LAPORAN AKHIR PROJEK PENYELIDIKAN GERAN PENYELIDIKAN YAYASAN FELDA

1. BUTIR-BUTIR PROJEK

Tajuk projek: Perkembangan Biopolielektrolit daripada Polisakarida Tak-Ionik

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2. RINGKASAN DAPATAN

Dalam kajian ini, biopolielektrolit dalam bentuk kanji sagu kationik telah dihasilkan dan potensinya sebagai alternatif kepada kitosan telah dikaji. Kanji sagu, sebagai polimer semulajadi dan sumber boleh diperbaharui, telah dipilih sebagai bahan mentah untuk pengkationan. Kanji sagu kationik telah disediakan dengan menggunakan proses alkali akueus dengan bahan kationik 3-kloro-2-hidroksipropiltrimetilammonium kloride (CHPTAC) (0.01 - 0.10 M), sodium hidroksida (0.03 - 0.86 M) dan suhu tindakbalas (30 - 62ºC) pada peringkat-peringkat berlainan. Setiap faktor telah dikaji pada lima peringkat berdasarkan Rekabentuk Komposit Pusat. Darjah penukargantian (DS), kecekapan tindakbalas, sifat-sifat terma dan pempesan, ciri-ciri pembengkakan dan keterlarutan serta sifat-sifat pengikatan lemak kanji kationik telah dikaji. Darjah penukargantian meningkat dengan penambahan dalam kepekatan CHPTAC, NaOH ataupun suhu tindakbalas. Darjah penukargantian yang paling tinggi tercapai ialah 0.06. Kecekapan tindakbalas adalah berkadar dengan kepekatan NaOH dan suhu tindakbalas tetapi berkadar songsang dengan kepekatan CHPTAC. Kecekapan tindakbalas yang paling tinggi tercapai ialah 78.8%. Walau bagaimanapun, purata pengurangan dalam suhu peralihan gelatinisasi, suhu pempesan dan entalpi gelatinisasi untuk kanji sagu kationik (DS 0.06) berbanding dengan kanji sagu asli ialah 11.5°C, 12.4ºC dan 7.8 J g^{-1}, masing-masing. Keterlarutan kanji telah bertambah daripada 17.3% untuk kanji sagu asli kepada 83.6% untuk kanji kationik (DS 0.06). Penambahan dalam keterlarutan (pada 75°C) untuk kanji kationik pada peringkat DS yang lebih tinggi telah mengakibatkan pengurangan dalam faktor pembengkakan. Pengkationan telah meningkatkan puncak viskositi sebanyak 1096.5 cP dan 'breakdown' sebanyak 880.5 cP, serta mengurangkan 'setback' sebanyak 129.5 cP. Pengenalan kumpulan-kumpulan kationik ke dalam kanji sagu asli telah berjaya meningkatkan kapasiti pengikatan lemak, kestabilan dan viskositi emulsinya. Walau bagaimanapun, kanji

kationik yang dihasilkan dalam projek ini masih tidak setanding dengan kitosan komersil. Kitosan memiliki kapasiti pengikatan lemak, kestabilan dan viskositi emulsi yang paling tinggi. Tiada perbezaan yang signifikan ($p < 0.05$) antara ketegangan permukaan (53 to 57 dynes cm^{-1}) kanji sagu kationik, kanji sagu asli dan kitosan pada pH 4 atau pH 7. Oleh itu, kanji kationik meningkatkan kestabilan emulsi dengan meningkatkan viskositi tetapi bukan sifat-sifat aktif-permukaan. Pengaruh pH terhadap sifat-sifat pengemulsian adalah tidak signifikan pada $p < 0.05$.

3. SUMMARY OF FINDINGS

In this study, cationic sago starch was produced and its potential as an alternative to chitosan was explored. Sago starch, being a natural polymer and renewable source, was selected as the raw material for cationization. Cationic sago starches were prepared using an aqueous alkaline process with different levels of cationic reagent 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) (0.01 - 0.10 M), sodium hydroxide (0.03 - 0.86 M) and reaction temperatures (30 - 62ºC). Each factor was studied at five levels based on a Central Composite Design. The degree of substitution (DS), reaction efficiency, thermal and pasting properties, swelling and solubility characteristics as well as fat binding properties of cationic starches were studied. Degree of substitution increased with an increased in either concentration of CHPTAC or NaOH or reaction temperature. The highest DS that can be achieved was 0.06. The reaction efficiency was proportional to the concentration of NaOH and reaction temperature but inversely proportional to the CHPTAC concentration. The highest reaction efficiency achieved was 78.8%. However, the average decrease in gelatinization transition temperatures, pasting temperature and gelatinization enthalpy for cationic sago starch (DS 0.06) compared to native sago starch were 11.5°C, 12.4ºC and 7.8 J g^{-1}, respectively. The starch solubility was increased from 17.3% for native sago starch to 83.6% for cationic starch (DS 0.06). The increase in solubility (at 75°C) for cationic starch at higher DS had caused a reduction of swelling factor. Cationization increased the peak viscosity of 1096.5 cP and breakdown of 880.5 cP, and reduced the setback of 129.5 cP. The introduction of cationic groups into native sago starch successfully increased its fat binding capacity, emulsion stability and viscosity. However, cationic starch produced in this project still cannot match with commercial chitosan. Chitosan had the highest fat binding capacity, emulsion stability and viscosity. There was no significant different ($p < 0.05$) between the surface tensions (53 to 57 dynes cm^{-1}) of cationic sago starch, native sago starch and chitosan neither at pH 4 nor pH 7. Thus, cationic starch improves emulsion stability by increasing viscosity but not surface-active property. The influence of pH on emulsifying properties was not significant at $p < 0.05$.

4. BACKGROUND AND RATIONALE

Many chemical polyelectrolytes cause environmental problems due to their resistance to biodegradation and their toxicity when allowed to accumulate in natural ecosystems. Concern about environmental protection has increased recently from a global viewpoint. Accordingly, chemical products should be used in harmony with environments. Under these circumstances, natural products are advantageous as substitutes of synthetic chemicals. On the other hand, natural chemical products and chemical modifiers of natural products have also received some attention as a substitute for synthetic chemicals (Fiechter, 1992; Ishigami & Suzuki, 1997).

Biopolyelectrolytes are natural polymeric polyelectrolytes produced by living-cells, they are biodegradable and biocompatible. Commercial biopolyelectrolytes include natural ionic polysaccharides (i.e. pectin, alginate, glucuronan, κ-carrageenan and chitosan), biosurfactants, bioflocculants and so on (Ikeda *et al.*, 1997; Ishigami & Suzuki, 1997; Bracccini *et al.*, 1999).

Cationic starch (biopolyeletrolytes) is produced by chemical reaction of starch with reagents containing amino, imino, ammonium, sulfonium, or phosphonium groups, all of which carry positive charges (Solarek, 1986). As the cationic starch has positive charges, it is attracted to, and retained by anionic substrates, making it useful as wet-end additives in paper making, as warp sizing agents in textile manufacture, act as flocculants in waste water treatment, as shampoo or detergents in cosmetic manufacture and as bactericides in pharmaceutical products (Rutenberg, 1980; Solarek, 1986; Khalil & Farag, 1998; Granö *et al.*, 2000).

Cationic starches have been produced commercially from corn, waxy corn, barley, pea, wheat, tapioca and potato with varying degrees of substitution (DS) and by a variety of cationizing agents and methods (Mentzer, 1984; Solerak, 1986; Sosulski *et al.*, 1997; Vasanthan *et al.*, 1997; Han & Sosulski, 1998; Khalil & Farag, 1998). Currently the commercially significant derivatives are the tertiary amino and quaternary ammonium starch ethers (Solarek, 1986). Commercial cationic starches typically have 0.2 to 0.4% nitrogen (Solarek, 1986). However, cationic starch has also been made by reaction with cyanamide or dialkylcyanamides, yielding pseudourea or imino disubstituted carbamates (Rutenberg, 1980). In 1998, Gruber and Bothor developed a method for cationization of starch granules by graft copolymerization using cationic methacrylic ester with a redox initiator, acrylamide. Each process of cationization has been discussed in details by Rutenberg (1980), Solarek (1986) and Gruber & Bothor (1998).

