UNIVERSITI PUTRA MALAYSIA

SWEET POTATO JUICE CLARIFICATION BY IMMOBILIZED PECTINASE ON SODIUM ALGINATE - CELLULOSE NAN OP ARTICLES

HO YIK JENG

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HO YIK JENG

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ABSTRACT

Sweet potato juice is the 7th most important food crop in the world. It is rich in vitamins, minerals, fibre, and antioxidants. However, its appearance is not appealing due to its turbidity. Thus, clarification of sweet potato juice is needed. Pectinase was used to clarify sweet potato juice by breaking down the pectin in sweet potato juice which can lead to agglomeration of cloud particle. Yet, pectinase cannot be recycled, only can be used once which makes the clarification of sweet potato juice to be very expensive. Thus, in this research, pectinase from Aspergillus aculeatus was immobilized separately on the surface of sodium alginate with/without cellulose nanoparticle. The method of immobilization used was entrapment technique using sodium alginate and cellulose nanoparticle as supports. 85.3% of pectinase was immobilized onto sodium alginate and had a recovery activity of 60.7%. 49.5% of pectinase was immobilized onto sodium alginate-cellulose nanoparticle and had a recovery activity of 69.0%. The strength of immobilized pectinase with cellulose nanoparticle is stronger than that without cellulose nanoparticle which are 20.97 N and 15.24 N respectively. The clarification of sweet potato juice by using immobilized pectinase with and without cellulose nanoparticle showed 26.9% and 78% in reduction of turbidity. The relative activity of immobilized pectinase with and without cellulose nanoparticle after 6 cycles of reactions are 54.3% and 33.3% respectively.

ACKNOWLEDGEMENT

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CHAPTER 1

INTRODUCTION

This thesis will highlight the immobilization of pectinase on the surface of sodium alginate with/without cellulose nanoparticles. After immobilization, beads with/without cellulose nanoparticles are formed. They are then used to clarify sweet potato juice to reduce its turbidity. The beads with/without cellulose nanoparticles are tested with their strength, ability in juice clarification and reusability.

1.1 Research Background

Pectinases catalyse the hydrolysis of the glycosidic linkage of polygalacturonic acid and they are abundantly used for wines, fruit and vegetable juices clarification.

In the early of 1930s, Germany and United States of America had introduced the method of fruit juice clarification by enzymes. The enzymatic clarification is influenced by few variables such as the temperature and incubation time of the treatment and the concentration of the enzyme (Baumann, 1981; Lanzarini et al., 1989; Neubeck, 1959). Pectinolytic enzymes can result in higher yield and clarified juice. It can also preserve the nutrients, original color and flavor. The clarification process of juice become harder because of the presence of pectin-their fiber-like molecular structure. Pectinase hydrolyzes pectins and cause pectin–protein complexes to flocculate. The resulting juice has a lower amount of pectins and a lower viscosity, which facilitates the subsequent filtration process. However, enzyme is very difficult to be recovered and reused.

Therefore, enzyme immobilization is required. Immobilization is defined as the imprisonment of enzyme in a distinct support that allows the exchange of medium containing substrate. (Sarioglu et al., 2016).

There are different techniques used for immobilization. For example, adsorption, covalent binding, affinity immobilization and entrapment. There are several reviews that discuss available strategies, their advantages and disadvantages have been published (Balcao et al., 2015; Cowan et al., 2013; Garcia-Galan et al., 2011; Hernandez et al., 2011; Khan et al., 2010; Sheldon, 2001; Sumitra et al., 2013). Yet, the selection of immobilization method will depend on the nature of enzyme, cost and its utilization. The matrix supports are grouped into three major categories that are natural polymers, synthetic polymers and inorganic polymers. At present, pectinases have been immobilized onto different types of supports such as silica-coated chitosan, reusable polymer, agar, and entrapped in polyvinyl alcohol sponge and alginate.

Entrapment is the imprisonment of enzymes in microcapsule or lattice type. (Singh, 2009) Efficient encapsulation has been achieved with alginate–gelatin–calcium hybrid carriers that prevented enzyme leakage and provided increased operational stability (Shen et al., 2011). However, sodium alginate is soft and easy to break From the researches from Safwan Sulaiman 2015, 2016 and 2017 (Sulaiman et al., 2015, 2016; Sulaiman, Mokhtar, Naim, Baharuddin, & Sulaiman, 2014), cellulose nanoparticle is highly potential to be applied as natural nanostructure support for enzyme immobilization. Therefore, in my studies, pectinase was entrapped on the surface of calcium alginate-cellulose nanoparticle beads.

1.2 Problem Statements

The strength of sodium alginate is poor. It is a soft setting gelling agent so the pearls will remain soft and jelly like ("Koerner-sodium alginate," n.d.). Therefore, cellulose nanoparticle is added to strengthen the sodium alginate structure.

Moreover, free pectinase can only be used once. It cannot be reused. Thus, enzyme immobilization is required.

1.3 Objective of Study

The objectives of this project are:

- To investigate the strength and reusability of immobilized pectinase on the surface of calcium alginate bead with and without cellulose nanoparticle.
- To evaluate the clarification of sweet potato juices by immobilized pectinase on calcium alginate bead with and without cellulose nanoparticle.

1.4 Scope of Study

In this research, pectinase was immobilized on the surface of sodium alginate-cellulose nanoparticle beads using entrapment method. Sodium alginate is used as the support as it is cheap and easily available. To strengthen the sodium alginate structure, cellulose nanofiber is added as a natural nanostructure support. Therefore, in this research, we study about the reusability of immobilized pectinase on the surface of calcium alginate bead with and without cellulose nanoparticle and also evaluate the clarification of sweet potato juice using sodium alginate with and without cellulose nanoparticle.

1.5 Thesis Structure

The flow of the thesis is as follow:

Chapter 1: Introduction

Chapter 2: Literature Review

Chapter 3: Methodology

Chapter 4: Result and Discussion

Chapter 5: Conclusion and Recommendations

CHAPTER 2

LITERATURE REVIEW

This chapter will highlight the new method of immobilization which is the immobilization on the surface of sodium alginate to form beads from a research "Microfluidic one-step synthesis of alginate microspheres immobilized with antibodies". Also, this chapter will discuss the origin and functions of cellulose nanoparticle which is used to strengthen the structure of sodium alginate in my project. Moreover, the mechanism of clarification of juice will be discussed.

2.1 Pectin

Pectin is important in food processing where it serves as food additives or for pharmaceutical purposes. Pectin is a class of polymeric carbohydrates that make up the cellular structure, which is found extensively in green plants (May, 2000). The bond of the pectin molecule consists of a galacturonic acid chain linked by α-1,4 glucosidic linkages (Flutto., 2003). The pectin molecular structure is shown in Figure 2.1 and the schematic structure of pectin is shown in Figure 2.2. The relative molecular mass of pectin molecules can be as high as 2000000, the corresponding degree of polymerization is more than 1000 units. Ester is the main component of galacturonic acid backbone, in addition to acetyl, amido. Commercial pectin is white or light beige powder, no smell and slightly fruity. Pectin is derived from plant extracts. Therefore, it is completely non-toxic and harmless, and has good gelling, thickening, and stabilizing, emulsifying and suspending functions in food processing.

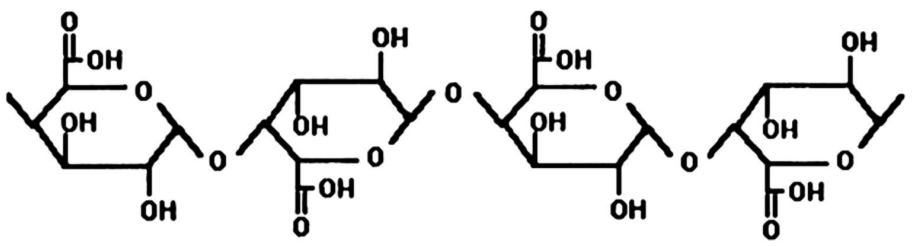


***Figure 2.1**: Pectin molecular structure* (Knee, n.d.)

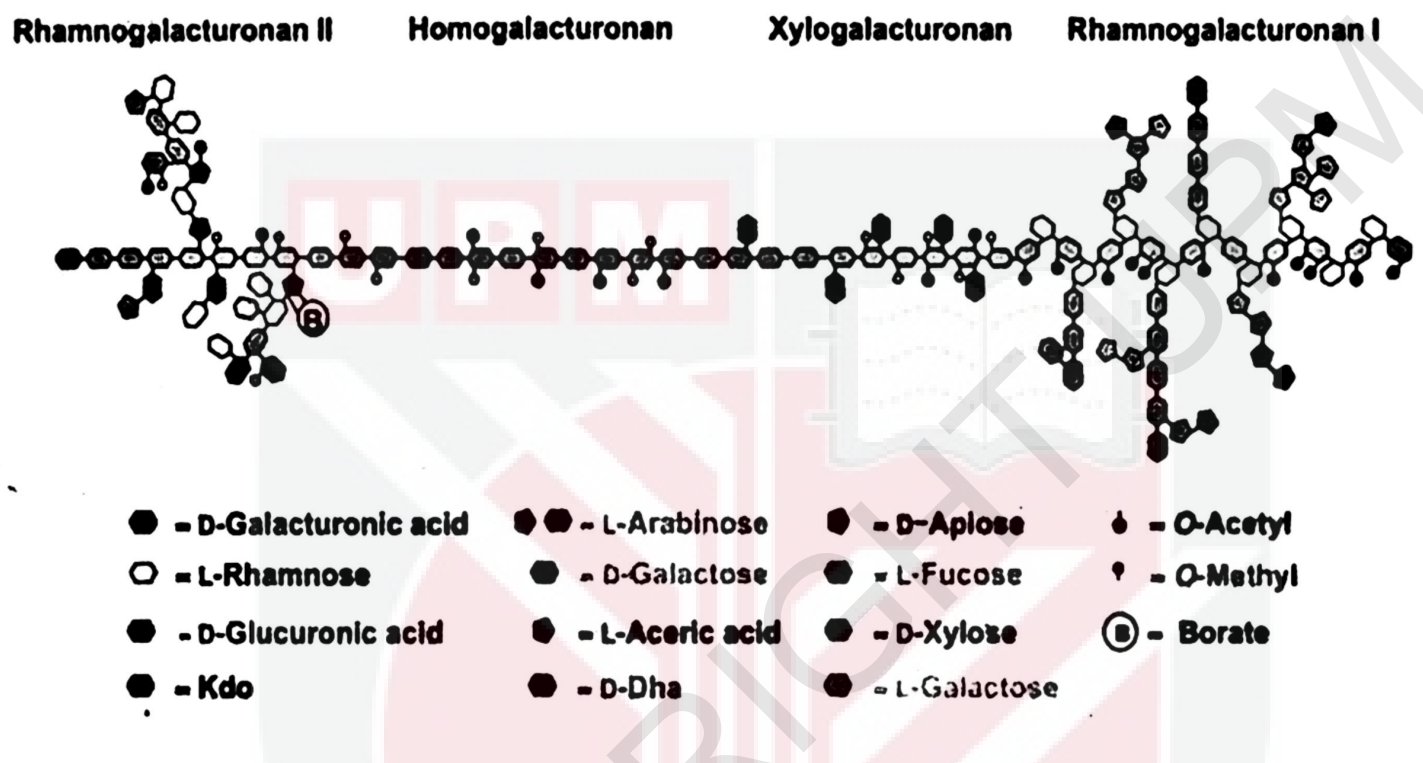


***Figure 2.2**: Schematic structure of pectin. Pectin consists of four different types of polysaccharides, and their structures are shown. Kdo, 3-DeoxyD-manno-2-octulosonic acid; DHA, 3-deoxy-D-lyxo-2-heptulosaric acid. HG and RGI are much more abundant than the other component* (Jesper Harholt, 2010)

Pectin has been widely used in the processing of jams, jelly, fruit products, confectionery, baking, fruit juice drinks, yogurt drinks, yogurt and frozen foods (Smith, 2003). One of the best known applications of the use of pectin is in the jams in traditional with high sugar and it is still one of the largest markets for pectin (May, 2000). The higher the quality of jam, the better the quality of fruits, thus, it requires significantly less pectin. Figure 2.3 indicates which fruits require high, moderate or low additions of pectin.

Relative Pectin Additions to Jams and Preserves

High	*Medium*	*Low*
Cherry	Apricot	Apple
Peach	Blackberry	Blackcurrant
Pear	Loganberry	Damson
Pineapple		Gooseberry
Raspberry		Greengage
Strawberry		Guava
		Plum
		Redcurrant

High fruit: lower pectin addition.
Low fruit: higher pectin addition.

***Figure 2.3**: Relative Pectin Additions to Jams and Preserve* (May, 2000)

Conventionally, immediate turbidity is presumed to be caused by pectin. Visual perception of turbidity and haze in fruit juices is the result of light scattering caused by suspended substances. The immediate turbidity in freshly pressed fruit juices is generally considered to be a result of suspended pectin particles stemming from the plant cell walls, but other disrupted cell walls and cell materials may also contribute to juice turbidity (Pinelo et. al. , 2009).

2.2 Immobilized Enzyme

Enzyme immobilization began during 1950s and 1960s (Abolpour Homaei, 2013). From 1970, the enzyme immobilization technology developed rapidly until 1980. At the beginning of the year, there are about 1,000 published articles and nearly 200 published annually. Patents, reported more than 100 methods of immobilization (Roseveal, 1984). After the mid-1980s, the rate of development of enzyme and cell immobilization research began to slow down. Therefore, some people think that the enzyme immobilization technology should be re-evaluated. The reason is that although a great deal of research work has been done, the practical application of industrial cases

are few (Klibanov, 1983). After nearly 20 years, researches really get the scale of the application of immobilized enzyme, but also limited in glucose isomerase, glucose oxidase and penicillin acylase and a few enzymes.

