhbhai Patel
Department of Fish Processing Technology, College of Fisheries Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Varsha Likhar
Department of Fish Processing Technology, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

Saiprasad Bhusare
Fish Nutrition, Biochemistry and Physiology Division, ICAR-Central Institute of Fisheries Education, Mumbai, Maharashtra, India

Corresponding Author:
Nidhi Dhansukhbhai Patel
Department of Fish Processing Technology, College of Fisheries Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India

Biochemical techniques used in fisheries: A review

Nidhi Dhansukhbhai Patel, Varsha Likhar and Saiprasad Bhusare

Abstract
Fishes are highly nutritious source of food it contains various types of major and micro nutrients. Due to its nutritional factor the demand for fish and fishery product is increasing rapidly worldwide. Further fishes are considered as highly perishable food, thus proper care is required in maintaining the quality of such products by preventing it from getting spoilt. The review focuses on the importance and types of biochemical techniques which are used in fish analysis or fish product analysis. Currently due to the growing importance of seafood analysing of the raw material and the products contributes as a very important aspect. These processes will help in checking of the product quality, spoilage level or presence of contaminants or pollutant. These processes will also help in determining the stage of spoilage on the basis of compound formed or compound present. Here in the review different processes such as chromatography, electrophoresis, spectroscopy, Titrimetry, Gravimetry are described along with their applications.

Keywords: Biochemical analysis, methods, fish, seafood, applications

Introduction
Biochemical techniques involves the use of methods for evaluating the composition and properties of the samples. Its applications are in broad areas of biological sciences including fisheries science. The information obtained from these kinds of analysis provide indication of substances that are present or they may also specify the exact amount of the substances present in the sample. Biochemical techniques depend upon the availability of appropriate analytical instrumentation, methods and applications. In recent years, a great number of methodologies for the widespread examination of a wide variety of component types have been developed. Further various kinds of instrumentations are also required for carrying out biochemical analysis. Also, now a days kits are available as a diagnostic tool which range from some simple assays to more sophisticated procedures. The kits include all the necessary standards which can be used in manual procedures or using an instrument. These techniques will help in determining various factors related to the fish products components and their shelf life.
The biochemical analysis technique involves the following methods

1. Titrimetry
2. Gravimetry
3. Chromatography
4. Electrophoresis
5. Spectrophotometry

Titrimetry
Titrimetry method was developed in 18th century but it is still being widely used today because of its high precision and versatility (Takeuchi, 2021) [27]. The determination of an analyte based on its stoichiometric reaction is made possible by the titrimetry methodology, which is a collection of methodologies for quantitative analysis. The procedure involves adding a reagent of known concentration to a sample very slowly and in increasing amounts until the analyte becomes consumed in measurable amounts. The reaction's end point is identified visually through the use of an indicator or instrumentally through the use of an appropriate procedure. The amount of reagent used in a reaction with an analyte can be used as an indicator for the concentration of the analyte in the sample. Titrimetry is one of the earliest methods of analysis that has survived to the present day. The method's widespread acceptance may be attributed to its many appealing qualities, including its ease of use, precision, accuracy, versatility, and application (Kozak & Townshend, 2018) [14].
In fisheries this technique is used for various purposes.

Applications
- Titration to determine dissolved oxygen (DO) in the water sample
- Titration of the water samples is a standard method for chloride determination
- For determining calcium in food (Siong *et al*, 1989) [26].
- For determining of the α-amino Nitrogen in Fish Protein Hydrolysates (FPH) (Wang *et al*., 2012) [33]

Gravimetry
When calculating the amount of an analytes based on the mass of a solid, the Gravimetry method is utilized as the method of choice.

Principle
It is based on the fact that it is possible to calculate the mass of an ion in a pure substance. Additionally, the same ion's mass percentage in a known amount of an impure compound may be determined using this method.

Procedure
1. The process of making a sample solution with a known sample weight
2. Separation of the desired constituent from the sample
3. Weighing of the isolated constituent
4. Quantifying the sample's concentration of a certain component based on the weight of the purified material.

