CHAPTER 1

INTRODUCTION

1.1 Phosphates in the seafood industry

Polyphosphate as one of food additives has been used widely in the seafood industry, for both of fresh and frozen products. The tissue shriveling and the ice cause the cell walls to be destroyed. When the ice melts, the liquid inside the cell will flow out together with the ice causing weight loss more than usual. Moreover, the structure of the tissue changes, this changes the taste of seafood [22]. Polyphosphate compounds are then used in the food industry in order to improve the quality of the food. The polyphosphate compounds help protein soluble in water because the water molecules will be surrounding the protein molecules. This would be due to the pH of the food for softy. In addition, it would help the better taste of the food [20, 21, 23]. The polyphosphate compounds will be hydrolyzed to orthophosphate [1, 8].

If humans take a lot of phosphate residues, it creates health hazard, such as skin itching, stimulating the parathyroid gland and to dissolve calcium from the bones. Phosphate may cause the bones fragile. The precipitation of phosphate and calcium may cause the risk of atherosclerosis [26, 27]. The Ministry of Public Health and Thai Industrial Standards Institute announces that the use of the phosphate should be less than 5000 mg/kg [8, 24]. There are reports on the determination of phosphate in seafood and frozen food by using spectrofluorimetry [5], spectrophotometry [7], ion chromatography [8, 9], thin-layer chromatography [1, 10], capillary isotachophoresis [11], gravimetry [6]. Those methods are cumbersome, also involve in high consumption of the reagent, relatively expensive instrument, long analysis time. Moreover the methods are not suitable for on-site analysis.

As a standard method, molybdenum blue method is employed for determination of phosphate in soil and water [19]. It is based on the reaction of phosphate with ammonium molybdate in acid condition to produce a phosphomolybdic acid. It is then reduced to phosphomolybdenum blue. Absorbance can be measured at 880 nm. However, it is for only orthophosphate. Currently, test kit [14] and test strips [16] have been developed based on molybdenum blue principle. They may be consider to be low cost versatile. It can be used on-site. Therefore it is to develope cost effective assay for phosphate contents in seafood and frozen food samples.

1.2 Determination of phosphate

Table 1.1 summarizes the procedures for the determination of phosphate in food industry.

Table 1.1 Analytical methods applied to the determine of phosphate in different samples

Analyte / Samples	Sample Preparation	Reaction	Technique	Ref.
Phosphorus in meat and fish products	Sample was digested by using HNO_3 and H_2O_2	Procedure A : phosphates form complex with morin-aluminium Procedure B: phosphates form complex with quinine sulphate solution	Spectrofluorimetry	[5]
Phosphorus in meat samples	Meat samples were digested by heating with a mixture of HNO_3 and HCl then diluted with distilled water.	The dilute sample was heated with a known amount of quimociac reagent to form precipitates of quinolinium phosphomolybdate.	Gravimetry	[6]
Phosphorus in meat	Sample was digested with H_2SO_4, $HClO_4$ and V_2O_5 solutions.	Phosphate reacts with molybdate in an acidic condition producing phosphomolybdic acid .It is then reduced by a reducing agent to become phosphomolybdenum blue.	Spectrophotometry	[7]
Polyphosphate in shrimp samples	A sample was prepared by extraction with distilled water and 8 M KOH was added to it .This was placed in an ultrasonic bath.	-	Ion-Chromatography	[8]

Table 1.1 Analytical methods applied to the determine of phosphate in different samples (Continued)

Analyte / Sample	Sample Preparation	Reaction	Technique	Ref.
Polyphosphate in sausage. (orthophosphate, diphosphate, triphosphates)	A sample was extracted with distilled water in an ultrasonic bath and then filtered prior to analyte	-	Ion-Chromatography	[9]
Condensed phosphate in the shrimp	The samples were extracted with distilled water.	Determine of phosphate by sprayed reagent (ammonium sulfate, ammonium molybdate and nitric acid) on the plate and develop color to yellow. Then sprayed a mixture consisting of sodium pyrosulfite, sodium sulfite and methylaminophenol the latter spray helps in reducing the yellow spot to blue spot.	Thin-Layer Chromatography	[1]
phosphate in shrimp	Samples were extracted with distilled water	The color developed on the TLC plate was based on the molybdenum blue reactions.	Thin-Layer Chromatography	[10]

Table 1.1 Analytical methods applied to the determine of phosphate in different samples (Continued)

Analyte / Samples	Sample Preparation	Reaction	Technique	Ref.
Polyphosphate in raw, cocktail prawns, squids, meat and different mixtures of seafood	Samples were extracted with distilled water before centrifuging and diluting with distilled water.	-	capillary isotachophoresis	[11]

From the above mentioned reports, the determination of phosphate contents in meat, seafood, frozen seafood and sausage samples seemed to be relatively complicate with relatively high reagent consumption, relatively high in cost for instrument and long analysis time and it is not suitable to be used for onsite analysis.

One of the common methods for the determination of phosphate in soil and water samples may refer to the molybdenum blue method which is also referred as the standard methods [12-14] as summarized in Table 1.2.

Table 1.2 Determination of phosphate in soil and water samples by molybdenum blue method

Analyte / Samples	**Sample Preparation**	**Reaction**	**Technique**	**Ref.**
Polyphosphate in soil	A sample was prepared using distilled water for the extraction of phosphate	The extract was analyzed for phosphate by employing the molybdenum blue method using ascorbic acid as a reducing agent	spectrophotometry	[18]
Phosphate in water	A sample was filtered by using cellulose membrane filter.	Molybdate react with phosphate followed by its reduction with sodium sulphide in aqueous sulfuric acid medium.	spectrophotometry	[19]
Phosphate in water	A sample was filtered using a glass filtration system.	N/A	test kits	[14]
Phosphate in nutrients essential for developing mosquito larvae	Unfiltered water samples	N/A	test strips	[16]

1.3 Principle of phosphate test

In sulfuric solution, orthophosphate ions (PO_4^{3-}) react with molybdate ions to form molybdophosphoric acid, which is reduced to phosphomolybdenum blue. The phosphate concentration is measured semiquantitatively by visual comparison of the reaction zone of the test strip with color scale.

1.4 Digital image – based colorimetry

The digital image-based colorimetry is useful to quantitative analysis. It is based on the red (R) green (G) and blue (B) values of a photo taken by a digital camera. It would involve charge couple detectors (CCD) or complementary metal oxide semiconductor (CMOS). The RGB intensity values can be read by using a computer program such as Matlab, Kylix, Visual basic and Adobe photoshop [25].

A smart phone such as an iphone4S can also be used as well as the digital camera and can be used. An application from an iphone4S can read the RGB intensity from the photo such as icolorsampler (for iphone version 1.0.0 @2012, YUSUKE GOTO).

It can be applied for determination concentration of phosphate in the sea food and frozen food.

1.5 The aims of this research

This research aims to development an assay for phosphate in the sea food and frozen food samples. The soluble form of phosphate that can be extracted with distilled water and can be analyzed by using the test kit and the test strips. A mobile phone will be used together with the test kit or strip for on-site determination.