Enzymes are in class of proteins that have catalytic function and are chemically catalyzed with the fast reaction rate (about 106 ~ 12 times), It has a molecular site selectivity, mild reaction conditions, substrate specificity strong and can be operated in aqueous solution and neutral pH, etc. The enzyme itself can be microbial degradation, in line with the requirements of green chemistry. It has been widely used in many fields such as food, medicine, light industry and agriculture application (OFagainC, 1995).

2.3 Immobilized Enzyme Properties

The behaviour of immobilized enzymes is different from that of dissolved enzymes. This is because of the effects of the support material, or matrix, as well as conformational changes in the enzyme. This modification in the properties may be caused either by the interaction between the immobilized enzyme and the substrate takes place in a micro-environment that is different from the bulk solution or the changes in the intrinsic activity of the immobilized enzyme (Beatriz Brena et. al., 2013). Properties of enzyme, for example specific activity, pH optimum, Km and stability change significantly upon immobilization ("Properties of Immobilized Enzyme," 2017).

Stability of immobilized enzyme refers to the retention of activity of an enzyme when in use. When an enzyme is immobilized onto a support material, a diffusion layer is formed around the particles, and this make the enzyme strong. From the existing

reports, most enzymes that are immobilized showed that their stability has increased, this advantage the industrial use of immobilized enzyme (Sumitra et. a;.,2003). Immobilized enzyme has improved its operational stability at higher temperature and in the presence of organic solvents (Mateo et. al., 2007). Therefore, the concept of stabilization has become one of the important driving force for enzyme immobilization. Studies have been carried out by several authors using different immobilization methods, and the results show there is a correlation between stabilization and the number of bonds to the matrix (Koch-Schmidt A, 1977).

Enzyme catalysts have lower activation energy, and more sensitive to heat and thus, the higher the temperature the faster the reaction ("Enzymes," n.d.). Since enzymes are made of proteins, their resistance to heat is very poor. They are very sensitive to heat, so adapting to the temperature range is narrow. Generally, enzymes cannot operate above 65 °C. After immobilizing the enzyme, its heat resistance will be improved, especially the use of cross-linking immobilized enzyme, the thermal stability can be greatly enhanced (Royhaila et. al., 2015). The schematic representation of thermal unfolding of an enzyme is shown as Figure 2.4.

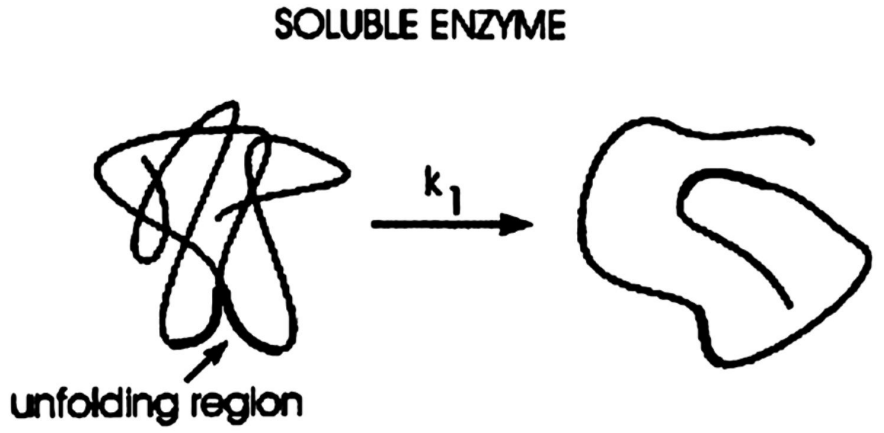


***Figure 2.4**: Schematic representation of thermal unfolding of an enzyme*

(RenateUlbrich et. al., 1999)

2.4 Method of Immobilization

Immobilized enzyme has been defined as enzymes that are confined or localized physically, which can be used repeatedly and continuously with the retention of catalytic activity (Chibata et al., 1978). There are several methods to immobilize enzyme (Messing et al., 1975). However, they can be classified as follows (Bowers, 1980):

1. Covalent bonding of enzyme.
2. Intermolecular cross-linking of enzyme molecules using multi-functional reagents.
3. Adsorption of the enzyme onto a water-insoluble matrix.
4. Entrapment of the enzyme inside a water-insoluble polymer lattice or semi-permeable membrane.

The techniques for enzyme immobilization is as shown in Figure 2.5 and the schematics diagram of the techniques is as shown in Figure 2.6.

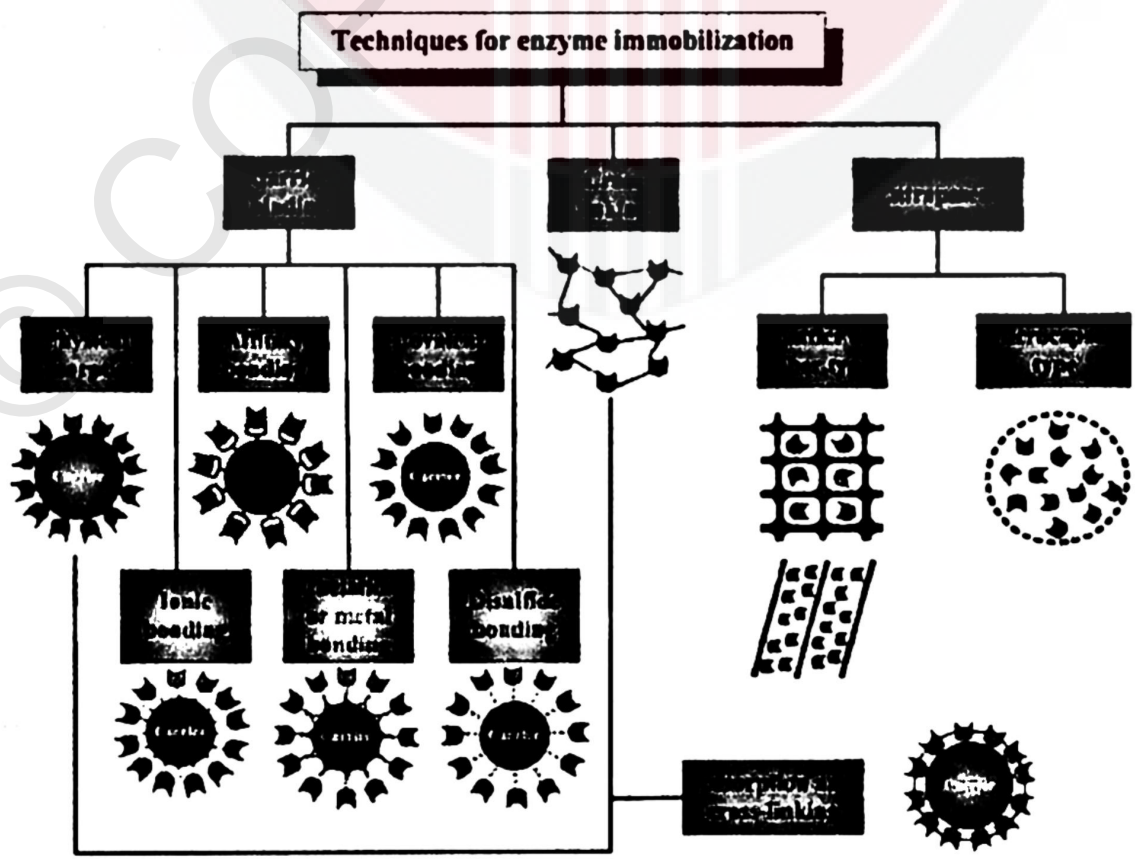


***Figure 2.5**: Techniques for enzyme immobilization* (Mokhtar, 2016)

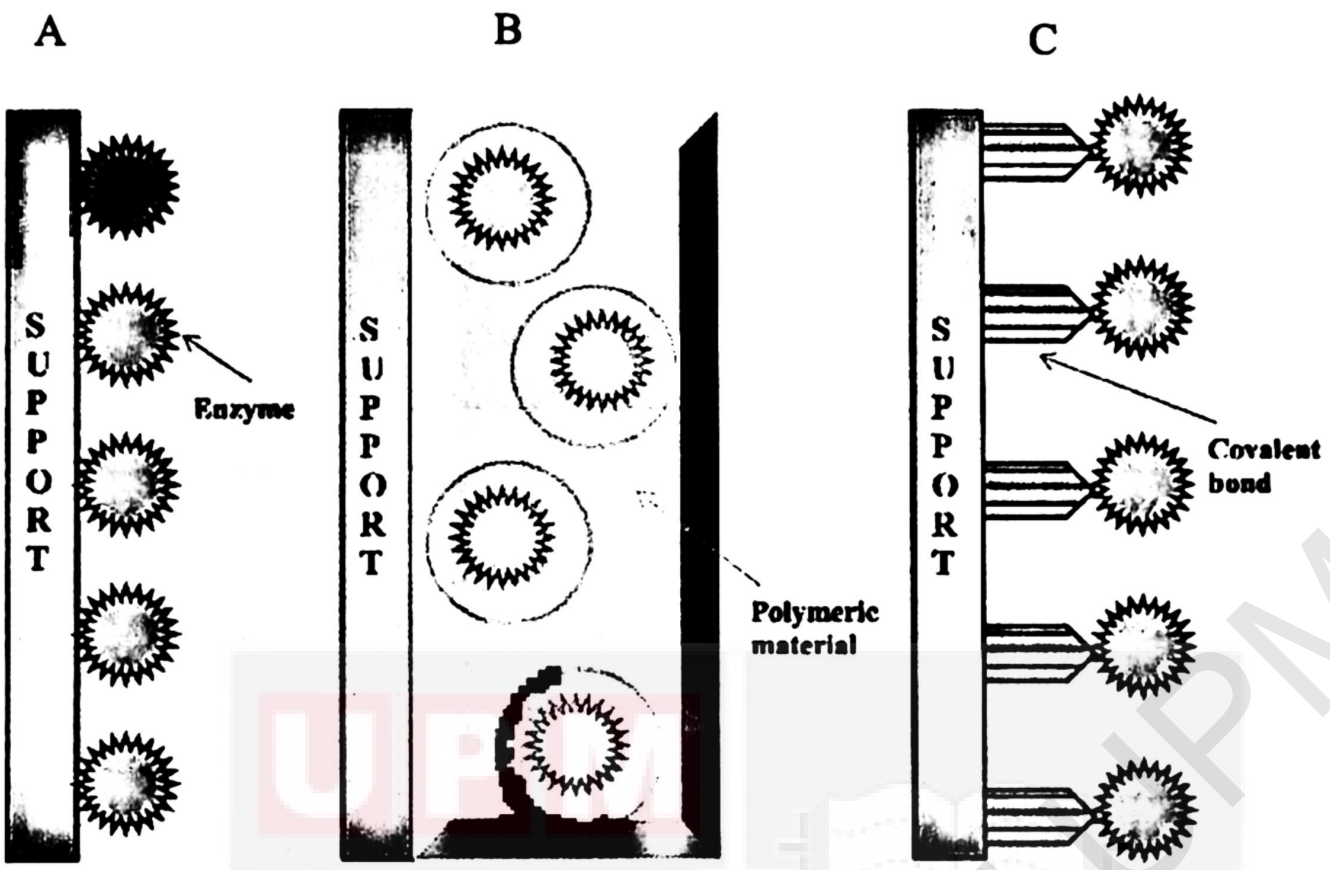


***Figure 2.6**: Schematics of the three most common enzyme immobilization techniques:(A) physical adsorption, (B) entrapment and (C) covalent attachment/cross-linking* (Spahn, 2008)

Enzyme immobilization refers to the use of organic or inorganic solid material as a carrier. The enzyme is entrapped or covalent bonded or adsorb to the surface and pores of the carrier to increase its catalytic activity, and can be recycled and reused. Enzymes can also be formed by cross-linking of the enzyme molecules to an immobilized enzyme called carrierless enzyme immobilization (cross - linkedenzymecrystals, CLEC) reported in the early 1990s (ClairNL et. al., 1992). However, each techniques has their own advantages and disadvantages. A summary has been published as shown in Table 2.1 (Beatriz Brena et. al., 2013).

Table 2.1: *Advantages and disadvantages of the main enzyme immobilization methods* (Beatriz Brena et .al., 2013)

Methods and binding nature	Advantages	Disadvantages
Physical adsorption: Weak bonds: hydrophobic, Van der Waals or ionic interactions.	• Simple and cheap • Little conformational change of the enzyme	• Desorption • Nonspecific adsorption
Affinity: Affinity bonds between two affinity partners	• Simple and oriented immobilization • Remarkable selectivity	• High cost
Covalent binding: Chemical binding between functional groups of the enzyme and support	• No enzyme leakage • Potential for enzyme stabilization	• Matrix and enzyme cannot be regenerated • Major loss of activity
Entrapment: Occlusion of an enzyme within a polymeric network	• Wide applicability	• Mass transfer limitations • Enzyme leakage
Cross-linking: Enzymes molecules are cross-linked by a functional reactant	• Biocatalyst stabilization	• Cross-linked biocatalysts are less useful for packed beds.

		• Mass transfer limitations Loss of activity

2.5 Entrapment Type

Entrapment method is to wrap the enzyme in a gel-forming mesh network structure, or semipermeable membrane polymer membrane to make it fixed where the enzyme activity is retained (Driskoll, 1976).

The entrapment method can be divided into two types: fibre entrapping and micro-encapsulation (Brena et. al., 2013). The former type is enzymes are embedded in the fine network of polymer gels; while the latter type is enzyme packets buried in a polymer semipermeable membrane prepared microcapsules.

Referring to a research, "Microfluidic one-step synthesis of alginate microspheres immobilized with antibodies" (Chen et. al., 2013), a new immobilization process was introduced using entrapment technique. From the research, antibodies were immobilized on the surface of support which is sodium alginate, and this comes to the objectives of my research. Immobilization on the surface is to overcome the difficulties of entrapment technique immobilization which is mass transfer limitation. The immobilization of antibodies on the surface of support from this research was shown in Figure 2.7.