Application of Gravimetric Analysis
It is one of the most common method used for estimating fecundity. It is based upon the relation between oocyte density in ovary and ovary weight
This technique can also be used to estimate total fecundity, batch fecundity and potential annual fecundity
- The method is used to determine fat content and compare samples.
- Gravimetric procedure for determining the glazing percentage in frozen fish (Vanhaecke *et al*., 2010) [31]
- Determination of SO_2, CO_2 and iodine
- Mg^{2+} levels in water and wastewater can also be determined

Chromatography
The word "chromatography" is derived from two words "chromo" means colour and "graphy" means writing (Rajasekar, 2018) [24]. It is a method that may be applied to the task of separating various kinds of components from a combined substance. Mikhail S. Tsvet first stumbled across the method in 1901. This separation is achieved by using a stationary phase in conjunction with a mobile phase. According to Tiwari and Talreja (2022) [29], the technology of chromatography may be used to separate, isolate, analyze, and purify many different kinds of components.

Types of chromatography techniques
- Paper chromatography
- Gas chromatography
- Thin layer chromatography (TLC)
- High Performance Liquid chromatography (HPLC)

Paper chromatography
Components are segregated according to their polarity, which might be toward the mobile phase or the stationary phase. The method used is mostly analytical in nature. Paper chromatography uses a cellulose filter paper as the stationary phase and a liquid as the mobile phase to separate solids from liquids. Synge and Martin first used paper chromatography in 1943 (Gupta *et al*., 2018) [7]. This method is also known as planar chromatography since it is performed on special paper (Tiwari & Talreja, 2022) [29].

Principle: The concept of partition is utilized to separate substances into their respective phases of liquid in this approach. The aqueous solvent is held in place by the filter paper, which is part of the stationary phase, while the mobile phase passes over it. The capillary action of the pores is responsible for the separation of the various substances. How much separation occurs depends on how well each component gets along with water. Adsorption phenomena can also play a role in the separation process. The paper's surface will function as the stationary phase, while the liquid solvent will play the role of the mobile phase in this adsorption phenomenon. Adsorption, then, is a process that takes place between the solid and liquid states.

Applications
- Identification of Amino acids in Fish
- For detecting adulterants.
- To detect the contaminants, present in various kinds of food.
- For determining the drugs present in cultured fishes.

Thin layer chromatography (TLC)
Thin layer chromatography (TLC) is a simple, easy-to-operate and cost-effective planar chromatographic technique that is being used in laboratories for separating chemical and biochemical compounds (Cheng *et al*., 2011). The technique uses a solid-liquid adsorption to separate non-volatile mixtures. In this particular example, the mobile phase is a liquid, and the stationary phase is silica gel that has been deposited onto a glass plate. In this method, the components are separated from one another based on their polarity towards the mobile and stationary phases. M. Tswettin first developed this chromatographic method in 1906 (Tiwari & Talreja, 2022) [29].

Applications
- TLC is used in identifying natural products such as the essential oils, fixed oil, glycosides, volatile oil, waxes, alkaloids, etc.
- It can also be used in the food industry, for separating and identifying colours, sweetening agent, and preservatives
- To determine biogenic amines, present in fish and squid (Lapa and Pickova, 2004) [16].
- Screening method for histamine in tuna fish (Lieber & Taylor, 1978) [18].
- Qualitative and quantitative analysis of lipid classes in fish oils (Indrasena, *et al*., 2005) [12].

High Performance Liquid chromatography (HPLC)
HPLC is a versatile and widely used technique. It is a type of chromatographic technique which is used in analytical and phytochemical chemistry for identifying, purifying and quantifying the individual components of the mixture (Cannell, 1998; Piana M *et al*., 2013) [2, 22]. It is absolutely versatile for the isolating of the peptides and proteins from a wide variety of sources. (Aguilar, 2004) [9].

Applications
- To analyze pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolation of valuable products
- Separation and purification of biopolymers such as, nucleic acids, enzymes etc.
- Water purification
- HPLC can be used for the determination of biogenic amines in fish implicated in food poisoning (Hwang *et al*., 1997) [11]
- Vitamin K can be determined in the edible part of fish (Ostermeyer & Schmidt, 2001) [19]

Gas chromatography (GC)
Gas chromatography, sometimes known as GC, is a technology that is both selective and sensitive. Its primary application is in the isolation of volatile substances. In the monitoring of air pollution, volatile organic molecules are determined quantitatively and identified qualitatively using GC paired with a Flame Ionisation Detector (FID). The "mobile phase" in this procedure is a carrier gas, which can be anything as inert as helium (He) or as unimaginative as nitrogen (N_2). A thin film of liquid or polymer on an inert solid substrate constitutes the stationary phase.