During the past few decades, chitosan as cationic substrate has been receiving increased attention for its commercial application in biomedical, food and various chemical industries. This natural, non toxic, biopolymer chitosan is now widely produced commercially from crab and shrimp waste shells (No *et al.*, 2000). In this study, we would like to produce cationic sago starch and explore its potential as an alternative to chitosan. This is based on the premise that cationic starch bearing positive charges should behave (theoretically) in similar manner as chitosan.

Sago starch was selected as the raw material for cationization due to several reasons: starch is the most commonly used natural biopolymers, easy to handle and widely available. In Malaysia, the largest sago starch processing industry is based in Mukah, Sarawak. It is envisage that by developing new applications for sago starch, large-scale farming of this crop in Malaysia will be enhanced. In addition, this is in line with the new government policy to industrialize the agricultural sector in order to reduce the nation's food import bill (RM 1.34 billion as per 1998).

5. OBJECTIVES

General objective

The general objective of this research work was to produce biopolyelectrolyte, in the form of cationic sago starch, and study their physical properties. Furthermore, the fat binding capacity as well as emulsion properties of cationic starches were compared to commercial chitosan and native sago starch.

Specific objectives

The specific objectives of the project were:

(1) to study the effect of cationic reagent 3-chloro-2 hydroxypropyltrimethyl ammonium chloride (CHPTAC) concentration, NaOH concentration and reaction temperature on DS and reaction efficiency.

(2) to study the physical characteristics of cationic sago starches such as swelling, solubility, thermal and pasting properties to get a better understanding of the effect of cationization on sago starch.

(3) to produce cationic starch with chitosan-like properties, for examples fat binding capacity as well as emulsion properties by using commercial chitosan as a comparison.

6. PROJECT PROTOCOL

The practical aspects of the project commenced with preliminary studies on the possibility to produce cationic sago starches by following the method described by Yook *et al.* (1994). The three most important factors were selected based on the literature review on the aqueous cationization method. The results of the preliminary studies were used as a basis to determine the practical range for each factor. Cationic sago starches were produced by using process conditions determined by Response Surface Methodology Experimental Design.

The effect of reaction temperature, CHPTAC and NaOH concentration on DS of cationic sago starches and reaction efficiency of cationization were studied. Then, the physical and functional properties of cationic sago starches such as swelling, solubility, pasting and thermal properties were studied to gain a better understanding of the effects of cationization on sago starch.

Finally, cationic sago starch with the highest DS was chosen and its chitosan-like properties such as fat binding capacity and emulsifying properties were compared to those of commercial chitosan and native sago starch. The emulsifying properties of each sample were studied at pH 4 and pH 7 to investigate whether they possess more positive charges at a lower pH. This is because degree of ionization of polybases (cationic starch and chitosan) increases with decrease in pH.

7. MATERIALS AND METHODS

Native sago starch was purchased from NITSEI Sago Industries Sdn. Bhd. (Penang, Malaysia). The starch was used as provided without any further treatment. Commercial chitosan was supplied by Hunza Lab Sdn. Bhd. (Penang, Malaysia). Degree of deacetylation for the chitosan is 94.5%. An aqueous solution of 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) (Aldrich Chemical Co., Milwaukee, WI), with 60% (w/w) active monomer concentration, was used as a cationizing agent. All other chemicals were of analytical grade.

Preparation of cationic sago starches

A total of 32 cationization experiments were carried out. For each experiment the aqueous alkaline solution was prepared by dissolving 120 g Na_2SO_4 and a selected amount of NaOH (Table 2) in 371 ml distilled water. Starch (200 g, dry basis) was mixed with the solution and the slurry was stirred with a torque stirrer (RW20.n S2, Ika Labortechnik, Malaysia) for 5 min in a thermostatically controlled water bath (HB4 Basic, Ika Labortechnik, Germany). The starch concentration in the slurry was 35% (w/w). The cationizing agent was then admixed with the slurry for 1 min. The reaction was allowed to proceed at the selected reaction temperature for 6 h followed by neutralization with 1 N HCl. The slurry was then centrifuged (Kubota 5100, Kubota Corporation, Japan) at 2, 300 × *g* for 8 min. The modified starch was washed with distilled water until chlorine-free (the supernatant was clear when tested with a few drops of 1 N $AgNO_3$), followed by air oven drying at 30°C overnight. The dry sample was then milled to fine powders (< 250 μm).

Analysis of Degree of Substitution (DS)

Nitrogen (N) content was determined by the macro-*Kjeldahl* method. A Digestion System 6 1007 digester (Tecator) was used for digesting the samples and a Kjeltec System 1002 distilling unit (Tecator) was used for distillation. The DS of each derivatized sample with the *N*-trimethyl-2-hydroxypropyl group as the substituent group was calculated on the basis of the increase in nitrogen concentration of the washed product as compared to the native starch using the formula described by Yook *et al.* (1994):

$$\mathrm{DS} = \frac{(162 \times \%\ \mathrm{Nitrogen})}{1400 - (117 \times \%\ \mathrm{Nitrogen})}$$

Duplicate determinations were performed for each starch sample in each response, and the results reported were of an average.

Determination of Reaction Efficiency (RE) of Cationization

Reaction efficiency measures the percentage of added cationic reagent that has potentially reacted with the starch, i.e. the amount of cationic reagent which has penetrated into the starch interior and bound with starch molecules (Kweon *et al.*, 1996).

Thermal properties

Thermal characteristics were studied using a Differential Scanning Calorimeter (DSC) (DSC 2910, Du Pont Instruments, USA) equipped with a standard DSC cell and a Thermal Analyst 2000 Controller. Starch (2.5 mg, dry basis) was weighed directly into an aluminium pan, and then 7.5 mg of deionized water was added directly into the pan using a microsyringe. After sealing, the pan was reweighed and left for 1 h to allow the sample to equilibrate. Then the sample was heated from 10 to 130°C at a heating rate of 10°C min^{-1}. An empty pan was used as a reference. Transition temperatures and gelatinization enthalpy were recorded from a plot of heat flow versus temperature. Standard parameters of onset, peak and completion temperatures were reported in °C and gelatinization enthalpy was expressed in $J\ g^{-1}$.

Functional Properties

Swelling factor of starch granules

Swelling factor was determined following the method described by Tester & Morrison (1990). Swelling tests were carried out at 75°C, based on a single point determination. Starch (200 mg) was weighed correct to 0.1 mg into a 35-ml screw-cap conical bottom centrifuge tube, 5.0 ml of distilled water was added, and the closed tube was incubated in a constant shaking waterbath (WB 22, Memmert, Germany) at 75°C for 30 min. The tube was then cooled rapidly to room temperature, 0.5 ml of blue dextran (Pharmacia, M_r 1 × 10^6, 5 mg ml^{-1}) was added, and the content was mixed by gently inverting the sealed tube several times. After centrifuging at 1, 500 × *g* for 5 min, the absorbance of the supernatant (A_S) was measured at 620 nm using a UV-Visible Spectrophotometer (UV-16101PC, Shimadzu, Japan). The absorbance of a reference tube (A_R) that contained no starch was also measured.