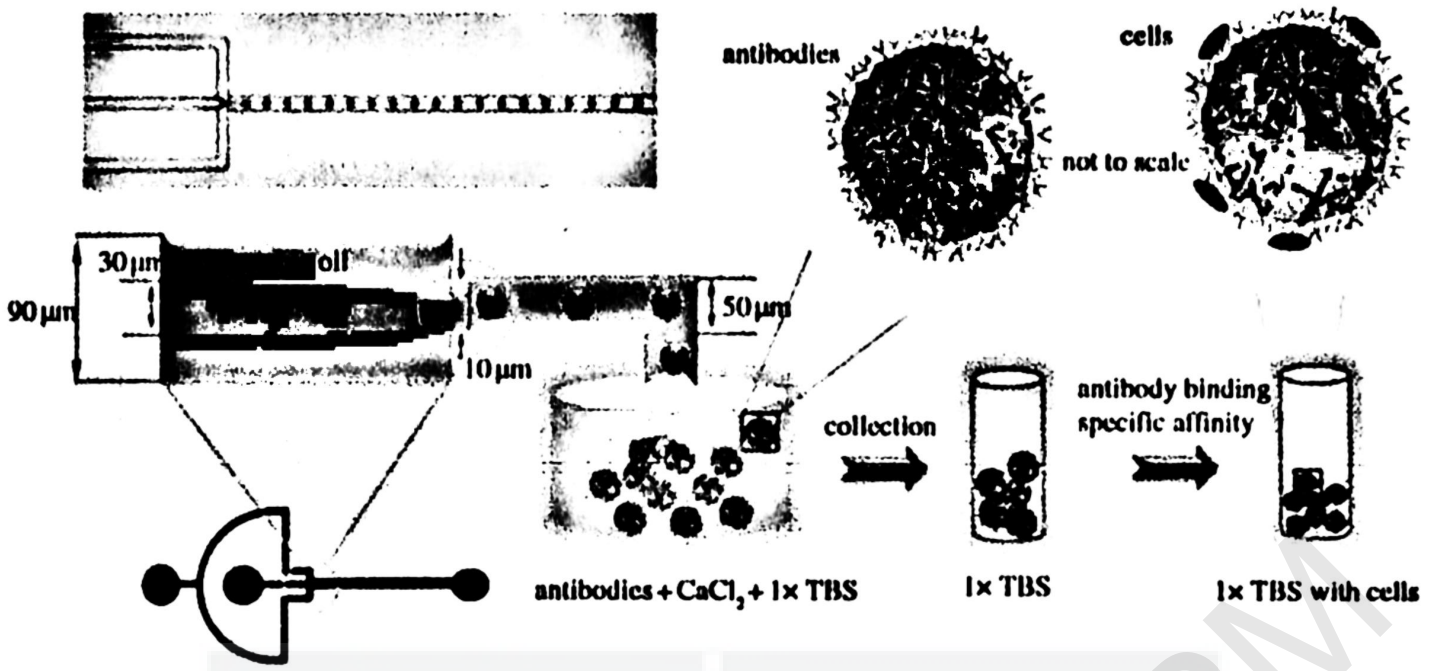


***Figure 2.7**: Immobilization of antibodies on the surface of sodium alginate* (Chen et. al., 2013)

2.6 Sodium Alginate

Alginate, commercially available as alginic acid commonly called as sodium alginate (Wang, n.d.). Sodium alginate is the sodium salt of alginic acid with a chemical formula of $(C_6H_7NaO_6)_n$. The structural formula of sodium alginate is as shown in Figure 2.7. ("Sodium Alginate," 1995)

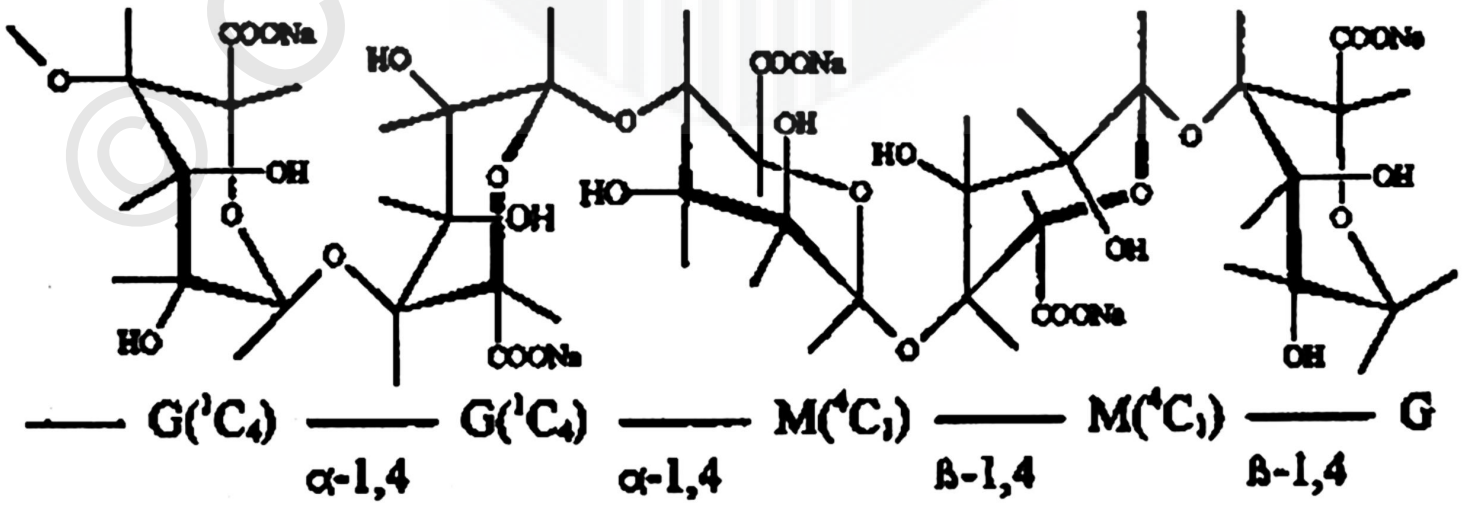


***Figure 2.8**: Structural Formula of Sodium Alginate* ("Sodium Alginate," 1995)

Sodium alginate occurs as white to yellowish brown filamentous, grainy, granular or powdered forms. It can be used as stabilizer, thickener, gelling agent and emulsifier. Sodium alginate can be either water soluble or insoluble in which depends on the type of associated salt (Wang, n.d.). It dissolves slowly in water, forming a viscous solution whereas insoluble in ethanol and ether ("Sodium Alginate," 1995). Equation 1 shows the reaction when sodium alginate reacts with calcium ions. The alginate polymer itself is anionic.

$$2\ Na(Alginate) + Ca^{++} \text{-------} > Ca(Alginate)_2 + 2\ Na^{+} \qquad (2.1)$$

Alginate is currently popularly used in food, pharmaceutical, textile, and paper products (Wang, n.d.). The properties of alginate utilized in these products are thickening, stabilizing, gel-forming, and film-forming.

Alginate can be found in brown algae's cell wall. Sodium alginate is recovered from seaweed by converting the alginate salts into sodium salt, then dissolve the salt in water, and finally removed the seaweed by filtration. The alginate must then be recovered from the aqueous solution. However, since the solution is very dilute and using evaporation method to recover is too costly, there are two different ways to recover the alginate (McHugh, 1987). The production of sodium alginate using the two different ways is shown in Figure 2.8.

The first is to add acid, which causes alginic acid gel to form, then some of the water must be removed from this. After that, alcohol followed by sodium carbonate are added which converts the alginic acid into sodium alginate. The second way of recovering the sodium alginate is by adding calcium salt. This leads to the formation of fibrous calcium alginate. Then, the calcium alginate is suspended in water and to convert it into alginic acid, acid is added. This fibrous alginic acid can be easily separated. Then, it was mixed with alcohol, followed by gradually added sodium

carbonate to the paste until all the alginic acid is converted to sodium alginate (McHugh, 1987).

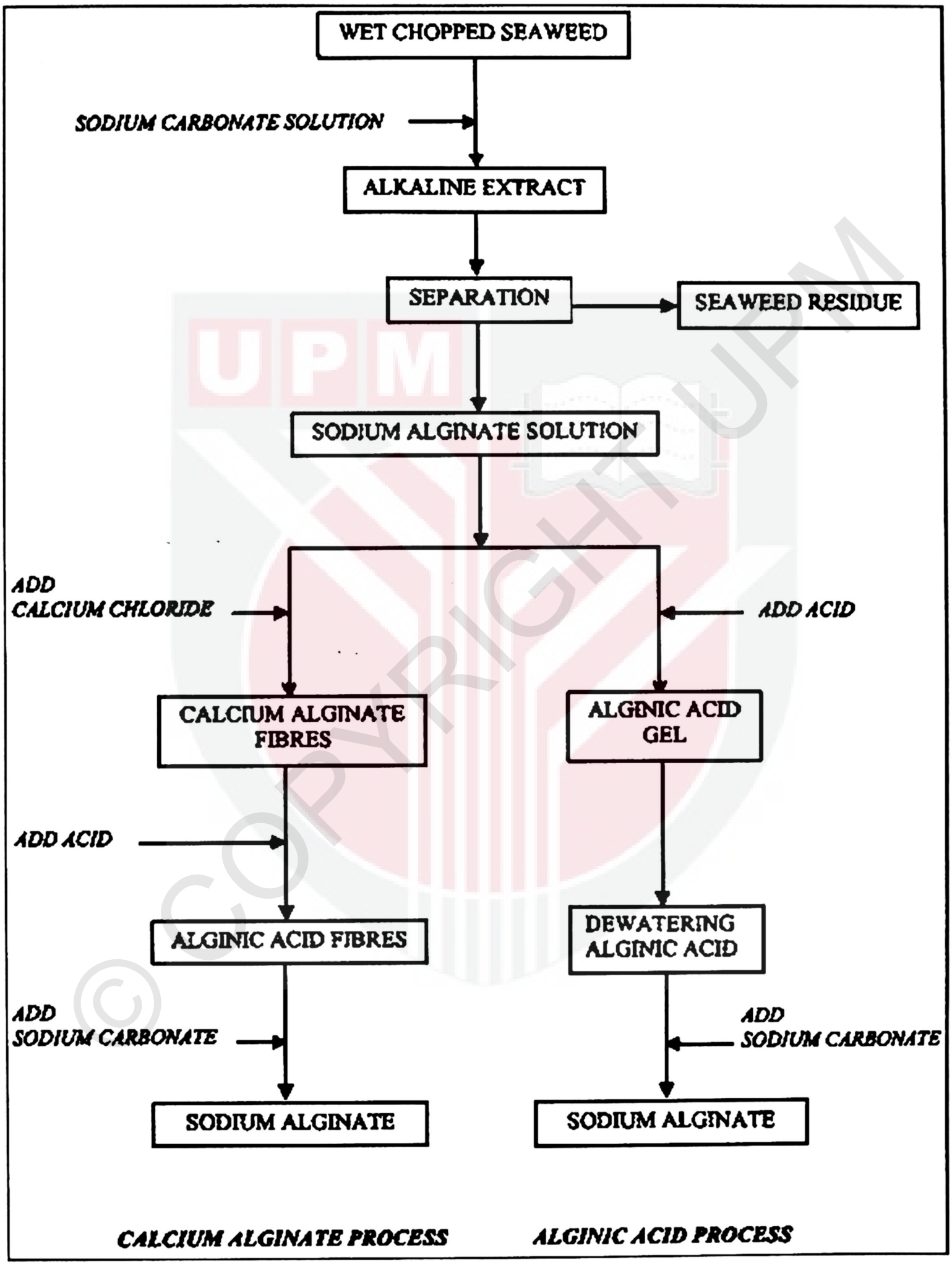


***Figure 2.9**: Flow chart for the production of sodium alginate* (McHugh, 1987)

2.6.1 Uses of sodium alginate for immobilization

As mentioned in 2.6, sodium alginate can be used as stabilizer, thickener, gelling agent and emulsifier. Sodium alginate can also be used as a support for enzyme. This can be achieved by entrapping them on a membrane. Calcium alginate beads were one of the first entrapment methods. This is done by suspending a cell in a solution of sodium alginate and this is added to a calcium chloride solution drop by drop. Free moving cells may have good activity for only 1 - 2 days, whereas an immobilized cell can lasts for 30 days (McHugh, 1987).

2.7 Cellulose nanoparticle (CNP)

Cellulose is the most abundant, renewable, and sustainable biopolymer on earth. It is present in plants, tunicates, and some bacteria (John Rojas, 2015). Nanocellulose is derived from natural materials. It can also be produced from sugars through biotechnological processes using bacterial nanocellulose. At present, there are various commercial applications for nanocellulose. For instant, it can be used as a barrier material in food packaging material by the treatment of wood and hardboard with nanocellulose. Nanocellulose can store large amount of waters which make it suitable to be used as hygiene products for medical applications. As for dietary supplements, nanocellulose is used as a thickening agent and stabiliser.

2.8 Sweet Potato

Sweet potato (Ipomoea batatas L.) Lam. is the 7th most important food crop in the world (FAO, 1997). There are more than two billion people in Asia, Africa, and Latin

America will depend on these crops for food, feed and income by 2020 (Scott et. al., 2000).

Several publications have been showing that the vitamins and minerals in sweet potatoes are comparable with a lot of different types of fruits. Sweet potatoes are rich in dietary fibre, minerals, vitamins, and antioxidants, such as phenolic acids, anthocyanins, tocopherol and β-carotene (Klibanov, 1983). Despite a healthy contributing crop, sweet potato is also claimed as a starchy staple in countries other than China and Japan (Padmaja et. al., 2012).

Nowadays, the demand for these drinks and beverages is largely based on their nutritive value, flavor, aroma and color (McLellan, 1990). Therefore, juice has been prepared out of sweet potato culls and has been reported that sweet potato juice may be consumed as a beverage or combined with other juices to form a variety of juice blends (Coggins et. al., 2003).

2.9 Clarification of juice using pectinase

Most of the industrial processed fruit based beverages, for example fruit juices and wines, are clarified during processing. This is to produce a final product with clear sight of vision. Suspended substances with the result of scattering of light have caused undesirable turbidity and haze in the final products. (Pinelo et. al. , 2009).