Further there are 2 types of gas chromatography
- Gas-solid chromatography (GSL)
- Gas-liquid chromatography (GLC)

Derivatives of volatile liquids can be separated using GLC. This method utilizes an inert gas as the mobile phase and a little quantity of liquid contained on a finely milled inert solid substrate as the stationary phase. In chromatography, components are separated based on their different solubilities in the inert substance that makes up the chromatography column. They continue to evaporate and travel through the column, aided by the flow of the inert gas. Most often, GLC is employed to determine the fatty acid composition of a phospholipid sample.
GSC is an uncommon method that finds its primary application in the isolation of gases found in the atmosphere. The fixed phase is an inert gas, while the mobile phase is an absorbent substance. Charcoal, molecular sieve, and hybrids of the two are the most often employed solids.

Applications
- Used for drug analysis
- Toxicological analysis
- Air samples can be analysed and level of pollutants can be determined
- Used in food analysis
- Used in forensic laboratories for various purposes
- For analysing encapsulated fish oils and fish oil ethyl esters (Joseph & Ackman, 1992) [13]
- For the determination of histamine content present in fish and its products (Hwang *et al*., 2003) [10]

Electrophoresis
Electrophoresis is a technique where in the components are separated from a mixture on the basis of the charge present in them.

Types of electrophoresis
a. Paper electrophoresis
b. Agarose Gel electrophoresis
c. Iso electric focusing

Paper electrophoresis
Zone electrophoresis includes the paper electrophoresis technique. In this step, the charge on a molecule is affected by the surrounding medium's pH.

Procedure: The sample might be applied in the form of a spot or a thin stripe. It is possible to apply the sample either before or after the buffer has been allowed the paper to become equilibrated with. Once the sample has been put to the paper and the buffer has reached equilibrium, the current may be turned on to continue processing. Phosphate, citrate, acetate, and similar compounds are frequently employed as buffers. A maximum of two hours is all that is required to finish the process. So, turn off the source of power and take away the paper after two hours. At 1100 ºC, the paper is dried in a hot air oven.

Detection & Quantitative assay: To determine the identity of the unknown electropherogram, one must first compare it to the standard electrogram acquired under standard conditions. The following techniques are often used to determine the identity of certain compounds based on their physical characteristics.
1. **Fluorescence:** Staining by using “Ethidium bromide” and subsequent visualization of the electrophoretic gram under UV light makes DNA & RNA fluoresce and thus facilitates their detection.
2. **UV absorption:** Proteins, Peptides & nucleic acids are absorbed.
3. **Staining:** here dye such as alcain blue, iodine, Sudan black can be used.

Agarose gel electrophoresis
Electrophoresis on an agarose gel is a common technique in molecular biology, biochemistry, and genetics for separating macromolecules like DNA, RNA, and proteins from a complex mixture. Separating DNA into fragments of varied sizes with this method is one of the most reliable methods available. Seaweed (of the genera Gelidium and Gracilaria) are the primary source for the natural linear polymer known as agarose. It creates a gel matrix by hydrogen bonding whenever heated in a buffer and then cooled as reported by (Lee *et al*., 2012) [17].

Principle: Gel electrophoresis is a technique for separating DNA fragments in a solid support medium (like agarose gel) according to their size.

Procedure: There are three primary steps to this procedure. In the first step, agarose is used for making a gel. The concentration of agarose used should be adjusted according to the size of the DNA fragments being purified. The DNA samples are then loaded into the sample wells in the second step. The separation is optimized by applying a voltage to the gel for a set amount of time. Final steps involve staining the gel, or direct visualization under UV light if ethidium bromide was included in the gel and electrophoresis solution (Voytas, 2000). When moving across the gel, DNA fragments pick up the dye and carry it along with them. When exposed to ultraviolet light, the intercalated dye fluoresces. It is possible to run in parallel a ladder set of DNA fragments that are all of

the same size. This allows one to estimate the sizes of the DNA fragments that are yet unknown.