Free or intersitial-plus-supernatant water (FW) is given by

$$\text{FW (ml)} = 5.5\left(\frac{A_R}{A_S}\right) - 0.5$$

the initial volume of the starch (V_0) of weight W_0 (mg) is

$$V_0\ \text{(ml)} = \frac{W_0}{1400}$$

and the volume of absorbed intragranular water (V_1) is thus

$$V_1 = 5.0 - FW$$

hence the volume of the swollen starch granules (V_2) is

$$V_2 = V_0 + V_1$$

and swelling factor (SF) is defined as

$$SF = \frac{V_2}{V_0}$$

This can also be expressed by the single equation

$$SF = 1 + \{(\frac{7700}{W_0} \quad \frac{(A_S - A_R)}{A_S} \quad]\}$$

Solubility

Starch solubility was determined following the method recommended by Leach *et al.* (1959), with some modifications. Solubility tests were carried out at 75°C, based on a single point determination. Starch (1 g) was weighed correct to 0.1 mg into a 250-ml screw-cap centrifuge bottle and 200.0 g of distilled water was added. The slurry was mixed by gently inverting the closed centrifuge bottle several times to keep the starch suspended. Then the closed bottle was incubated in a constant shaking waterbath at 75°C for 30 min. The bottle was then cooled rapidly to room temperature. After centrifuging at 1, 500 × *g* for 5 min, 25.0 ml of the supernatant was transferred using a 25-ml pipette into a weighed moisture dish. The moisture dish and soluble starch were reweighed after drying in the air oven at 120°C overnight.

The starch solubility (S) is calculated to dry basis:

$$\% S = \frac{W_{SS}}{W_0} \times 100$$

where W_{SS} is the weight of soluble starch (g) and W_0 is the initial weight of the sample (g).

Pasting properties

A Rapid Visco-Analyzer (RVA) (RVA-4, Newport Scientific, Australia) was used to determine the pasting properties of starches. Distilled water (25 ml) was added to a starch sample (2.5 g) and mixed in an aluminium sample canister. The standard 1 programmed heating and cooling cycle was used, where the sample was held at 50°C for 1 min, heated to 95°C in 3.7 min, held at 95°C for 2.5 min, cooled to 50°C in 3.8 min, and then held at 50°C for 2 min; the paddle speed was 960 rpm for the first 10 s, and then maintained at 160 rpm for the rest of 13 min. Recorded values were peak viscosity, trough 1, breakdown, final viscosity, setback and pasting temperature.

Fat Binding Properties

Fat binding capacity

Fat binding capacity was measured using a method proposed by No *et al.* (2000). Starch (1 g) was weighed correct to 0.1 mg into a weighed 15-ml conical bottom centrifuge tube, 5.0 ml of corn oil (Mazola, CPC/AJI (Malaysia) Sdn. Bhd.) was added, and mixed on a vortex mixer for 1 min to disperse the sample. The content was left at ambient temperature for 30 min with shaking for 5 s every 10 min. After centrifuging at 1, 610 × *g* for 25 min, the supernatant was decanted and the tube was weighed again.

Fat binding capacity (FBC) was calculated as follows:

$$\% \text{ FBC} = \frac{W_F}{W_0} \times 100$$

where W_F is the weight of fat bound (g) and W_0 is the initial weight of the sample (g). Quadruplicate measurements were performed, and the results reported were of an average.

Emulsion stability

In this study, the influence of pH towards the emulsifying properties of sago starch, cationic starch (with highest DS) and chitosan was studied at pH 4 and 7. However, chitosan is practically insoluble at pH 7 (Hwang & Damodaran, 1995), therefore chitosan emulsion was prepared only at pH 4. The emulsions prepared consisted of 1% (w/w) sample and 20% (w/w) oil phase.

Sample (0.5 g) was weighed correct to 0.1 mg into a 100 ml-beaker and about 38 g of distilled deionized water was added. Then the slurry was stirred with a magnetic stir bar (4 cm length) on a hotplate stirrer (Jenway 1000, UK) to suspend the sample in the solution. The pH of the solution was adjusted using a pH Meter (CyberScan pH 2500, Eutech Instruments, Singapore) to pH 4 or 7 by adding a few drops of 0.05 N HCl or 0.05 N NaOH, respectively. The solution was then added with distilled deionized water to a total weight of 40.5 g. This was followed by heating in a constant shaking waterbath at 95°C for 30 min. The solution was cooled rapidly to room temperature.

For chitosan, which is insoluble in water but soluble in acid (Koide, 1998), 0.05 N HCl (instead of deionized water) was added to dissolve the chitosan. The solution was adjusted to pH 4 by adding a few drops of 1 N HCl. Hydrochloric acids (0.05 N) was then added to make a total solution weight of 40.5 g. In this case, heat treatment was not applied.

Emulsions for each sample were prepared at pH 4 and 7 by adding 10.0 ml of corn oil (Mazola, CPC/AJI (Malaysia) Sdn. Bhd.). The solution was mixed and homogenized with a homogenizer (Ultra Turrax®, T25 Basic, Ika Labortechnik, Malaysia) at 22,000 rpm for 5 min. The stability, surface tension and viscosity of the homogenized emulsion were measured.

The effectiveness of each sample paste in maintaining the emulsion stability was monitored by an acquiescent electrical resistivity measurement (Atkins, 1982). After homogenization of the emulsion, the electrode of a Bench Conductivity Meter (CyberScan Con 500, Eutech Instruments, Singapore) was placed in a beaker (8 cm height) containing the emulsion, to a depth of 3.5 cm from the surface. The electrical resistivity of the upper part of emulsion was measured at 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min.

Resistivity measurements followed apparent first-order kinetics for the first 15 min of analysis and became linear after that. Thus, the pseudo zero-order linear regression slope (resistivity versus time) of the 15 through 60 min data was reported. Duplicate determinations of emulsifying properties were performed for each sample emulsion, and the results reported were of an average.

Surface tension of emulsion

Surface tension of emulsions was measured using a Surface Tensiomat (Surface Tensiomat® 21, Fisher Scientific, USA), with a 6 cm ring. The unit of surface tension was in dynes cm^{-1}. Three measurements were performed for each sub-emulsion.

Viscosity of emulsion

Viscosity of emulsions was measured using a Digital Viscometer (DV-II, Brookfield, USA), at the speed of 30 rpm. The spindle numbers for sago starch, cationic starch, and chitosan were 0, 1 and 2, respectively. The unit of viscosity was in cP. The data of each sub-emulsion was taken at 20, 40, 60, 80, 100, 120 s and the average viscosity of the emulsion was calculated.

Experimental Design

Response surface methodology

Response surface methodology is a collection of mathematical and statistical techniques that are for the modelling and analysis of problems in which a response of interest is influenced by several variables. It is useful for developing, improving, and optimising process (Myers & Montgomery, 1995; Montgomery, 1997).

In the industrial world, several input variables potentially influence some performance measure or quality characteristic of the product or process. These input variables are called independent variables and the performance measure or quality characteristic is called response (Myers & Montgomery, 1995).

Central composite design

A five-level three-factor fractional design was used. There were 32 experimental runs which included 16 factorial points, 12 axial points and 4 centre points. Experiments were completed in random order. The three independent variables considered the most important factors having the greatest effects on cationization process were: CHPTAC concentration (X_1); NaOH concentration (X_2) and reaction temperature (X_3). The levels for each factor were chosen from preliminary experiment and also from works reported in the literature. These coded variables (X_1 to X_3) each at five different levels were: -1.68 (lowest level); -1; 0 (middle level); 1 and 1.68 (highest level). Correspondence between these coded and actual values are presented in **Table 3.1**.

The central composite design treatments for cationization of starch are shown in **Table 3.2**. The experiments were run and the response values were entered following the run numbers in randomized order. Standard order was only used as a convenience for entry of the pre-existing designs. The highest and lowest values of each variable were chosen from the results of a preliminary experiment. The dependent variables were DS, reaction efficiency of cationization, swelling factor, solubility, gelatinization transition temperatures, gelatinization enthalpy, peak viscosity, breakdown, setback and pasting temperature of cationic starches. Duplicate determinations were performed for each starch sample in each response, and the results reported were of an average.

Simple comparative design

Single factorial design as shown in **Table 3.3** was adopted to evaluate the fat binding capacity of sago starch, cationic starch (sample with highest DS) and chitosan using Design Expert version 5.0 (Stat-Ease Inc.). Fat binding capacity of each sample was determined in quadruplicate following the run order as in **Table 3.3**.

On the other hand, emulsion stability, surface tension and viscosity of the emulsions prepared by sago starch, cationic starch (sample with highest DS) or chitosan were analysed using Multivariate Tests of General Linear Model, SPSS version 10.0 software. Duplicate determinations were performed for each emulsion, and the results reported were of an average. Fisher Least Significant Difference method was adopted to compare pairs of treatment means.