The use of enzymes in clarification of various types of fruit juices has contributed in increasing the yield and production of them in the industry. In the early thirties, the practice of fruit juice clarification using enzymes was introduced in German and the United States of America (Tapre et. al., 2013).

The application of pectinase in fruit juice technology has been dealt in detailed by Neubeck (Neubeck, 1959a). The use of pectinase to extract juice from apple is a well-known school practical investigation. Pectinase was first used industrially to clarify apple juice (Ncbe, 2000). Pectinases are able to reduce viscosity and forming a less gel juice by degrading pectin-containing substances into smaller fractions (Screenath et. al., 1987).

To produce a clear juice, depectinization has to be done. Even though centrifugation of fruit juice has been carried out to remove the suspended particles in the fruit juice, there are still some soluble pectin remains in the juice. The pectin remained is one of the biggest factor of turbid juice. Depectinization has two effects. It will degrade the viscous soluble pectin and also cause the aggregation of cloud particles. Referring to Figure 2.10, a pectin coating is formed around proteins. Since the juice is acidic, therefore when pectin molecules are in the environment, it will carry a negative charge which causes them to repel one another. Then, pectinase degrades this pectin and thus part of the positively-charged protein inside is exposed. Finally, the electrostatic repulsion between the cloud particles reduced and thus they clump together. This is the reason why the juice will become clearer as the large cloud particles can be easily eliminated using centrifugation method or filtration method (Ncbe, 2000).

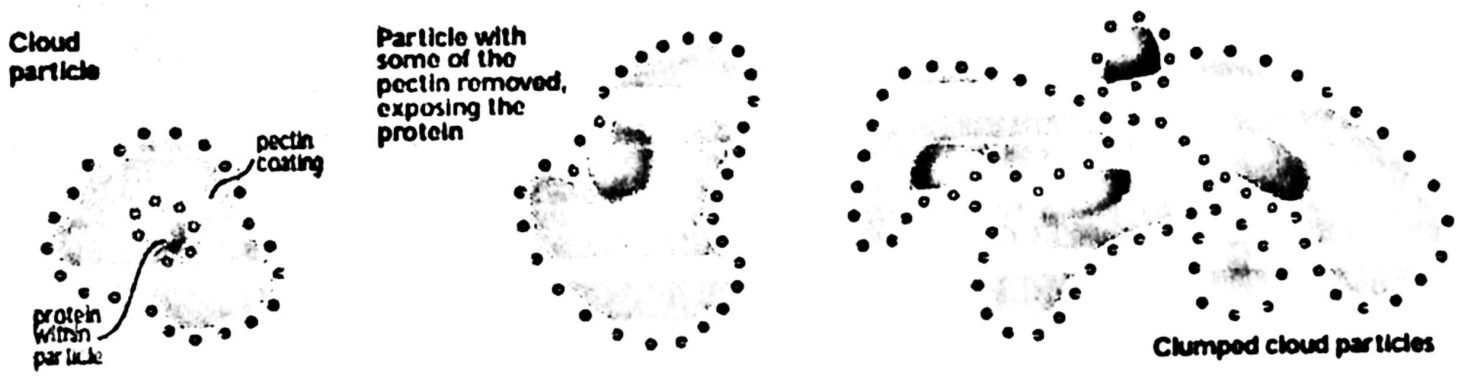


Figure 2.10: *The breakdown of pectin which leads to agglomeration of cloud particle* (Ncbe, 2000)

2.10 Concluding Remark

Since the immobilization method is using the immobilization of antibodies on the surface of sodium alginate, thus in my research, immobilization of pectinase on the surface of sodium alginate and cellulose nanoparticle was done.

CHAPTER 3

METHODOLOGY

This chapter will highlight the method of pectinase immobilization on the surface of sodium alginate and cellulose nanoparticle to form beads. After the beads were formed, the method of clarify sweet potato juice using the beads were shown. Then, the method to identify immobilization yield were shown using pectinase assay and pectinase loading. The method to test the reusability of the beads were shown too.

3.1 Materials

Pectinase, Sodium alginate, Calcium chloride and albumin bovine serum were purchased from Sigma, USA. Cellulose nanoparticle was borrowed by a phd student in UPM, Safwan. Sweet potatoes were purchased from local super market. Coomassie Bue G-250 was purchased from Fisher scientific, UK. Pectin powder was bought online from Take it global Sdn. Bhd. Sodium Hydroxide was purchased from R&M Chemical, Malaysia. Phosphoric acid, phenolphthalein, etil alcohol 95%. Monopotassium phosphate and Dipotassium phosphate was purchased from Systerm, Malaysia.

Table 3.1:Material used and its source

No	Item	Source
1	Peptinase (from *Aspergillus aculeatus*)	Sigma-P2611, USA
2	Sodium alginate	Sigma-W201502, USA
3	Calcium Chloride, $CaCl_2$	Sigma C1016, USA
4	Cellulose nanoparticle	Phd UPM- Safwan
5	Sweet potatoes (gendut)	Local super market
6	Coomassie Blue G-250	Fisher scientific, UK
7	Pectin powder	Take it global Sdn. Bhd.
8	Albumin bovine serum	Sigma A3059, USA
9	Phosphoric acid	Systerm, Malaysia
10	Phenolphthalein	Systerm, Malaysia
11	Etil Alcohol 95%	Systerm, Malaysia
12	Sodium Hydroxide, NaOH	R & M Chemicals, Malaysia
13	Monopotassium phosphate, KH2PO4	Systerm, Malaysia
14	Dipotassium phosphate, K2HPO4	Systerm, Malaysia

3.1.1 Cellulose Nanoparticle

0.1 g cellulose nanoparticle was added to strengthen the structure of sodium alginate. It is because when < 0.05 g of cellulose nanoparticle was used, the bead formed is jelly-like which is only calcium alginate bead was seen. When the amount of cellulose nanoparticle used > 1 g, the agglomeration of beads happened as shown in Figure 3.1.

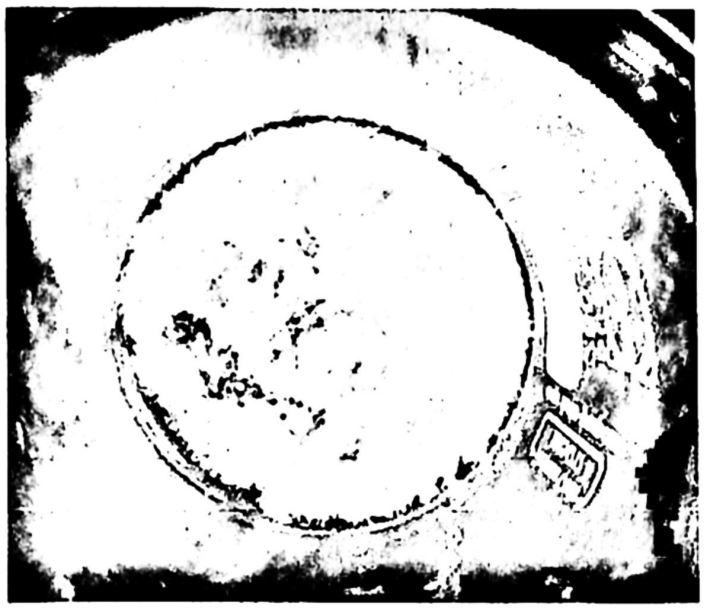


Figure 3.1: Agglomeration of bead

3.2 Instruments and Apparatus

Weighing balance model ER-120A was used to weigh 0.1 g cellulose nanofiber, 0.02 g calcium chloride and 0.15 g sodium alginate and so on. UV/VIS spectrophotometer with model Ultrospec 3100 pro was used to measure absorbance values. Centrifuge was used for sedimentation. Incubator with model New Brunswick Scientific was used to incubate enzyme with juice at 55°C which is pectinase favourable temperature for 1 hour. Texture analyzer with model TA. Xt plus was used to analyse the strength of beads formed.

Table 3.2: Instruments used and its source and model

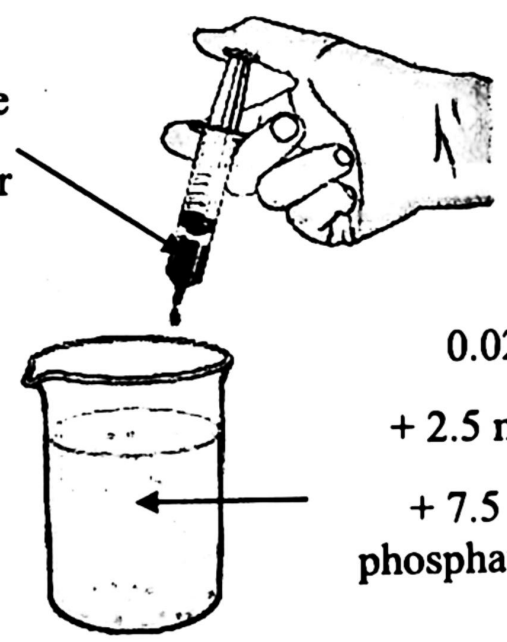
No	Item	Model	Source
1	Weighing balance	ER-120A	A&D, Japan
2	UV/VIS Spectrophotometer	Ultrospec 3100 pro	Artisan Technology Group, US
3	Centrifuge	Universal 320	Hettich, Germany
4	Incubator	New Brunswick Scientific	Lab Companion, USA

5	Texture analyzer	TA. XT plus	Stable Micro Systems, USA

3.3 Immobilization of Pectinase

Sodium alginate will be dissolved in distilled water to give a final concentration of 1.5% (w/v). 0.1 g of cellulose nanoparticle will be prepared according to our previous studies (Safwan et al., 2015, 2016, 2017), and then it will be added to the alginate solution. The alginate-nanoparticle solution will be dropped through a nozzle of the 2 mm using syringe pump through into 2% $CaCl_2$ solution with 2.5 mL pectinase solution and 0.05 M potassium phosphate buffer (pH 6.0) which leads to the formation of calcium-alginate-nanoparticle beads. The process is shown as in Figure 3.2.A. The mixture was gently stirred and stored overnight at 4 °C. The mixture was centrifuged for 10 minutes. The supernatant was removed as in Figure 3.2.D and the measurement was done by using UV/VIS spectrophotometer after adding Bradford reagent. To make sure that there is no enzyme activity detected in the filtrates, the immobilized enzyme was washed with the same phosphate buffer and the measurement was done by using UV/VIS spectrophotometer. The overall process of pectinase immobilization is as shown in Figure 3.2.

A) Step 1:



B) Step 2:

The mixture was gently stirred and stored overnight at 4 °C.

C) Step 3:

The mixture was centrifuged for 10 minutes.

D) Step 4:

The supernatant was removed and the measurement was done by using UV/VIS spectrophotometer after adding Bradford reagent.

E) Step 5:

The immobilized enzyme was washed with the same phosphate buffer and the step 4 was repeated until no enzyme activity was detected.

***Figure 3.2**: Immobilization of pectinase A) Formation of alginate beads; B) Storage of mixture; C) Centrifugation of mixture; D) Removal of supernatent*

3.3.1 Immobilization of Pectinase on the surface of sodium alginate and cellulose nanoparticle

Referring to Figure 3.3, pectinase was immobilized on the surface of sodium alginate and cellulose nanoparticle and form calcium alginate-nanoparticle beads.