Applications
- The method is used to separate proteins, DNA or RNA.
- For Analyzing of PCR products, e.g., genetic fingerprinting
- Identification of fish species (Hill, *et al*., 1966) [8]
- Estimation of the size of DNA molecules
- Used to find sources of contamination by *Listeria monocytogenes* in a cold-smoked rainbow trout processing plant (Autio *et al*., 1999) [1].

Isoelectric focusing (IEF)
IEF was established for the purpose of distinguishing proteins on the basis of the variations in their isoelectric pH value, which is also referred to as their pI value. Proteins are isolated and analyzed using this technique (Garfin, 1990) [6]. For this method, an electric field is applied to a solution with a pH gradient, and the ampholytes migrate to the area where their net charge is zero (the isoelectric point, or pI) (Pergande & Cologna, 2017) [21]. Proteins move toward the electrode with the opposite charge when put in a liquid with a variable pH and exposed to an electric field. Proteins approach their pI values at varying speeds, but once there, they tend to stay put for long periods of time. The creation of reliable electric field-based pH gradients is key to this technique (Garfin, 1990) [6].

Application
The technique used for isolation of proteins, to separate a specific component from contaminants of slightly different pI. The method is also used for the preparation of isoforms of proteins.

Spectrophotometry
Biochemists and molecular biologists routinely utilize the spectrophotometric technique of analysis to calculate the concentration of biomolecules in a given solution. DNA, RNA, and protein concentrations may be calculated using the Beer-Lambert-Bouguer Law (Trumbo *et al*., 2013) [30].
Spectrophotometer is made up of two different units: a spectrometer, which is responsible for producing light with a certain wavelength, and a photometer, which is responsible for measuring the intensity of the light that is either transmitted or absorbed. The device measures the light intensity of a sample by sending a beam of light through it. These devices are utilized in the procedure of measuring color, as well as in the monitoring of the color accuracy throughout the production process.

Atomic Absorption spectroscopy
The atomic absorption technique is a spectroscopy-based analytical method for determining element concentrations. Because of its element-selectivity and its ability to give analytical sensitivities at the parts-per-million level and below, AAS method has widespread acceptance. The process of AAS may be split into two distinct phases. First, the process in which an analyte molecule is broken down into its component atoms in the gas phase, also known as atomization; and second, the subsequent process in which free atoms absorb radiation (Lagalante, 2004) [15].

Principle: This technique relies on the fact that ionizing radiation of varying wavelengths may be absorbed by the free electrons created in an atomizer. When UV or visible light is absorbed by these free electrons, it leads the electrons to go into orbits with a higher energy level. During this step of the process, the absorption spectrum is allowed to be released, which the photodetectors then use to make a measurement. Free electrons in the gas phase can be measured using the resulting absorption spectra. When radiation, in the form of photons, is absorbed, a spectrum is produced that may be quantified in terms of absorbance. According to research (Garcia & Baez, 2012) [5], the absorbance of a sample changes as the concentration of molecules changes.

Applications
- The atomic absorption spectrometer, or AAS, is utilized in the process of quantitative and qualitative analysis of metallic elements.
- It has been put to use in the process of decontaminating environmental samples such as water and soil.
- Determination of heavy metals such as Cadmium, lead and trace elements such as copper, zinc in sediments and fish (Dalman, 2006) [4].

Mass spectroscopy
To analyze a sample's biomolecules and investigate protein-protein interactions, mass spectrometry is an invaluable tool. This is according to (Rajawat & Jhingan, 2019) [25]. In addition, it may be used to determine the type and amount of substances in a sample by measuring the ions' mass-to-charge ratio.

Principle: The basic idea is to produce positively (+) charged ions by hitting an organic molecule with an electron beam. The electron energy is used to disrupt the bonds of the molecular ion, resulting in positively charged species or fragment ions. According to their mass and charge, the newly created positive ions are then accelerated and deflected along a circular route by a magnetic field. Each ion is recorded as a distinct line on the plate and its peak intensity. The charge, mass, and velocity of an ion determine its deflection, while the mass to charge (m/z) ratio determines how the ions are separated and the quantity of the ions determines how easily they can be detected (Rajawat & Jhingan, 2019) [25].

Applications
- Mass spectrometry is a technique that may be utilized for the purpose of quantifying known materials.
- The structure and chemical content of diverse substances may be determined, and previously undiscovered compounds can be identified.