8. RESULTS AND DISCUSSION

8.1 Effect of Processing Factors on Degree of Substitution (DS) and Reaction Efficiency (RE)

Tables 3a and 3b show the coefficients of the quadratic regression model for determination of *DS*, *RE*, T_o, T_p, T_c, ΔH and *PT* of cationic starches. Response surfaces (Figs 1-7) show the effects of CHPTAC and NaOH concentration on these seven responses, while the reaction temperature factor was held at the mid-point temperature (46ºC). Reaction temperature of 46ºC was chosen for discussion because of the value of regression coefficient for reaction temperature factor was the smallest among the three factors (Table 3a and 3b).

Fig 1 shows *DS* values increased when the concentration of cationic reagent and NaOH increased. The highest *DS* that can be achieved was 0.06, which means there are six cationic groups attached per 100 glucose units in starch. However, the influence of NaOH concentration was higher than that of CHPTAC concentration on *DS* (Table 3a). This is probably due to the fact that high alkaline condition may lead to a higher degree of starch granules swelling. Swelling of starch granules will result in an increase in the granular surface area, which will then increase the chemical contact area and thereby enhance chemical binding to the hydroxyl sites in starch. In addition, increased starch granular surface area may improve the surface porosity that will facilitate easy penetration of chemicals into the granules.[1,5,14]

As shown in Fig 2, at a lower NaOH concentration (0.20 M), *RE* value decreased gradually when the concentration of cationic reagent increased. From the *RE* equation (equation 2), both the decrease in *DS* and the increase in CHPTAC concentration tend to cause a reduction in the *RE* values. At low alkaline condition, the starch granules swelled insufficiently and resulted a decrease in *DS*, which subsequently decreased the *RE*. On the other hand, at a higher NaOH concentration (0.69 M), the *RE* value increased slowly up to 0.07 M CHPTAC but decreased at the highest CHPTAC concentration (0.08 M). Although high alkaline condition may lead to a higher degree of starch granules swelling, and resulted in an increase in *DS*, the increment in CHPTAC concentration is more predominant which leads to a net increase of *RE* values. The highest *RE* achieved in this study was 78.75%. These results were consistent with those reported by Kweon *et al.*[13] These authors reported that *DS* for cationic corn and pea starch increased in proportion to CHPTAC concentration up to 0.2 M but the *RE* values decreased at the highest CHPTAC concentration (0.2 M).

8.2 Thermal and Pasting Properties of Cationic Sago Starch

Figs 3-7 show the effects of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the T_o, T_p, T_c and ΔH, *PT* of cationic starches. Evidently, the gelatinization transition temperatures (T_o, T_p, T_c), ΔH and *PT* of cationic starch decreased significantly ($p < 0.05$) when the concentration of cationic reagent and NaOH increased (Tables 3a and 3b). The lowest *PT*, T_o, T_p, T_c, and ΔH for cationic starches (*DS* 0.06) were 62.8ºC, 52.1ºC, 60.0ºC, 73.6ºC and 7.2 J g^{-1} compared to 75.2ºC, 64.8ºC, 72.9ºC, 82.5ºC and 15.0 J g^{-1} for native sago starch, respectively. The average decrease in gelatinization transition temperatures, *PT* and ΔH for cationic starch compared to native sago starch were 11.5°C, 12.4°C and 7.8 J g^{-1}, respectively. Hence, cationization tends to decrease the T_o, T_p, T_c, ΔH and *PT* of starch. This can be attributed to the positive charge on the free hydroxyl sites that improved the water uptake and thermal properties to a marked degree.[10] The introduction of cationic groups into the starch results in a weakening of the starch granule structure due to repulsion between neighbouring groups inhibiting interchain associations. This structural loosening will serve to increase the hydrophilicity of the starch and thus increase its ability to attract water. As the water uptake can take place more rapidly, the gelatinization can occur more easily and at a lower temperature. In addition, cationization disrupts the crystalline structure of native starches as shown by progressively diminished X-ray diffraction patterns with increasing *DS*.[1]

Fig 8 shows typical RVA pasting curves for native and cationic sago starches. In general, cationization increased the *PV* and *BD*, decreased the *SB* and *PT*. The highest *PV* for cationic sago starch (*DS* 0.06) was 2121.0 cP, as compared to 1024.5 cP for native sago starch. This was consistent with those observed by Liu *et al.* These authors found that after cationization, all the cationic corn starches showed a higher *PV* as compared to the corresponding native starches. These are probably because the introduction of cationic groups into the starch results in a weakening of the starch granule structure which tend to permit greater uptake of water with a consequent increase in the swelling of the granule that leads to a higher *PV*. The highest *BD* for cationic starch (*DS* 0.06) was 1576.0 cP whereas for native sago starch was only 695.5 cP. This was similar to those observed by Liu *et al.* Accordingly, the increment in *BD* of cationic corn starch (*DS* 0.04) was 4488 cP. This phenomenon could be explained by the looser swollen granule structure (due to repulsion between neighbouring cationic groups) when heated in excess water will permit the more rapid loss of granule integrity. After cooling, the solubilized starch polymers and the remaining insoluble granular fragments have a tendency to reassociate. The process involves reassociation of the molecule named retrogradation. The steric repulsion and electrostatic effects of cationic starches preventing the retrogradation of starch molecules. In this study, cationization reduced the *SB* of starches from 219.0 cP (native sago starch) to 89.5 cP (*DS* 0.06). From the work done by Solarek, the dispersion of cationic starches showed improved clarity and stability (resistance to retrogradation).

8.3 Emulsifying and Fat binding Properties

The degree of substitution for cationized sago starch was 0.06, which means there were six cationic groups attached per 100 glucose units in the cationic starch.

Emulsifying Properties

Chitosan is an unbranched cationic polymer. It is a weak polybase. The pKa of the amino group of glucosamine (C-2) residues is about 6.3, and hence chitosan dissolves and develops positively charged sites along its entire polymeric structure below pH 6.3. Cationic starch is also classified as a weak polybase. Degree of ionization for polybases increases with a decrease in pH. In this study, the emulsions were prepared at pH 4 (< pH 6.3) and 7 to investigate whether it possesses more positive charges at a lower pH. However, since chitosan is practically insoluble at pH 7, chitosan emulsions were prepared only at pH 4. Emulsifying properties of the samples were not investigated in alkaline and high acidic pH range because under high acidic condition, starch hydrolysis occurs, on the other hand, under alkaline condition, the chitosan cannot be solubilized and the starch tends to swell and gelatinize instantaneously.

In general, from the Multivariate Tests, the differences between the emulsifying properties of the three samples were significant at $p < 0.01$, but the effect of the pH on the variables were not significant ($p > 0.05$).

Emulsion stability of cationic starch

Emulsion stability is a measure of the ability to remain durable and unchanged for a serviceable lifetime. Conductivity is often used to determine the nature of the emulsion and to control its stability during time. The continuous phase is electrically conductive and the disperse phase is non-conductive. Conductivity of emulsion is proportional to the degree of dispersion of the emulsion. Apparatus using this principle for the measurement of the stability of emulsions is based on a pair of electrodes used to measure conductivity of the emulsion at a specified height in a test cell. In this study, resistivity measurements followed apparent first-order kinetics for the first 15 min of analysis and subsequently became linear. The pseudo zero-order linear regression slope (resistivity versus time) of the 15 through 60 min data was reported.

From Fig 9 and Table 4, it is evident that cationization significantly ($p < 0.05$) reduced the emulsion strength rate constant (ESRC) of sago starch by an average of 76.7%. According to Bangs and Reineccius, the smaller the ESRC values, the more stable the emulsion formed. This is due to the fact that the smaller the ESRC values, the higher is the ability of the emulsion to remain unchanged for a serviceable lifetime. Therefore the emulsions of cationic sago starches were significantly ($p < 0.05$) more stable than those of native sago starch. The ESRC value of chitosan emulsion was the lowest among these three samples. However, the differences in the ESRC values of chitosan and cationic starches were not statistically significant ($p > 0.05$). In addition, the influence of pH on ESRC values was also not significant ($p > 0.05$) for all samples.