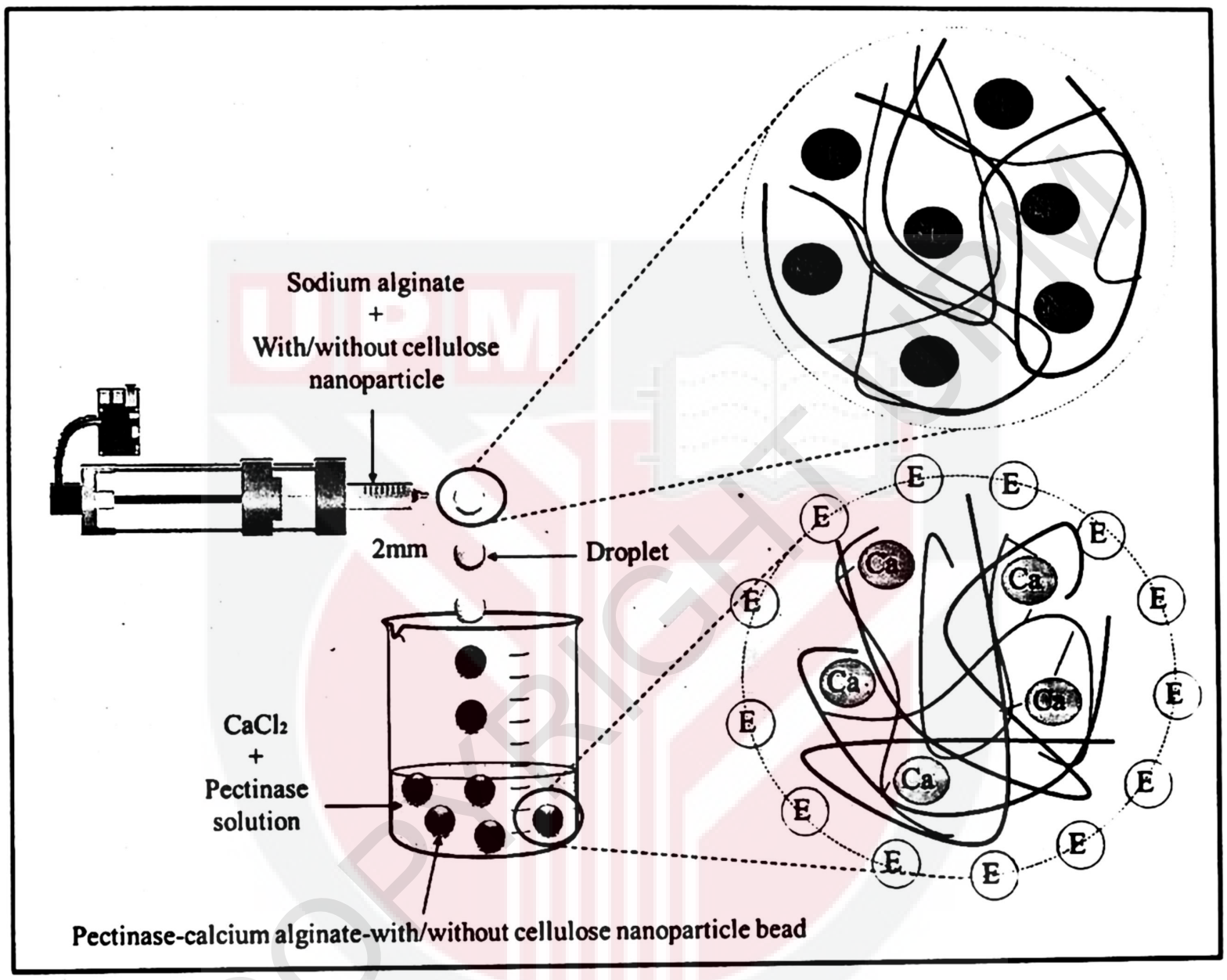


***Figure 3.3**: Immobilization of pectinase on the surface of sodium alginate and cellulose nanoparticle*

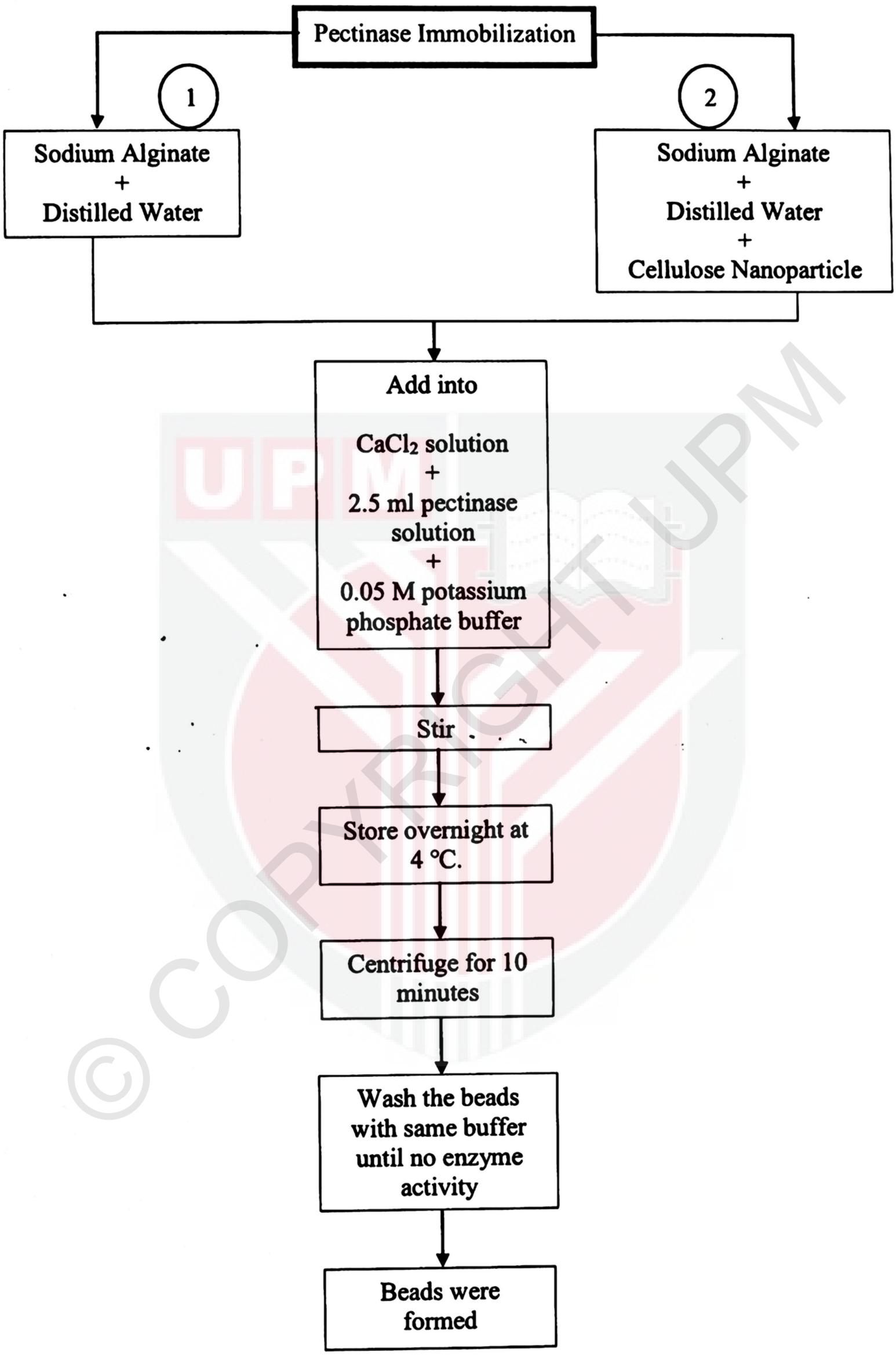


***Figure 3.4**:Steps for pectinase immobilization*

3.4 Pectinase Assay

Pectinase activity was based on the increase of the free carboxyl group of galacturonic acid due to pectin degradation. In this work, it was measured by NaOH titration in the presence of phenolphthalein as indicator. 20 mL of 1% (w/v) pectin was dissolved in 0.15 M NaCl (pH 7) and 2 mL solution containing pectinase was added and incubated for 1 h at 55 °C. After that, the solution was titrated by 0.02 M NaOH to reach pH 7, when the color of phenolphthalein changed from colorless to pink. The heated enzyme solution was also used as control (blank). The pectinase activity can be expressed as Eq.3.1. One unit of enzyme activity (U) was defined as the amount of enzyme that produced 1μmol H^+ (from galacturonic acid); 1μmol H^+ required 1μmol OH^- to be neutralized.

$$H^+ + OH^- \rightarrow H_2O$$

$$\text{Pectinase activity } \left(\frac{\mu mol-H+}{ml.min} \text{ or } \frac{U}{ml}\right) = \frac{1000 \times (Vs-Vb) \times c}{V \times t} \quad (3.1)$$

Where,

V_s = NaOH used to titer the sample (ml)

V_b= NaOH used to titer the blank (ml)

c = NaOH concentration (N or mmol-OH^{-1} /ml)

V = Reaction volume (ml)

t = Reaction time (min)

3.5 Pectinase Loading

Protein loading was measured by using Bradford method, where bovine serum albumin was used as the standard protein. Bradford reagent was prepared where 100 mg of Coomassie Blue G250 was dissolved in 50 mL of 95% ethanol. The solution

was then mixed with 100 mL of 85% phosphoric acid and made up to 1 L with distilled water. Bradford reagent was added to the protein solution, and the measurement was done by using UV/VIS spectrophotometer at wavelength of 595 nm. The protein loading after enzyme immobilization was calculated as:

$$\text{Enzyme Loading (\%)} = \frac{Po - Pe}{Po} \times 100 \qquad (3.2)$$

Where,

PL = protein loading on the support (%)

P_0 = amount of protein added before immobilization (mg/ support)

P_e = total amount of protein in all washing after immobilization (mg/support)

3.6 Clarification of sweet potatoes

3.6.1 Preparation of sweet potato juice

Sweet potatoes purchased from local super market were cut into small pieces. Then, they were ground in a household blender. The mash was squeezed through a muslin cloth and centrifuged at 9,000 rpm for 10 min. The hazy sweet potato juice was stored in refrigerator.

3.6.2 Turbidity of sweet potato juice

The wavelength of sweet potato juice was screened by UV/VIS Spectrophotometer at wavelength 600 nm. Then, 0.1 mL of free pectinase was added to sweet potato juice and incubated for 1 h at 55 °C. Then, each juice sample was taken at different reaction times (10 min intervals) and then centrifuged at 5,000 rpm for 10 minutes. The absorbance of supernatant was measured by using UV/VIS Spectrophotometer at wavelength 600 nm. The steps were repeated with 0.1 g of immobilized pectinase. The steps of clarification of sweet potato juice were shown in Figure 3.5.

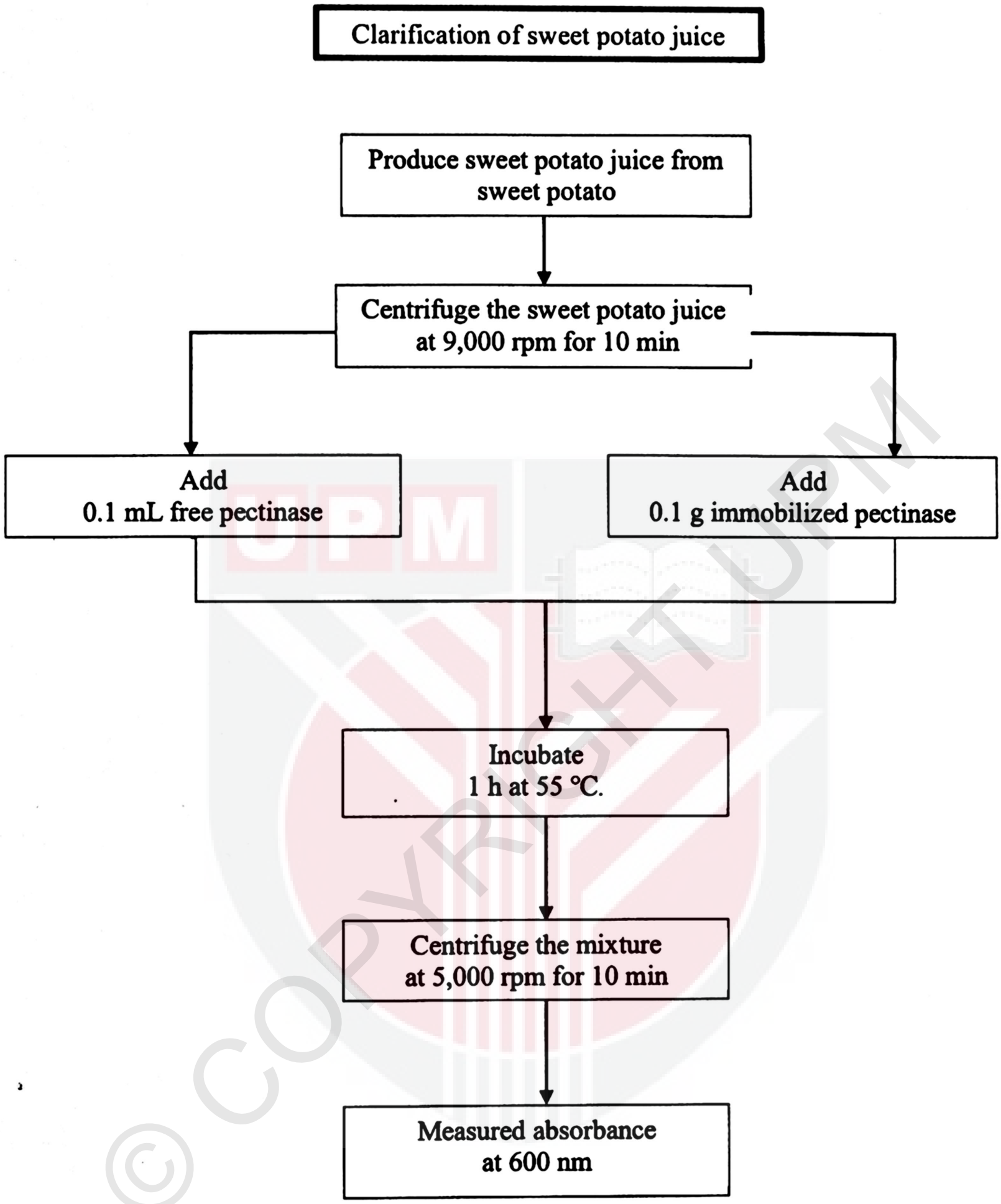


***Figure 3.5**:Steps for sweet potato juice clarification*

3.7 Recyclability of immobilized pectinase

To study the reusability of immobilized pectinase, the enzyme was separated by filtration through a sieve and reused with fresh sweet potato juice. The enzyme recycle was repeated for 6 cycles. The figure is as shown as Figure 3.6. The enzyme entrapped bead in the first run of freshly prepared was defined as 100%.