Ultraviolet and visible spectroscopy
This method makes use of electromagnetic radiation in the ultraviolet (UV) and nearby visible (visible) ranges. The ultraviolet-visible spectrum extends from around 190 nm to 900 nm. Furthermore, UV-Vis spectroscopy is particularly useful for quantitative measurements since there is a linear connection between absorbance and absorber concentration (Tissue, 2002) [28].
Principle: It is predicated on the idea that the material absorbs radiation at a variety of frequencies, which then causes it to form an absorption spectrum with a range of absorbances that correspond to those frequencies. The atomic and molecular composition of a substance determines its absorption spectrum. The energy gap between the two energy levels of

the molecule determines the wavelength of light radiation absorbed by the substance. An absorption line is created as a byproduct of the process, which, along with other lines, makes up what is known as an absorption spectrum. The spectrometer receives incoming light in the ultraviolet (UV) and visible (VIS) regions of the electromagnetic spectrum. Therefore, when a photon with enough energy travels to an object, the energy that is absorbed by the electrons will allow them to leap into a higher energy state. This occurs when a photon having sufficient energy travels to an object. The number of photons, or other forms of radiation, that are taken in will produce an absorption spectrum, which may be evaluated in terms of absorbance. Number of excited electrons, which is a function of molecule concentration, determines the absorbance of a sample.

Applications

- Some proteins and nucleic acid molecules can be identified in their purified form or in complex biological mixtures using this technique.
- Biological samples can be quantified directly or indirectly via colorimetric tests, both of which are performed using this technique.
- Using ultraviolet-visible spectra of ocular fluid with multivariate analysis, it may be utilized to categorize fresh and rotten fish (Rahman *et al*., 2016) [23].
- The UV and visible light sensitivities of cyprinid fish (*Danio aequipinnatus*) cones may be measured (Palacios *et al*., 1996) [20].

Conclusion

The biochemical techniques are very important for analysing various aspects and component. This technique is also important as consumer point of view as fishes are one of the highly demanding food items. Fishes are delicacy in various parts of the world. Thus, proper care during culture and processing is must and for that there is a need of biochemical analysis at various steps. Several analyses such as species identification, checking for contamination or presence of any unwanted material, to check the quantity of the components present, removal of unwanted components, etc. These techniques are also important and useful at industrial level. These techniques are useful for both aquaculture and fish processing sector for various purposes.