Surface tension of emulsion

Surface tension is a manifestation of intermolecular forces. It is related to other properties derived from intermolecular forces, such as internal pressure, compressibility and cohesion energy density. Surface tension is an important tool for measuring the interaction capacity of the solvent(s) with the polymer. This value is minimum when the polymer and solvent are highly compatible.

However, the surface tension values (53 to 57 dynes cm^{-1}) obtained in this study (Table 5) shows that there was no significant difference ($p > 0.05$) between the emulsions formed by native sago starch, cationic sago starch or chitosan at both pH 4 and 7. According to Tesch *et al*, the abilities to reduce surface tension and to stabilize the emulsion were the two requirements for an emulsifier. Since the increase in the emulsion stability of cationic starch compared to its native starch is not caused by its surface active property, we can postulate that it is caused by high viscosity of the continuous phase as discussed in the following section.

Viscosity of emulsion

According to Gundersen *et al*, viscosity of an emulsion depends upon several factors: the viscosity of the continuous and the dispersed phase, the volume fraction of the dispersed phase, the average particle size and particle size distribution, shear rate, the nature of the emulsifier and temperature.

Cationization substantially ($p < 0.05$) increased the viscosity of native sago starch emulsion by an average of 6 times (Table 6). However, the viscosity of emulsion formed by chitosan showed the highest value, which was 13 times higher than that of cationic starch. This was consistent with the results obtained in the analysis of emulsion stability, i.e., the higher the viscosity of the emulsion, the greater is the stability of the emulsion formed. This was in agreement with those observed by Acton and Saffle. The increase in viscosity of the stronger polybases is probably because the cationic groups tend to weaken the granule structure due to repulsion between neighbouring groups inhibiting interchain associations. This structural loosening permits greater uptake of water with a consequent increase in the swelling of the granule which leads to a higher viscosity.

High viscosity of the dispersion medium favours emulsification possibly by slowing down the drainage of the liquid between two colliding droplets, and thus retarding break-up mechanisms such as creaming and coalescence. It is well known that the viscosity of the continuous phase can be increased by addition of hydrophilic polysaccharides such as starch (stabilizers). Most polysaccharides are predominantly hydrophilic and therefore not very surface active (so do not act as emulsifiers). The main stabilizing action of high-molecular-weight polysaccharides is by viscosity modification or gelation in the aqueous dispersion medium. It is postulated that stabilization of the emulsion formed by cationic starch is predominantly due to this similar mechanism.

As evident in the emulsion stability analysis, the influence of pH on viscosity of native and cationized sago starch emulsions was also not significant ($p > 0.05$). Thus, cationized sago starch emulsions did not bear more positive charges at a lower pH.

Fat binding capacity of cationic starch

Fig 10 shows that fat binding capacity of chitosan was the highest, followed by cationic starch and then native sago starch. The differences in fat binding capacity between all the samples were highly significant ($p < 0.01$). This is consistent with the emulsion stability analysis, i.e., the higher the fat binding capacity of the sample, the greater is the stability of the emulsion formed. However, the fat binding capacity of cationic starch is still inferior to chitosan which is widely known as an excellent fat binder. Higher fat binding capacity values of chitosan (314-535%) were observed by Cho *et al.* According to Knorr, lipid (fat) binding of chitosan can range up to four to five times its weight for chitosan under optimum conditions. The differences in fat binding capacity of cationic starch and chitosan could be attributed to the difference in charge density or distribution in the two samples.

9. SUMMARY AND CONCLUSIONS

(1) The results obtained from this research showed that it is possible to produce cationic sago starch using an aqueous-alkaline process and a cationic reagent, CHPTAC.

(2) Using adequate combinations of cationic reagent concentration, NaOH concentration and reaction temperature, it was possible to obtain different DS, reaction efficiency and, consequently, different functional properties.

(3) DS was proportional to CHPTAC concentration, NaOH concentration and reaction temperature whereas reaction efficiency was proportional to NaOH concentration and reaction temperature but inversely proportional to CHPTAC concentration. The highest reaction efficiency achieved was 78.8%.

(4) The introduction of cationic groups in starch during the aqueous-alkaline process, with a maximum DS of 0.06, caused increased solubility (75°C), peak viscosity and breakdown, and decreased gelatinization transition temperatures, gelatinization enthalpy, swelling (75°C), pasting temperature and setback. The introduction of cationic groups into the starch results in a weakening of the starch granule structure due to repulsion between neighbouring groups inhibiting interchain associations. This structural loosening permits greater uptake of water with a consequent increase in the swelling of the granule which lead to a higher peak viscosity. As the uptake can take place more rapidly, the gelatinization can occur more easily and at a lower temperature. After the granules swollen, the looser structure also permits the more rapid loss of granule integrity, and this was observed in the higher values for breakdown with the cationized starch. Steric repulsion and electrostatic effects tend to prevent the retrogradation of starch molecules, and results a reduction in setback. The increase in solubilization of high DS cationic starches at 75°C has caused a reduction of swelling factor.

(5) The introduction of cationic groups into native sago starch has successfully increased its fat binding capacity, emulsion stability and emulsion viscosity. Hence, we can suggest that there are interactions occurred between the positive charges in cationic starch and the negative charges in fat. However, the fat binding properties of cationic starch still cannot match with chitosan which is famous as a fat binder nowadays.

(6) However, there was no significant difference ($p < 0.05$) between the surface tensions (53 to 57 dynes cm^{-1}) of cationic sago starch, native sago starch and chitosan neither at pH 4 nor pH 7 obtained in this study. Thus, cationic starch improves its emulsion stability by increasing its viscosity but not its surface-active properties.

(7) The effect of pH (pH 4 and pH 7) on emulsifying properties of the native sago starch, cationic sago starch and chitosan was not significant at $p < 0.05$.

PUBLICATIONS FROM THIS STUDY

C.L. Siau, A. Abd Karim, M.H. Norziah, W.D. WanRosli & C.C. Seow (2001). Effect of cationization on functional properties of sago starch using an aqueous alkaline process. In *Abstract of BioThailand 2001: From Research to Market.* Bangkok, Thailand.

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C. L. Siau, A. Abd Karim, M.H. Norziah & W.D. Wan Rosli (2002). Response surface methodology study of cationization on thermal and pasting properties of sago starch. Submitted to *Journal of Agricultural and Food Chemistry.*

C. L. Siau, A. Abd Karim, M.H. Norziah & W.D. Wan Rosli (2002). Effect of cationization on the emulsifying and fat binding properties of cationic sago starch. Submitted to *Journal of Agricultural and Food Chemistry.*

Table 1. Coded and actual levels of the three variables used in the cationization process of sago starch.

Variable	Coded level of variable				
	-1.68	-1	0	1	1.68
CHPTAC concentration (X_1, M_{CHPTAC})	0.01	0.03	0.06	0.08	0.10
NaOH concentration (X_2, M)	0.03	0.20	0.45	0.69	0.86
Reaction temperature (X_3, ^0C)	30	36	46	56	62

* CHPTAC concentration was calculated as CHPTAC to starch molar ratio (M_{CHPTAC} = CHPTAC mole.mole^{-1} starch).

Table 2. Central composite design treatments for cationization of starch.