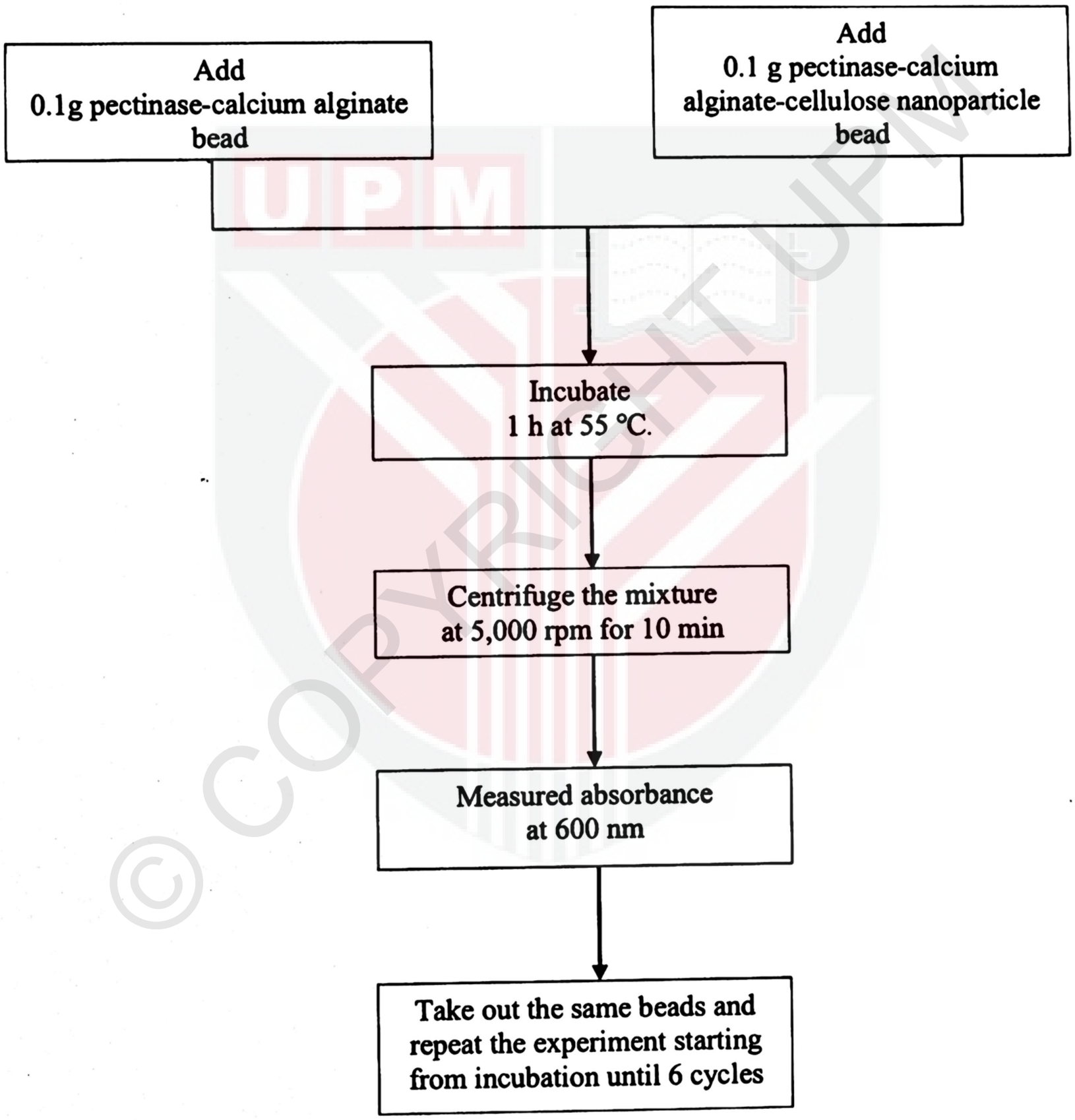


***Figure 3.6**:Reusability of immobilized pectinase*

3.8 Summary

Pectinase was immobilized on the surface of sodium alginate and cellulose nanoparticle using entrapment technique to form beads. Cellulose nanoparticle was used to strengthen the structure of sodium alginate. The immobilized beads formed were then added into sweet potato juice to test with its reusability and its ability to clarify sweet potato juice.

CHAPTER 4

RESULT AND DISCUSSION

In this chapter, the immobilization activity of calcium alginate beads with/without cellulose nanoparticle was evaluated using pectinase assay. The strength of calcium alginate beads with/without cellulose nanoparticle was tested using texture analyzer. Then, the recyclability and clarification of the beads with/without cellulose nanoparticle were evaluated.

4.1 Pectinase Immobilization

In this study, pectinase was immobilized onto the surface of supports – sodium alginate with/ without cellulose nanoparticle using entrapment technique. Two different beads were formed, which were calcium alginate beads and calcium alginate-nanoparticle beads.

4.1.1 Pectinase Immobilization onto Sodium Alginate with/ without cellulose nanoparticle

The percentage of immobilization yield is correlated to the interaction of pectinase with sodium alginate and cellulose nanoparticle. As depicted in Table 4.1, 85.3% of pectinase was immobilized onto sodium alginate and had a recovery activity of 60.7%. The pectinase activity was determined using titration method as shown in Chapter 3, section 3.4 and the calculation is based on equation 3.1. The result of titration is shown in Appendix E. The immobilization yield is considered high. This indicates the successfully entrapment of pectinase onto sodium alginate with 60.7% activity

retained. The calcium-alginate bead formation is as shown in Figure 4.1. The bead was like jelly. This is because sodium alginate is soft, therefore the bead formed is soft. The force needed to break the bead was 15.24 N as shown in Table 4.2 and a graph was drawn as shown in Figure 4.3. The test was carried out using texture analyzer.

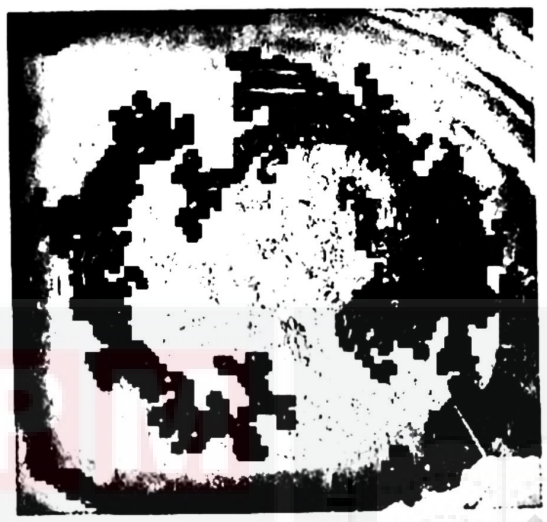


***Figure 4.1**: Immobilized Pectinase with Sodium Alginate*

To strengthen the structure of sodium alginate, cellulose nanoparticle was added. Pectinase was entrapped onto the surface of sodium alginate and cellulose nanoparticle to form calcium alginate-nanoparticle beads. Referring to Table 4.1, 49.5% of pectinase was immobilized onto calcium alginate-cellulose nanoparticle and had a recovery activity of 69.0%. The calcium-alginate-nanofiber beads formed is shown in Figure 4.2. The force needed to break the bead was 20.97 N as shown in Table 4.2 and a graph was drawn as shown in Figure 4.3.

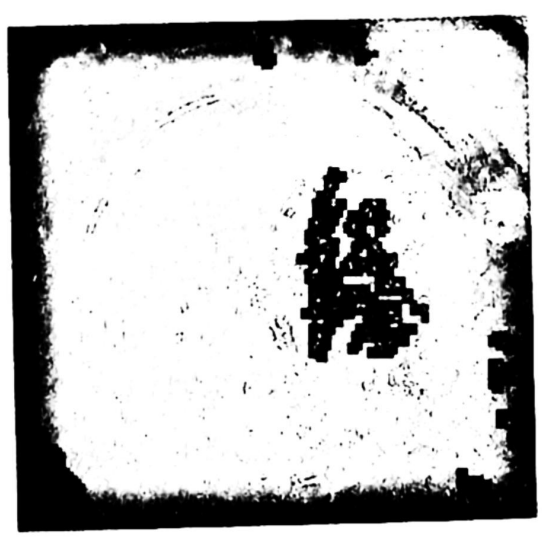


***Figure 4.2**: Immobilized Pectinase with Sodium Alginate and Cellulose Nanoparticle*

Table 4.1: Immobilization of Pectinase enzyme with alginate

Enzyme added		Unbound enzyme		Immobilized enzyme		Immobilization yield	
Protein (Po) (mg/g support)	**Activity (A)** (U/g support)	**Protein** (mg/g support)	**Activity (U)** (U/g support)	**Protein (P)** (mg/g support)	**Activity (I)** (U/g support)	**Binding (%)** (P/Po) x 100	**Activity (%)** (I/A) x 100
Without Cellulose Nanoparticle (0.15 g support)							
464.57	1613.67	68.26	361.50	396.31	978.83	85.31	60.66
With Cellulose Nanoparticle (0.25 g support)							
464.57	968.20	48.82	805.80	229.92	668.10	49.49	69.00

Table 4.2: Strength analysis of immobilized pectinase on sodium alginate with/without cellulose nanoparticle

Type of support	Force (N)
With cellulose nanoparticle	20.97
Without cellulose nanoparticle	15.24

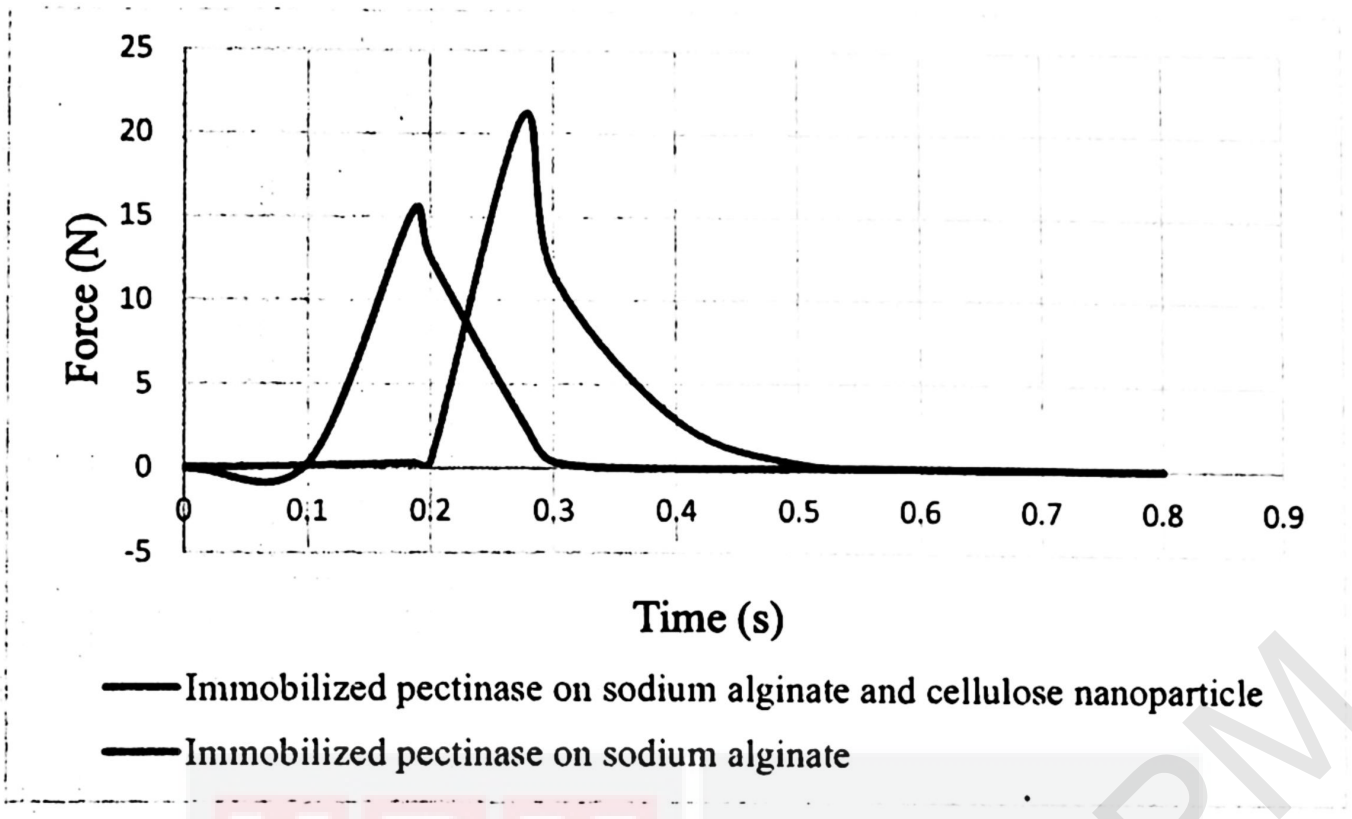


***Figure 4.3**: Strength analysis of immobilized pectinase on sodium alginate with/without cellulose nanoparticle*

4.2 Comparison between the entrapment of pectinase onto sodium alginate with/ without cellulose nanoparticle

Comparing both, entrapment onto calcium alginate-nanoparticle beads (69.0%) has a higher immobilization activity than onto calcium alginate beads (60.66%). This indicates that the calcium alginate beads are more active with the addition of cellulose nanoparticle.

The strength of calcium alginate-cellulose nanoparticle beads (20.97 N) is higher than calcium alginate beads (15.24 N). This is because sodium alginate forms a soft gel ("Sodium Alginate," n.d.). The physical structure is weak. Therefore, cellulose nanoparticle is added as a natural nanostructure support to strengthen the structure of sodium alginate.

4.3 Bound Pectinase Determination

Protein loading was measured by using Bradford method, where bovine serum albumin was used as the standard protein. Thus, a standard curve was drawn as shown in Figure 4.4. A linear set intercept of the line produced the following equation:

$$y = 0.0127\ x \qquad (4.1)$$

The value of R^2 shown in graph which is 0.982 indicate a high accuracy of protein concentration estimation. Therefore, the protein concentration of samples can be determined using the standard curve and equation.