References

1. Auti T, Hielm S, Miettinen M, Sjöberg AM, Aarnisalo K, Björkroth J, *et al*. Sources of Listeria monocytogenes contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Applied and Environmental Microbiology. 1999;65(1):150-155.
2. Cannell RJP. Natural Products Isolation. Human Press Inc. New Jersey; c1998. p. 165-208.
3. Cheng, Sy-Chyi, Min-Zong Huang, Shiea J. Thin layer chromatography/mass spectrometry. Journal of Chromatography A. 2011;1218-19:2700-2711.
4. Dalman O, Demirak A, Balcı A. Determination of heavy metals (Cd, Pb) and trace elements (Cu, Zn) in sediments and fish of the South-eastern Aegean Sea (Turkey) by atomic absorption spectrometry. Food chemistry. 2006;95(1):157-162.
5. García R, Báez AP. Atomic absorption spectrometry (AAS). Atomic absorption spectroscopy. 2012;1:1-13.
6. Garfin DE. Isoelectric focusing. In Methods in enzymology. 1990;182:459-477.
7. Gupta M, Kapoor B, Gupta R. Paper Chromatography: A Review. Journal of Emerging Technologies and Innovative Research. 2018;5(10):2349-5162.
8. Hill WS, Learson RJ, Laxe JP. Identification of fish species by agar gel electrophoresis. Journal of the Association of Official Analytical Chemists. 1966;49(6):1245-1247.
9. Aguilar MI. HPLC of Peptides and Proteins. 2004;251:3-8.
10. Hwang BS, Wang JT, Choong YM. A rapid gas chromatographic method for the determination of histamine in fish and fish products. Food Chemistry. 2003;82(2):329-334.
11. Hwang DF, Chang SH, Shiua CY, Chai TJ. High-performance liquid chromatographic determination of biogenic amines in fish implicated in food poisoning. Journal of Chromatography B: Biomedical Sciences and Applications. 1997;693(1):23-30.
12. Indrasena WM, Henneberry K, Barrow CJ, Kralovec J. A. Qualitative and Quantitative Analysis of Lipid Classes in Fish Oils by Thin-Layer Chromatography with an Iatroscan Flame Ionization Detector (TLC-FID) and Liquid Chromatography with an Evaporative Light Scattering Detector (LC-ELSD). Journal of liquid chromatography & related technologies. 2005;28(16): 2581-2595.
13. Joseph JD, Ackman RG. Capillary column gas chromatographic method for analysis of encapsulated fish oils and fish oil ethyl esters: collaborative study. Journal of AOAC International. 1992;75(3):488-506.
14. Kozak J, Townshend A. Titrimetry Overview; c2018.
15. Lagalante AF. Atomic absorption spectroscopy: A tutorial review. Applied Spectroscopy Reviews. 2004;34(3):173-189.
16. Lapa-Guimaraes J, Pickova J. New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. Journal of Chromatography A. 2004;1045(1-2):223-232.
17. Lee PY, Costumbrado J, Hsu CY, Kim YH. Agarose gel electrophoresis for the separation of DNA fragments. Journal of Visualized Experiments. 2012;(62): e3923.
18. Lieber ER, Taylor SL. Thin-layer chromatographic screening methods for histamine in tuna fish. Journal of Chromatography A. 1978;153(1):143-152.
19. Ostermeyer U, Schmidt T. Determination of vitamin K in the edible part of fish by high-performance liquid chromatography. European Food Research and Technology. 2001;212:518-528.
20. Palacios AG, Goldsmith TH, Bernard GD. Sensitivity of cones from a cyprinid fish (*Danio aequipinnatus*) to ultraviolet and visible light. Visual neuroscience. 1996;13(3):411-421.
21. Pergande MR, Cologna SM. Isoelectric point separations of peptides and proteins. Proteomes. 2017;5(1): 4
22. Piana M, Zadra M, de Brum TF, Boligon AA, Gonçalves AF, da Cruz RC. Analysis of rutin in the extract and gel of Viola tricolor. Journal of Chromatographic Science. 2013;51:406-411.
23. Rahman A, Kondo N, Ogawa Y, Suzuki T, Shirataki Y, Wakita Y. Classification of fresh and spoiled Japanese dace (*Tribolodon hakonensis*) fish using ultraviolet–visible spectra of eye fluid with multivariate

analysis. Engineering in agriculture, environment and foo. 2016;9(1):64-69.
24. Rajasekar S. Idiosyncratic Apporch for an Erratic and Stunning Management of Aegle Marmelos Linn. Leaves on Photosensitivity by using Zebra Fish Embryo Model (Doctoral dissertation, College of Pharmacy, Madurai Medical College, Madurai; c2018.
25. Rajawat J, Jhingan G. Mass spectroscopy. In Data processing handbook for complex biological data sources; c2019. p. 1-20.
26. Siong TE, Choo KS, Shahid SM. Determination of calcium in foods by the atomic absorption spectrophotometric and titrimetric methods. Pertanika. 1989;12(3):303-311.
27. Takeuchi M. Titrimetry. Analytical Science. 2021;37(2):227-227.
28. Tissue BM. Ultraviolet and visible absorption spectroscopy. Characterization of Materials; c2002.
29. Tiwari S, Talreja S. Thin Layer Chromatography (TLC) VS. Paper Chromatography: A Review. Acta Scientific Pharmaceutical Sciences. 2022;6(9).
30. Trumbo TA, Schultz E, Borland MG, Pugh ME. Applied spectrophotometry: analysis of a biochemical mixture. Biochemistry and Molecular Biology Education. 2013;41(4):242-250.
31. Vanhaecke L, Verbeke W, De Brabander HF. Glazing of frozen fish: analytical and economic challenges. Analytica Chimica Acta. 2010;672(1-2):40-44.
32. Voytas D. Agarose gel electrophoresis. Current protocols in molecular biology. 2000;51(1):2-5.
33. Wang H, Zhang F, Cao J, Zhang Q, Chen Z. Comparison of Chromatographic and Titrimetric Methods for the Determination of the [alpha]-amino Nitrogen in Standard Solution and Fish Protein Hydrolysates. Journal of Food Research. 2012;1(4):174.