Std	Run	Block	Factor A: CHPTAC M_{CHPTAC}	Factor B: NaOH M	Factor C: Temperature °C
22	1	Block 1	0.06	0.03	46
14	2	Block 1	0.03	0.69	56
13	3	Block 1	0.03	0.69	56
20	4	Block 1	0.10	0.45	46
3	5	Block 1	0.08	0.20	36
9	6	Block 1	0.03	0.20	56
6	7	Block 1	0.03	0.69	36
5	8	Block 1	0.03	0.69	36
17	9	Block 1	0.01	0.45	46
16	10	Block 1	0.08	0.69	56
29	11	Block 1	0.06	0.45	46
30	12	Block 1	0.06	0.45	46
24	13	Block 1	0.06	0.86	46
7	14	Block 1	0.08	0.69	36
12	15	Block 1	0.08	0.20	56
18	16	Block 1	0.01	0.45	46
4	17	Block 1	0.08	0.20	36
19	18	Block 1	0.10	0.45	46
2	19	Block 1	0.03	0.20	36
28	20	Block 1	0.06	0.45	62
15	21	Block 1	0.08	0.69	56
23	22	Block 1	0.06	0.86	46
11	23	Block 1	0.08	0.20	56
32	24	Block 1	0.06	0.45	46
10	25	Block 1	0.03	0.20	56
1	26	Block 1	0.03	0.20	36
31	27	Block 1	0.06	0.45	46
25	28	Block 1	0.06	0.45	30
26	29	Block 1	0.06	0.45	30
27	30	Block 1	0.06	0.45	62
21	31	Block 1	0.06	0.03	46
8	32	Block 1	0.08	0.69	36

* CHPTAC concentration was calculated as CHPTAC to starch molar ratio (M_{CHPTAC} = CHPTAC mole.mole^{-1} starch).

Table 3a. Coefficients of the quadratic regression model for determination of *DS*, *RE*, T_o, T_p and T_c.

Effect [a]	Regression coefficients				
	DS	*RE*	T_o	T_p	T_c
A. Intercept	0.043	71.63	56.46	67.26	77.51
X_1	8.701E-03*	-7.90*	-2.42*	-2.14*	-1.84*
X_2	0.015*	22.97*	-2.93*	-2.38*	-1.76*
X_3	3.337E-03*	6.16*	-1.03*	-0.89*	-0.70*
B. Quadratic					
X_{11}	-6.759E-03*	-6.01*	1.51*	0.98*	0.92*
X_{22}	-8.315E-03*	-13.14*	1.39*	0.83*	0.92*
X_{33}	-3.303E-03*	-4.79*	1.05*	0.23	0.50*
C. Interaction					
X_1X_2	0.013*	12.96*	-2.51*	-2.35*	-1.87*
X_1X_3	-7.500E-05	-1.75	3.750E-03	-0.16	-0.097
X_2X_3	-5.125E-04	-2.92	-6.250E-03	-0.18	-0.16
R^2	0.9632	0.9701	0.9344	0.9482	0.9206
Adj-R^2	0.9482	0.9579	0.9076	0.9270	0.8881
Pred-R^2	0.9208	0.9344	0.8592	0.8884	0.8294

[a] X_1 = CHPTAC concentration; X_2 = NaOH concentration; X_3 = reaction temperature

* Significant at level $p < 0.05$.

Table 3b. Coefficients of the quadratic regression model for determination of *ΔH* and *PT*.

Effect [a]	Regression coefficients	
	ΔH	PT
D. Intercept	12.99	67.48
X_1	-0.93*	-1.98*
X_2	-2.06*	-2.80*
X_3	-0.24	-0.81*
E. Quadratic		
X_{11}	9.375E-03	1.33*
X_{22}	-0.38*	1.27*
X_{33}	-0.14	0.73*
F. Interaction		
$X_1 \times X_2$	-1.19*	-2.33*
$X_1 \times X_3$	-0.22	0.014
$X_2 \times X_3$	-0.18	-0.091
R^2	0.9421	0.9548
Adj-R^2	0.9184	0.9363
Pred-R^2	0.8762	0.9026

[a] X_1 = CHPTAC concentration; X_2 = NaOH concentration; X_3 = reaction temperature

* Significant at level $p < 0.05$.

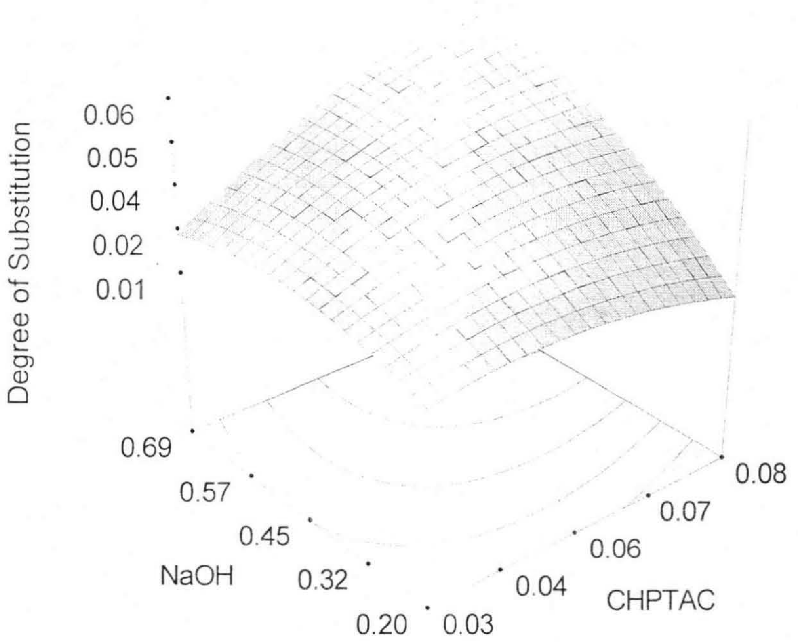

Figure 1. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the degree of substitution of cationic starches.

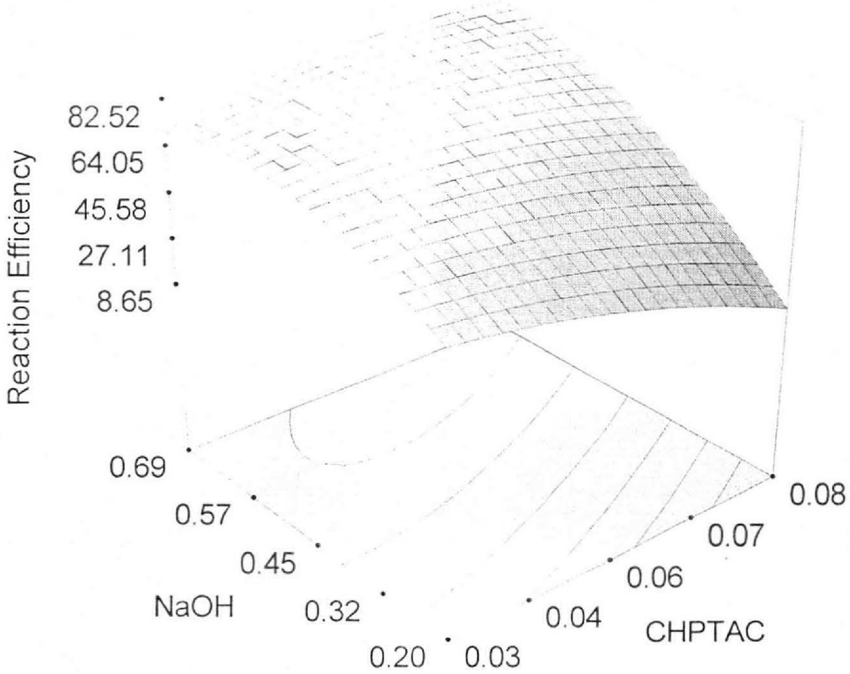

Figure 2. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the reaction efficiency of cationization.

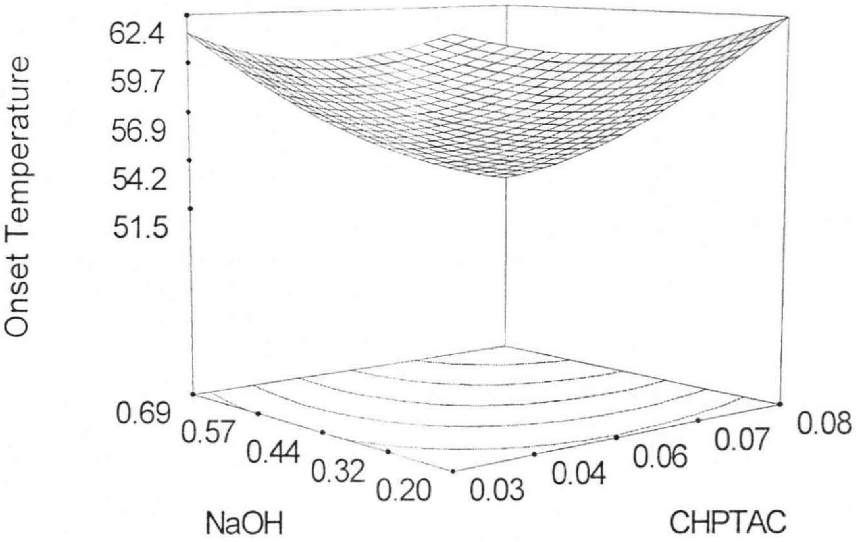

Figure 3. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the onset temperature of cationic starches.