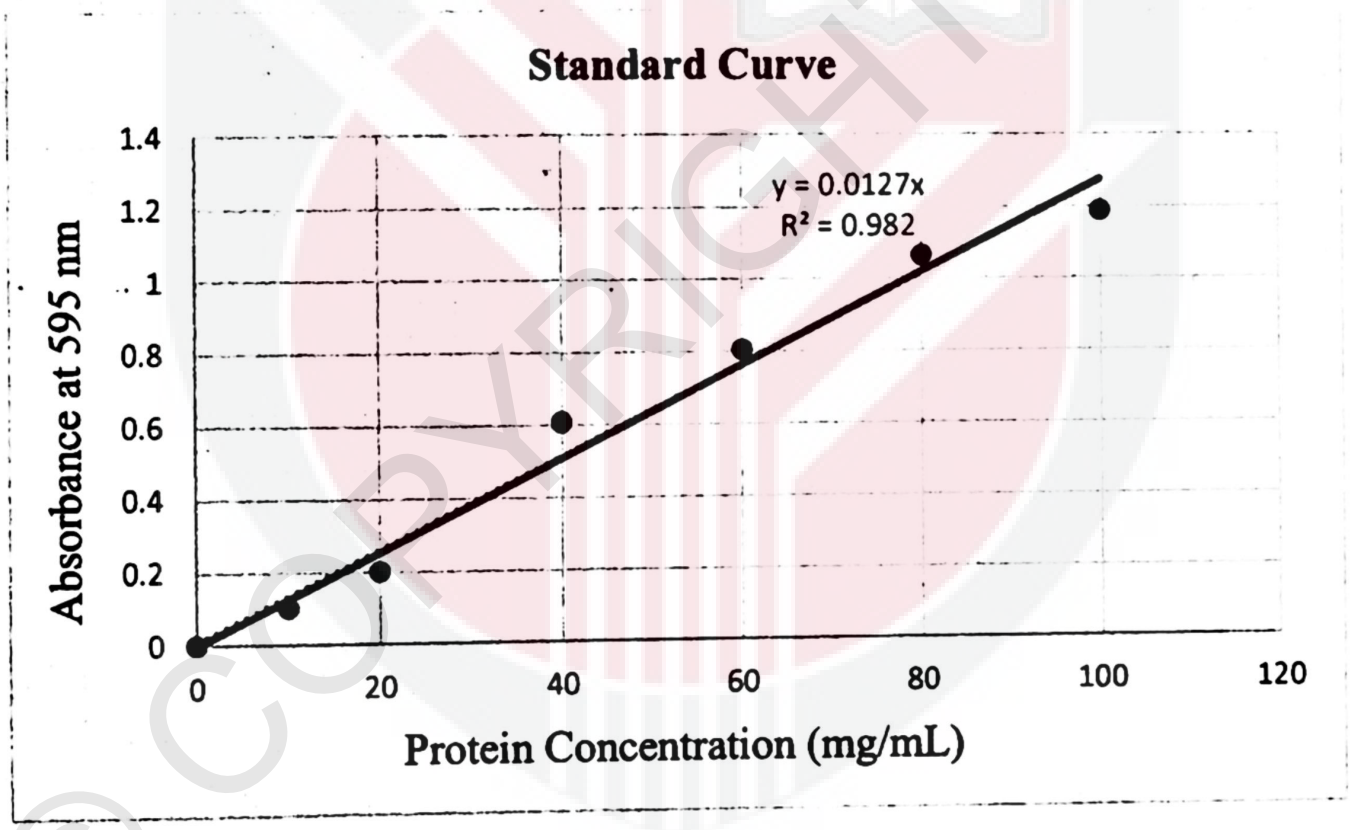


***Figure 4.4**: Standard Curve of Micro Assay Bradford Analysis by using BSA as standard*

4.4 Reusability of Immobilized Pectinase

The reusability of immobilized enzyme is highly important, because it is a key quality for the financial feasibility of bioprocess fixed in immobilized enzyme system (Goradia, 2006). Sodium alginate with/ without cellulose nanoparticle beads (0.1 g each) were added to sweet potato juice to clarify the juice. The experiment was carried

out at 55 °C which is the optimum temperature of pectinase. Then, the same beads were repeated to use to clarify sweet potato juice for another 5 times. The process was done in batch relations and the same reaction conditions were used for all batches.

Referring to Appendix H, the absorbance value is increasing. This simply means that the turbidity of the sweet potato juice is increasing.

Figure 4.5 shows the reusability of immobilized pectinase onto calcium alginate beads and onto calcium alginate-cellulose nanoparticle which is 6 cycles of repeated batch process. The result shows that the residual activity of both immobilized pectinases gradually decreased until cycle 6. The reusability profile of immobilized pectinase onto calcium alginate-cellulose nanoparticle has reduced from to 63%. As for immobilized pectinase onto calcium alginate beads, the reusability profile has reduced to 33.3%. The loss of activity of enzyme may be due to leakage of enzyme from the beads as results of washing of beads at the end of each cycle or conformation changes by repeated uses (Alagöz et.al., 2016). Moreover, the beads might break when transferring the same bead from one beaker of sweet potato juice to another beaker of sweet potato juice. However, the immobilized enzyme offers repeated uses whereas free enzyme can only be used one time unless it can be recovered from the reaction mixture without any denaturation.

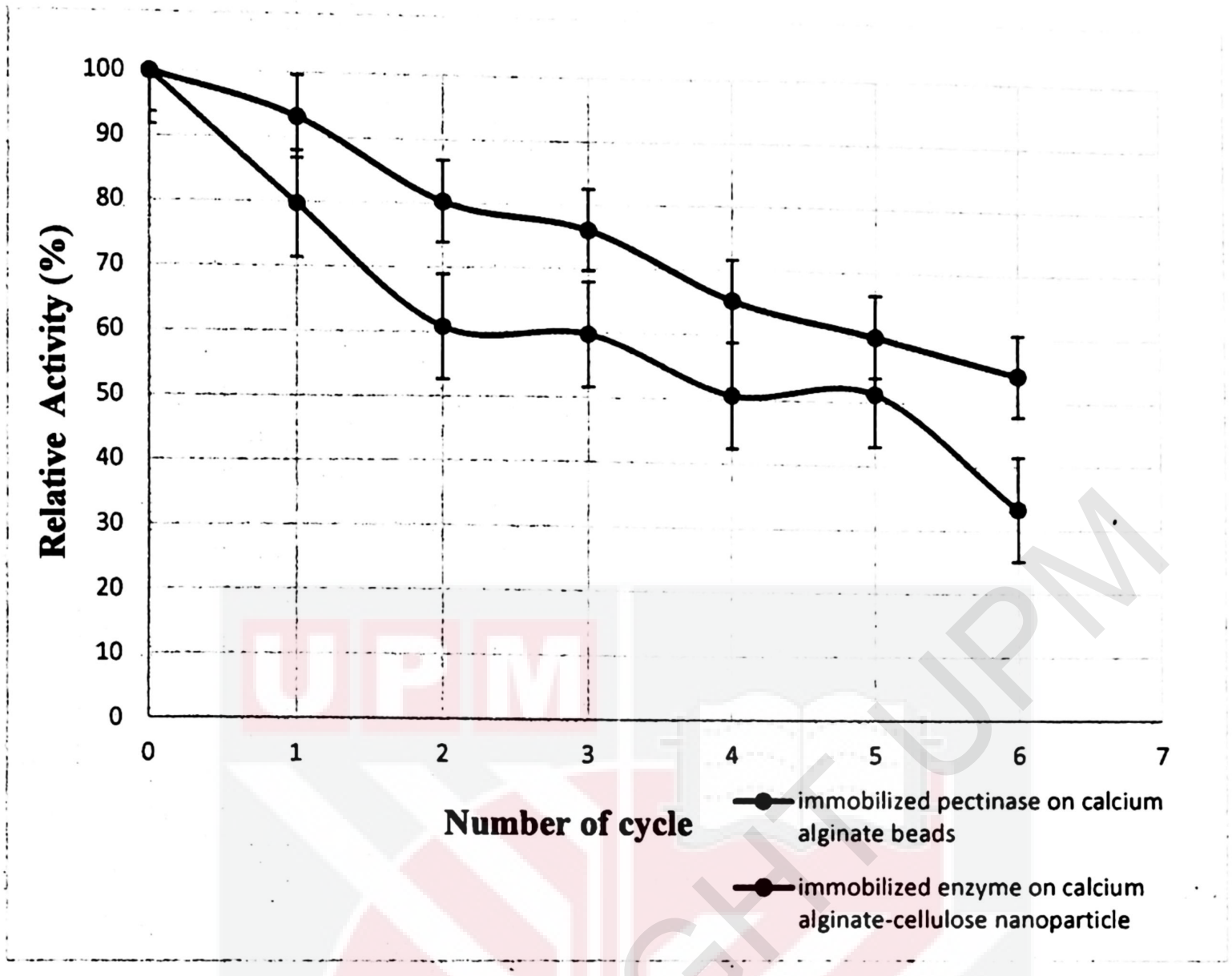


***Figure 4.5**: Reusability of immobilized enzyme onto sodium alginate with/ without cellulose nanoparticle in batch relations*

4.5 Clarification of Sweet Potato Juice

Figure 4.6 shows a graph of wavelength screening of free pectinase, sweet potato juice and sweet potato juice reacted with pectinase by using UV-VIS spectrophotometer. Sweet potato juice and enzyme was diluted to obtain a suitable wavelength of juice for turbidity test in measuring the absorbance when sweet potato juice reacts with immobilized pectinase and free pectinase.

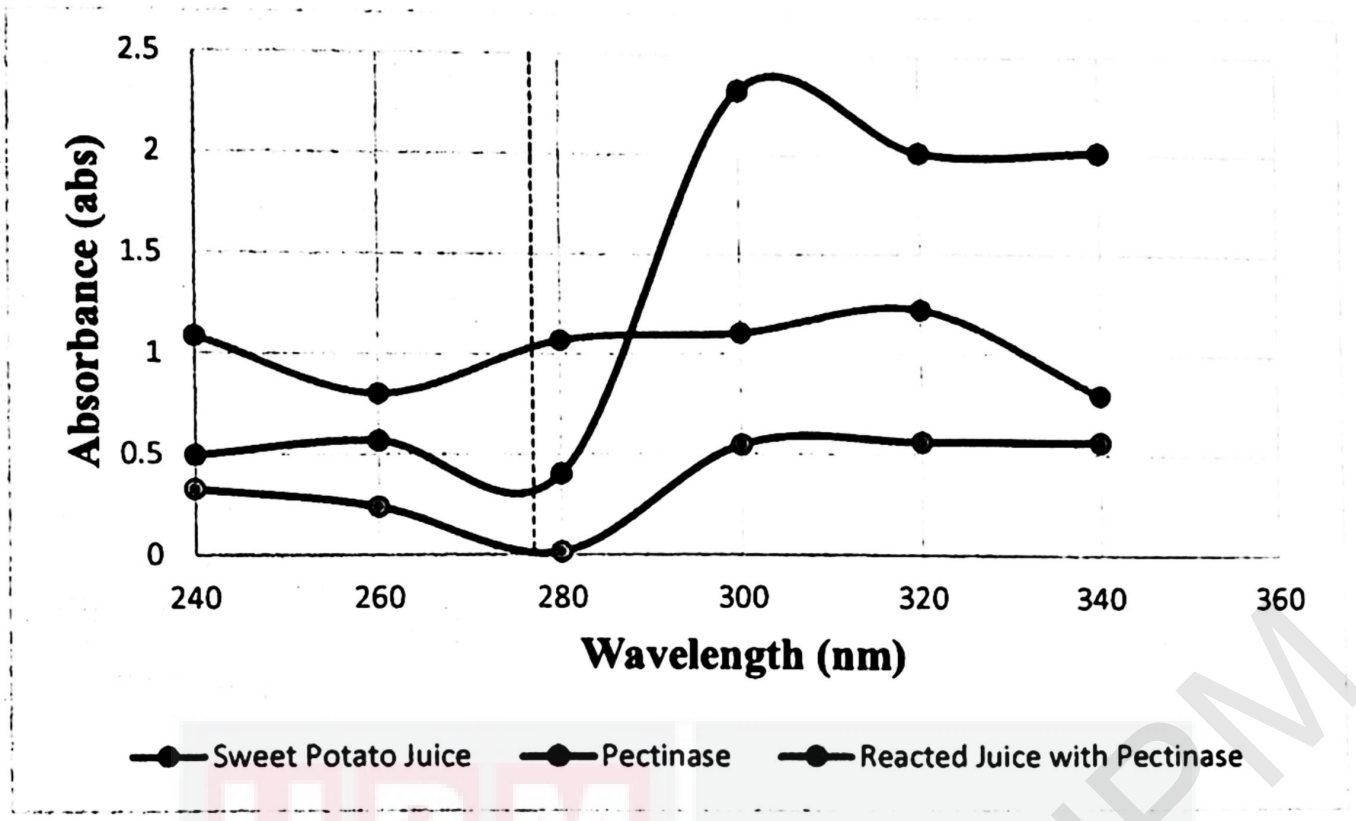


***Figure 4.6**: Graph of wavelength screening of Sweet Potato Juice, Pectinase and reacted juice with pectinase*

Based on the graph, wavelength 275 nm is the most suitable wavelength. It is because it has significant difference in the absorbance between enzyme and juice. It also shows the reduction of absorbance when juice react with enzyme. The wavelength shown was then used for turbidity test.

After the pectinase has been immobilized into beads, the bead was added into sweet potato juice to clarify it. The process was carried out at 55 °C.

Turbidity in fruit juice is generally resulted from the suspended pectin particles stemming from plant cell wall (Weiss., 1987). The turbidity reducing action of pectinase is mainly via electrostatic destabilization of suspended, negatively charged, pectin particles (Endo, 1965). Agglomeration occurs once this occurs to a significant extent and the particles may be removed by centrifugation or filtration of the juice (Pinelo et al., 2009).

Experiments of clarification of sweet potato juice with free pectinase, immobilized pectinase onto sodium alginate, immobilized pectinase onto sodium

alginate-cellulose nanoparticle were carried out. The results were tabulated in Appendix B. All the results show a decreasing absorbance value. This means that the turbidity of sweet potato juice has decreased and more light intensity passes through the sample. A comparison graph between this three clarification of juice was drawn as shown in Figure 4.7.

The absorbance value of juice clarified with free pectinase shows the greatest reduction with time. Immobilized pectinase onto sodium alginate also shows greater in reduction with time than immobilized pectinase onto sodium alginate-cellulose nanoparticle. However, Table 4.1 shows that immobilized activity of immobilized pectinase onto sodium alginate is higher than immobilized pectinase onto sodium alginate-cellulose nanoparticle. Therefore, the weak in clarification of immobilized pectinase on calcium alginate-nanoparticle beads may due to the reason that pectinase is hard to be released from the bead to carry out clarification.

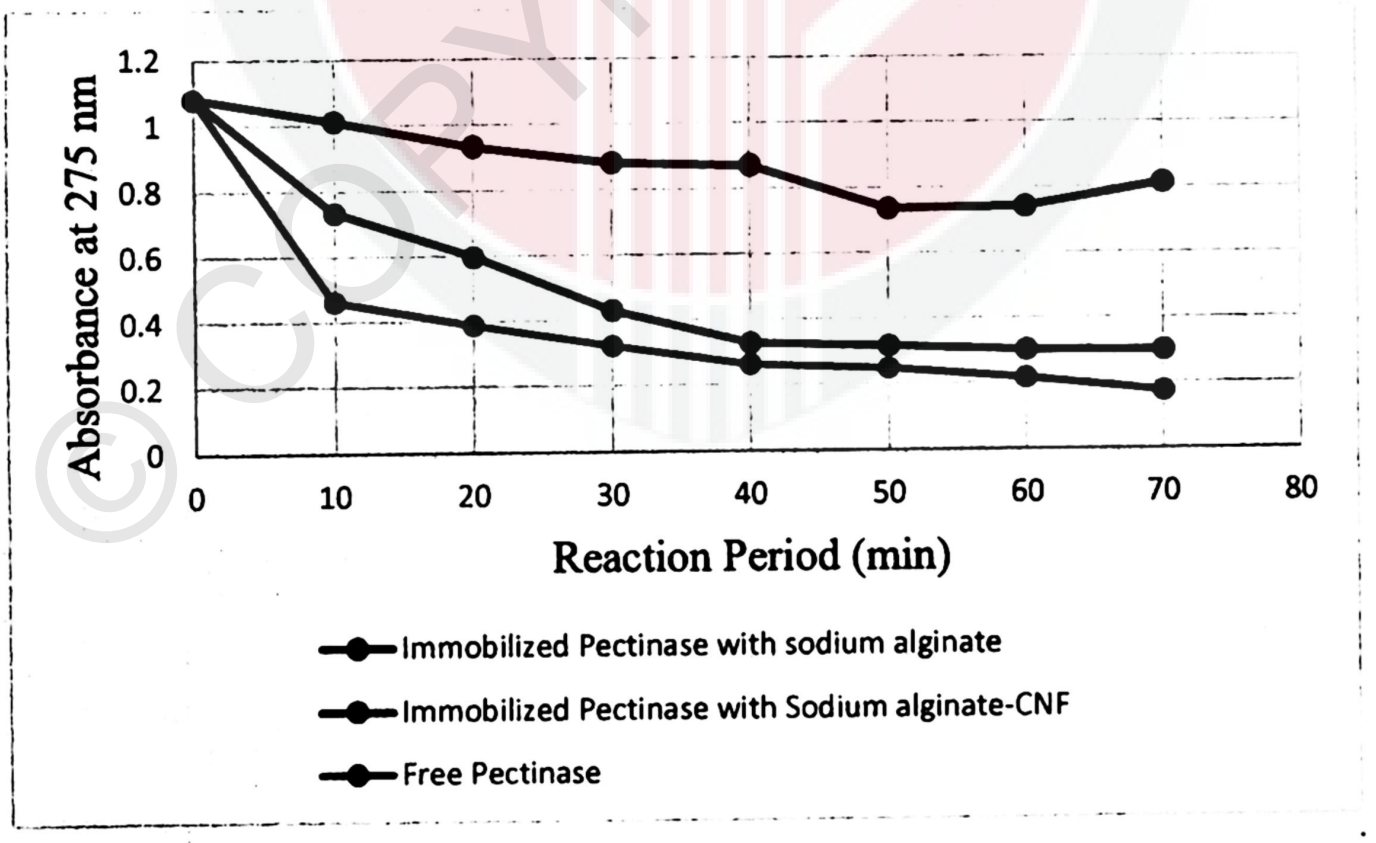


***Figure 4.7**: Clarification of Sweet Potato juice*

4.6 Concluding Remark

The immobilization of pectinase on the surface of sodium alginate and cellulose nanoparticles has a higher immobilization activity, strength and can be recycled more than the beads without cellulose nanoparticle. However, the beads with cellulose nanoparticles show a lower strength in clarify sweet potato juice.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

This chapter will highlight the conclusion of this project and recommendations to improve this project in the future.

5.1 Conclusion

Immobilization of pectinase using entrapment method is considered success. 85.3% of pectinase was immobilized onto sodium alginate and had a recovery activity of 60.7%. 49.5% of pectinase was immobilized onto sodium alginate-cellulose nanoparticle and had a recovery activity of 69.0%. The immobilization yield of pectinase is higher when immobilized on the surface of calcium alginate-cellulose nanoparticle bead than immobilized on calcium alginate bead.

The strength of immobilized pectinase with cellulose nanoparticle is stronger than that without cellulose nanoparticle which are 20.97 N and 15.24 N respectively. This indicated that cellulose nanoparticle can strengthen the structure of sodium alginate.

The clarification of sweet potato juice by using immobilized pectinase with and without cellulose nanoparticle showed 26.9% and 78% in reduction of turbidity. This might due to the difficulties of pectinase to be released from the calcium alginate-cellulose nanoparticle beads.

The relative activity of immobilized pectinase with and without cellulose nanoparticle after 6 cycles of reactions are 54.3% and 33.3% respectively. This is because of the stronger calcium alginate beads formed when added together with cellulose nanoparticle.

Therefore, based on the results, the immobilized pectinase on calcium alginate-cellulose nanoparticle has a stronger strength which indicates that cellulose nanoparticle can strengthen the structure of sodium alginate. Moreover, the immobilized pectinase on calcium alginate-cellulose nanoparticle has a higher immobilization yield which indicates the successfully immobilization of pectinase.

5.2 Recommendation

Since the immobilization yield of pectinase-calcium alginate-cellulose nanoparticle beads is higher but makes a weaker clarification of sweet potato juice as compared to pectinase-calcium alginate beads, it is recommended that the amount of cellulose nanoparticle added as support should be researched. It is important to know how will the amount of cellulose nanoparticle affect the binding of pectinase onto sodium alginate. It is also recommended to identify the kinetic reaction between cellulose nanoparticle and sodium alginate. It is to identify whether it will help in enhancing the binding strength which in turn weaken the clarification ability.

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APPENDICES

Appendix A: Bovine Albumin Serum Standard Curve

Absorbance value for Standard BSA using UV/VIS Spectrophotometer at wavelength 595 nm with dilution 1:6.

Protein Concentration (mg/mL)	Absorbance value at wavelength 595 nm			
	1	2	3	Average
0	0.000	0.000	0.000	0.000
10	0.101	0.103	0.103	0.102
20	0.204	0.20	0.201	0.202
40	0.610	0.602	0.613	0.608
60	0.802	0.798	0.817	0.806
80	0.991	1.101	1.103	1.065
100	1.197	1.183	1.182	1.187

Appendix B: Strength analysis of immobilized pectinase on sodium alginate with/without cellulose nanoparticle

Time (s)	Force (N)	
	Pectinase on sodium alginate	Pectinase on sodium alginate with cellulose nanoparticle
0	0.056	0.00532
0.1	0.1547	0.346
0.185	0.325	15.27
0.2	0.399	12.56
0.275	20.97	2.79
0.3	11.59	0.4
0.4	2.87	0.0021
0.5	0.3	0.0003
0.6	0.0042	0.0000

Appendix C: Absorbance value for Pectinase

a) Absorbance value for Free Pectinase

Absorbance value at wavelength 595 nm				Concentration (mg/mL)	Amount of protein loaded (mg/g support)
1	2	3	Average		
2.365	2.363	2.352	2.360	185.8	$185.8 \, x \, 2.5 = \mathbf{464.57}$

b) Pectinase detected in all washings after immobilization onto Sodium Alginate

Washing Filtrates	Absorbance value at wavelength 595 nm				Concentration (mg/mL)	Amount of protein loaded (mg/g support)
	1	2	3	Average		
1	0.142	0.140	0.139	0.140	11.02	$\frac{11.02}{0.15} x \, 2.5 = 183.72$
2	0.084	0.083	0.083	0.083	6.54	$\frac{6.54}{0.15} x \, 2.5 = 108.92$
3	0.079	0.078	0.079	0.079	6.22	$\frac{6.22}{0.15} x \, 2.5 = 103.67$
Total						**396.31**

c) Pectinase detected in all washings after immobilization onto Sodium Alginate and Cellulose Nanoparticle

Washing Filtrates	Absorbance value at wavelength 595 nm				Concentration (mg/mL)	Amount of protein loaded (mg/g support)
	1	2	3	Average		
1	0.206	0.206	0.207	0.206	16.22	$\frac{16.22}{0.15+0.1} x \, 2.5 = 162.2$
2	0.086	0.085	0.087	0.086	6.77	$\frac{6.77}{0.15+0.1} x \, 2.5 = 67.72$
Total						**229.92**

Appendix D: Titration of Pectinase Immobilization

a) *Titration of Pectinase Immobilization onto Sodium Alginate*

Titre Result	Free Enzyme	Unbound Enzyme				Immobilized Enzyme
		1	2	3	Average	
Volume of NaOH used to titre sample (V_s), mL	26.80	16.00	9.50	15.10	13.53	18.70
Volume of NaOH used to titre blank (V_b), mL	11.30	11.30	11.30	11.30	11.30	11.30
Concentration of NaOH (c), N	0.02	0.02	0.02	0.02	0.02	0.02
Volume of incubation mixture (V), mL	20.20	20.20	22.20	22.20	21.53	22.20
Reaction time (t). s	9.51	7.43	3.35	6.40	5.73	6.81
*Pectinase Activity, (U/mL)	96.82	21.69				58.73
**Activity with support (U/g support)	**1613.67**	**361.50**				**978.83**

*Pectinase Activity = $\frac{1000\ x\ (Vs-Vb)x\ c}{V\ x\ t}$

**Activity with support (U/g support) = $\frac{Pectinase\ Activity\ x\ 2.5\ mL}{0.15}$

b) ***Titration of Pectinase Immobilization onto Sodium Alginate-Cellulose nanoparticle***

Titre Result	**Free Enzyme**	**Unbound Enzyme**			**Immobilized Enzyme**
		1	**2**	**Average**	
Volume of NaOH used to titre sample (V_s), mL	26.80	20.10	27.30	23.70	20.10
Volume of NaOH used to titre blank (V_b), mL	11.30	11.30	11.30	11.30	11.30
Concentration of NaOH (c), N	0.02	0.02	0.02	0.02	0.02
Volume of incubation mixture (V), mL	20.20	20.20	22.20	21.20	22.20
Reaction time (t). s	9.51	6.92	10.50	8.71	7.12
*Pectinase Activity, (U/mL)	96.82	80.58			66.81
Activity with support (U/g support)	**968.20**	**805.80**			**668.1**

*Pectinase Activity $= \frac{1000\ x\ (Vs-Vb)x\ c}{V\ x\ t}$

**Activity with support (U/g support) $= \frac{Pectinase\ Activity\ x\ 2.5\ mL}{0.15+0.1}$

Appendix E: Reusability of Immobilized Pectinase onto sodium alginate

Testing the reusability of Immobilized Pectinase onto Sodium Alginate after 6 cycles using absorbance method with dilution 1:4.

i) The reusability of Immobilization of Pectinase with Sodium Alginate

Sample	Absorbance value at wavelength 600 nm			
	1	2	3	Average
1	0.206	0.206	0.207	0.206
2	0.399	0.399	0.401	0.399
3	0.407	0.408	0.412	0.409
4	0.449	0.529	0.530	0.502
5	0.499	0.494	0.495	0.496
6	0.690	0.672	0.673	0.678

ii) Absorbance value of sweet potato

Absorbance value at wavelength 600 nm			
1	2	3	Average
1.016	1.017	1.018	1.017

iii) Residual activity of immobilized pectinase after 6 cycles

Residual activity (%)					
1	2	3	4	5	6
79.74	60.77	59.78	50.64	51.23	33.33

$$\text{Residual activity} = \frac{1.017 - \text{absorbance value}}{1.017} \times 100\%$$

Appendix F: Reusability of Immobilized Pectinase onto sodium alginate-cellulose nanoparticle

Testing the reusability of Immobilized Pectinase onto Sodium Alginate- cellulose nanoparticle after 6 cycles using absorbance method with dilution 1:4.

i) The reusability of Immobilization of Pectinase onto Sodium Alginate- cellulose nanoparticle

Sample	Absorbance value at wavelength 600 nm			
	1	2	3	Average
1	0.070	0.069	0.069	0.069
2	0.200	0.200	0.200	0.200
3	0.245	0.241	0.243	0.243
4	0.356	0.352	0.348	0.352
5	0.405	0.404	0.405	0.405
6	0.465	0.464	0.464	0.464

ii) Absorbance value of sweet potato

Absorbance value at wavelength 600 nm			
1	2	3	Average
1.016	1.017	1.018	1.017

iii) Residual activity of immobilized pectinase after 6 cycles

Residual activity (%)					
1	2	3	4	5	6
93.21	80.33	76.1	65.3	60.14	54.33

Appendix G: Absorbance value of Juice with free and immobilized pectinase

a) *Absorbance value of Juice with free pectinase at 275 nm*

Time (min)	**Juice with immobilized pectinase onto sodium alginate at 275 nm**			**Average**
0	1.102	1.087	1.057	1.082
10	0.474	0.464	0.451	0.463
20	0.390	0.389	0.388	0.389
30	0.323	0.323	0.324	0.323
40	0.266	0.265	0.265	0.265
50	0.249	0.249	0.249	0.249
60	0.219	0.218	0.218	0.218
70	0.176	0.176	0.176	0.176

b) *Absorbance value of Juice with immobilized pectinase onto sodium alginate at 275 nm*

Time (min)	**Juice with immobilized pectinase onto sodium alginate at 275 nm**			**Average**
0	1.102	1.087	1.057	1.082
10	0.728	0.731	0.746	0.735
20	0.607	0.594	0.593	0.598
30	0.442	0.431	0.423	0.432
40	0.329	0.328	0.329	0.329
50	0.318	0.318	0.317	0.318
60	0.303	0.303	0.303	0.303
70	0.302	0.302	0.302	0.302

c) Absorbance value of Juice with immobilized pectinase onto sodium alginate-cellulose nanoparticle at 275 nm

Time (min)	Juice with immobilized pectinase onto sodium alginate- cellulose nanoparticle at 275 nm			Average
0	1.102	1.087	1.057	1.082
10	1.042	1.021	0.976	1.013
20	0.942	0.923	0.936	0.934
30	0.901	0.889	0.856	0.882
40	0.874	0.869	0.870	0.871
50	0.730	0.738	0.740	0.736
60	0.740	0.742	0.742	0.742
70	0.808	0.817	0.813	0.813