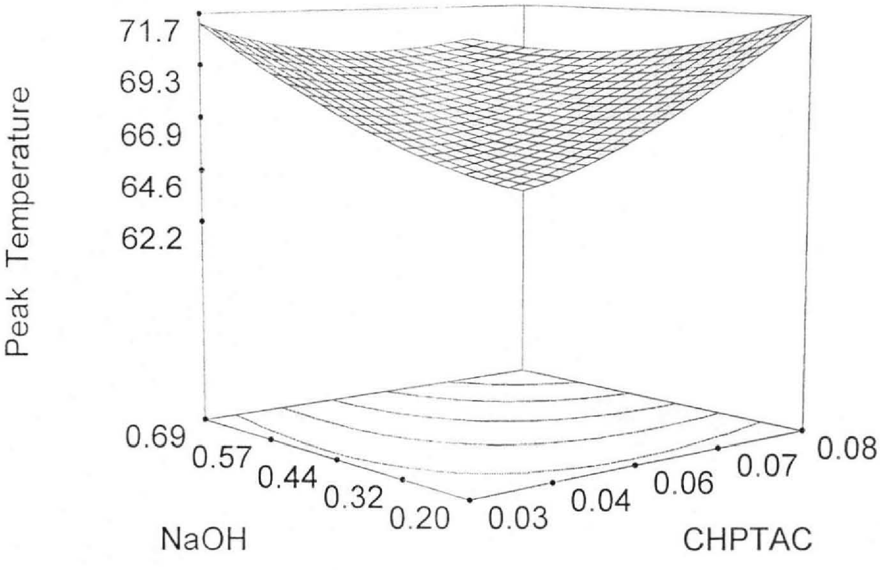

Figure 4. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the peak temperature of cationic starches.

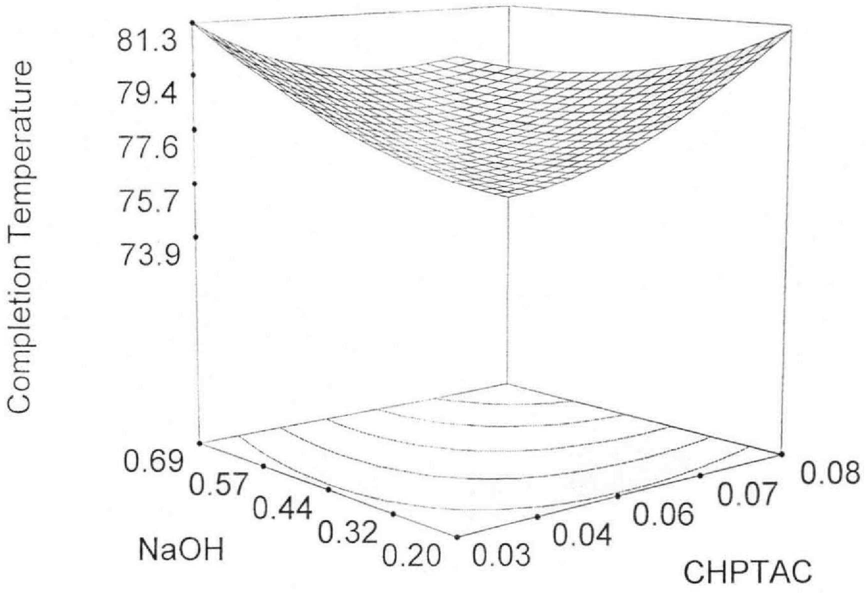

Figure 5. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the completion temperature of cationic starches.

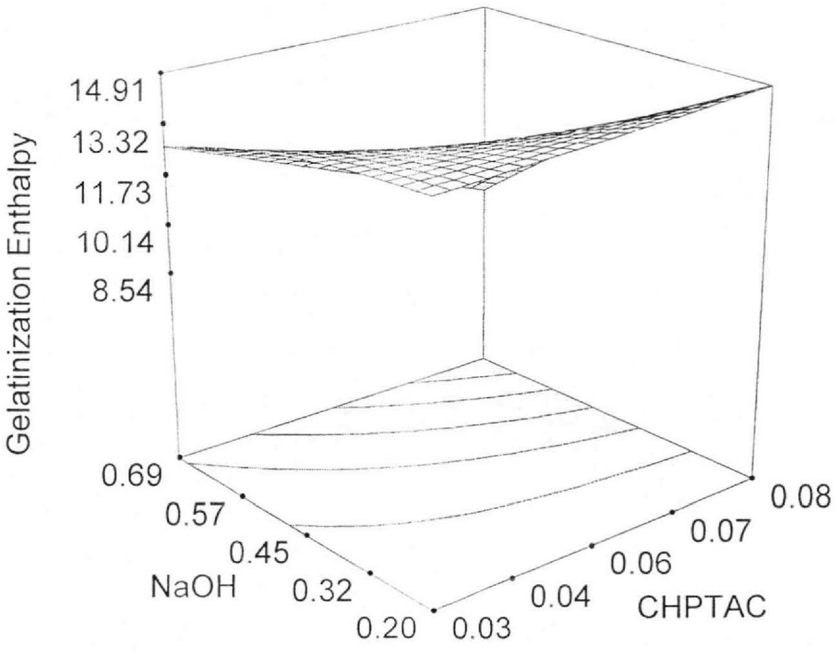

Figure 6. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the gelatinization enthalpy of cationic starches.

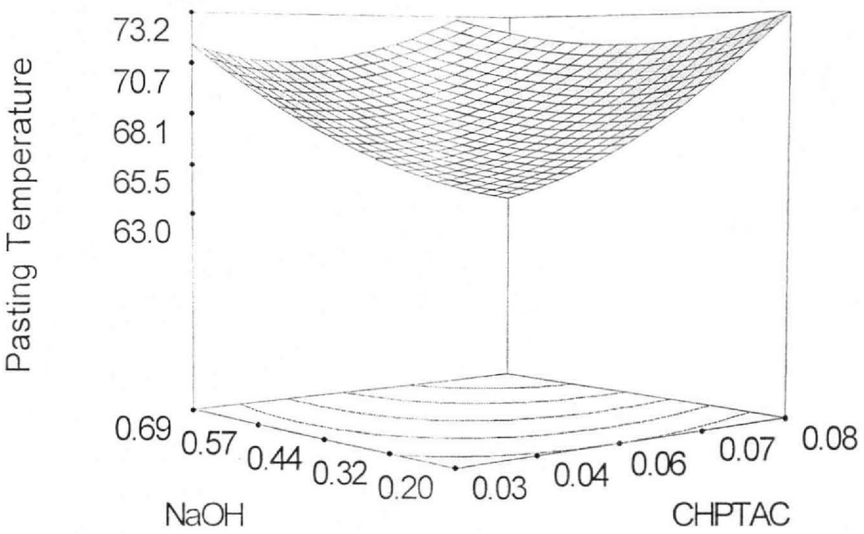

Figure 7. Effect of NaOH and CHPTAC concentration at reaction temperature of 46ºC on the pasting temperature of cationic starches.

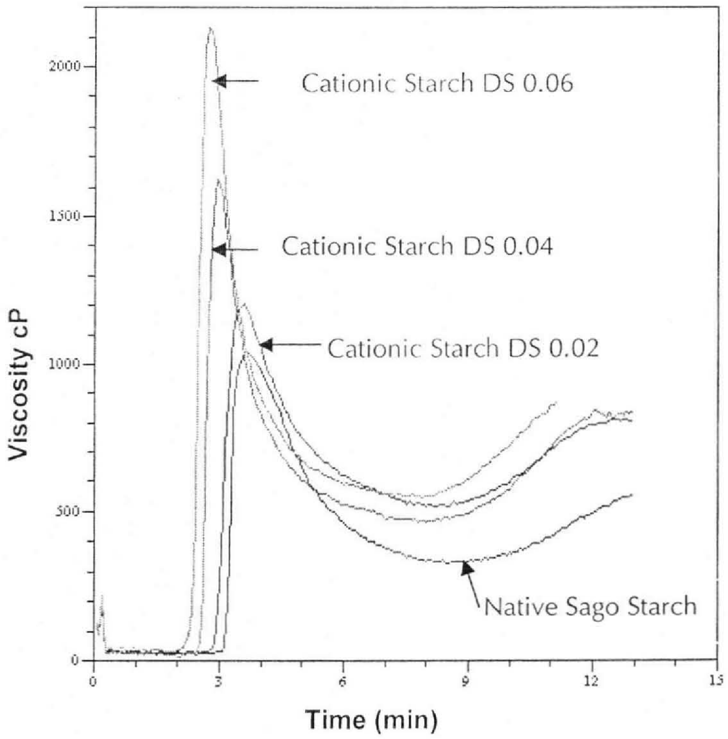

Figure 8. RVA pasting curves for native sago and cationic starches.

Table 4. One factor design for fat binding capacity of native sago starch, cationic starch and chitosan.

Std	Run	Block	Samples
12	1	Block 1	Chitosan
5	2	Block 1	Cationic starch
7	3	Block 1	Cationic starch
10	4	Block 1	Chitosan
11	5	Block 1	Chitosan
2	6	Block 1	Native sago starch
8	7	Block 1	Cationic starch
4	8	Block 1	Native sago starch
1	9	Block 1	Native sago starch
6	10	Block 1	Cationic starch
3	11	Block 1	Native sago starch
9	12	Block 1	Chitosan

Table 5. Linear regression coefficients of the dependence of resistivity ratio (y) on time (x), $y = cx + d$.

Samples	Emulsion strength rate constant, c (min^{-1})	Resistivity ratio at time zero, d	R^2
pH 4			
Native sago starch	0.0181^a	0.0930	0.9973
Cationic starch	0.0042^b	0.0171	0.9968
Chitosan	0.0011^b	0.1288	0.9327
pH 7			
Native sago starch	0.0192^a	-0.0592	0.9990
Cationic starch	0.0045^b	-0.0582	0.9774

Means within the emulsion strength rate constant column with the same letter are not significantly different at the 5% probability level.

Table 6. Emulsion surface tension (mean ± SD) of native sago starch, cationic starch and chitosan at pH 4 and 7.

Samples	Surface tension (dynes cm^{-1})	
	pH 4	pH 7
Native sago starch	54.2 ± 1.4^{a}	53.5 ± 0.4^{a}
Cationic starch	54.6 ± 1.7^{a}	56.2 ± 1.4^{a}
Chitosan	56.2 ± 0.1^{a}	-

Means within the same column or the same row with the same letter are not significantly different at the 5% probability level.

Table 7. Emulsion viscosity (mean ± SD) of native sago starch, cationic starch and chitosan at pH 4 and 7.

Samples	Viscosity (cP)	
	pH 4	pH 7
Native sago starch	4.13 ± 0.10^{a}	4.42 ± 0.37^{a}
Cationic starch	28.55 ± 0.78^{b}	30.50 ± 1.70^{b}
Chitosan	406.50 ± 2.12^{c}	-

Means within the same column or the same row with the same letter are not significantly different at the 5% probability level.

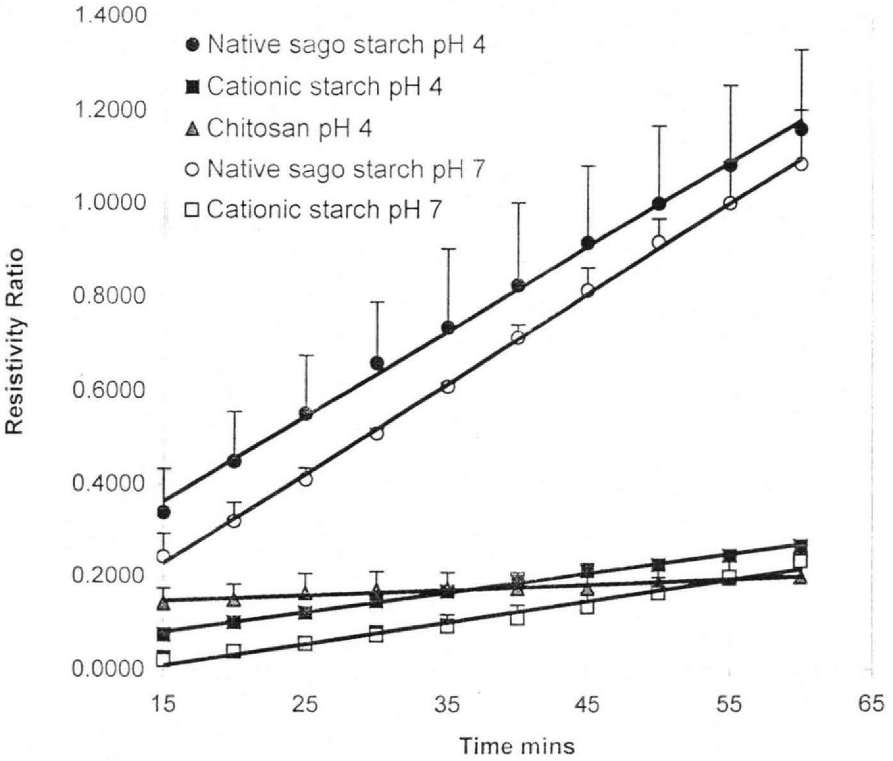

Figure 9. Resistivity ratio as a function of time for native sago starch, cationic starch and chitosan emulsions at pH 4 and 7. Resistivity ratio = $(\text{Time}_i - \text{Time}_0)\ (\text{Time}_0)^{-1}$; Time_0 = resistivity value (ohms) at time zero; Time_i = resistivity value (ohms) at time in experiment; Emulsion strength rate constant = slope.

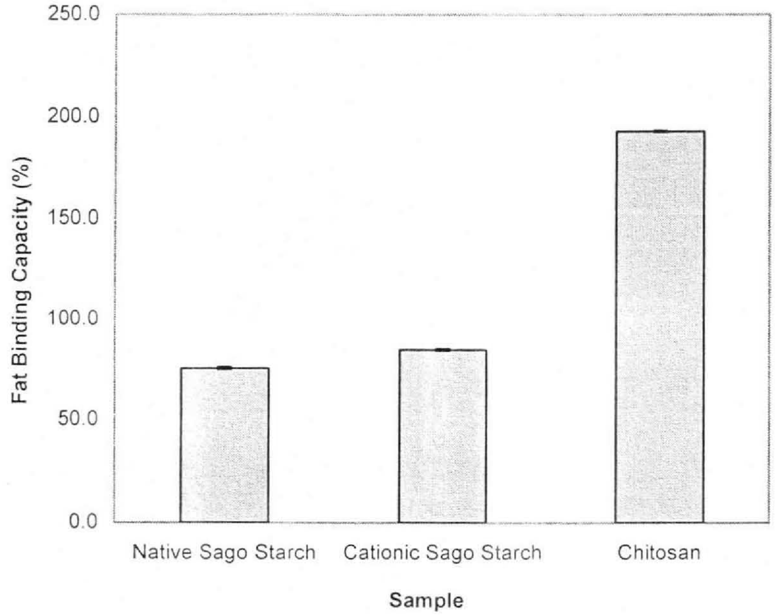

Figure 10. Fat binding capacity of native sago starch, cationic sago starch and chitosan.