

CHARACTERISATION AND SOLUBILITY STUDIES ON MICROENCAPSULATION OF CLINACHANTUS NUTANS (BELALAI GAJAH) UTILIZING SAGO STARCH

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Abstract

This study was undertaken to evaluate the potential of gelatinized sago starch combination with different wall materials in the microencapsulation of Clinachantus nutans or C.nutans essential oil by freeze drying method. C.nutans essential oils were encapsulated either with only one wall material (gelatinized sago starch only) or the combination of gelatinized sago starch and gum Arabic in order to maximize encapsulation performance. The performance of C.nutans essential oil encapsulation was evaluated by Fourier transform infrared (FT-IR) spectroscopy, total phenolic content, thermal transition properties (DSC), Scanning electron microscopy (SEM) analysis, and Particle Size Analysis. The combination of dual core materials (gum Arabic and gelatinized sago starch) is the best encapsulation efficiency featured as microparticles that exhibit the retention percentage at 50.7%, smaller diameter of particle size distribution around 34.16 μm and higher endothermic at 86.8°C.

Keywords: Clinacanthus nutans; Essential oils; Microencapsulation;
Gelatinized sago starch; Gum arabic

Introduction

Plants and herbs have been used to treat health problem and ailments for many years [1]. Many fields of studies mostly in pharmacological and chemical have been conducted in various plant extracts to analyse their chemical constituents and therapeutic benefits. Herbal products have played a crucial role today because the purpose was not only to heal the diseases but also to prevent the diseases from occurring. Moreover, it also possesses a complete biodegradability. Furthermore, the human body can metabolize chemical constituents at a faster rate by using plant material compared to artificial drug. The chemical constituents also show few side effects due to low or absent toxicity. Public demand for the usage of effective evidence herbal medicine have been increased with time as a consequence of greater health issue promoted by the government, therapeutic effects, fewer adverse effects and a growing distrust in conventional synthetic medicine.

Clinacanthus nutans belong to the Acanthaceae family. The Acanthaceae family includes about 250 genera and 2500 species of dicotyledomous flowering plants that are generally herbs and shrubs [2]. *C.nutans*, also known as “Sabah Snake Grass” or “Belalai Gajah”, have distributed widely in tropical Asia and subtropical region such as Malaysia, China, Indonesia, Vietnam and Thailand. It is a fast-growing, small shrub herb, about one meter tall,

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and can multiply through stem cuttings [3]. *C.nutans* is commonly consumed by local ethnic in Malaysia in its raw form or blended with water as juice or herbal tea. In Thailand, Malaysia and China, *C.nutans* has been recognized as famous folklore medicinal properties. *C.nutans* is a medical plant with promising therapeutic characteristics for treatment and prevention of diseases such as treating inflammation and viral infection [4]. It can also be an anti-hepatitis, antioxidant, anti-herpes, antibacterial and antitumorigenic agents [4-6]. *C.nutans* essential oils are volatile, evaporate easily and can decompose when exposed to light, heat and pressure [7].

The encapsulation of essential oils aims to preserve and protect their functional properties and provide a controlled release in a given medium. Most encapsulation processes involve the microencapsulation method which is one of the most effective techniques for protecting essential oils compound that are very volatile from oxidation and thermal degradation. Microencapsulation means the encapsulation around microscopic particles having a size range of 1 to 800 μ m in which active core material such as essential oils is surrounded by protective sheath of wall materials such as gum Arabic (acacia gum), modified starch and maltodextrin [8]. Microencapsulation can be achieved by various techniques such as by spray drying, coacervation, extrusion, inclusion in cyclodextrins, centrifugal extrusion and freeze drying [8]. Freeze drying is one of the most popular techniques especially when handled with heat sensitive volatile compound because the operation takes place at very low temperatures. Freeze drying is a process widely used for high quality microencapsulation of oils and flavours although it is less attractive than other methods of drying due to their high energy consumption and long processing time [9]. Since freeze drying is applied at very low temperatures and water is removed from the system by sublimation of ice using vacuum, oxidation and other chemical changes are limited [10]. It also results in powders with good quality, no water activity as well as easier handling and storage.

Choosing suitable wall material for a specific core material is an important step in microencapsulation. Starch has been recognized as a water-soluble, biodegradable, food grade and nontoxic biopolymer which is universally available at a low cost [11]. Hence, gelatinized sago starch was used as a wall material in microencapsulation for *C.nutans* essential oils. However when water is added to dry starch granules, the volume of the granules increases due to hydration. As the temperature rose, the starch granules broke down and became gelatinized. The gelatinized sago starch becomes high in viscosity at high solid concentrations. Therefore, it is desirable to use sago starch in combination with other surface-active biopolymer such as gum Arabic. Gum Arabic has more unique properties compared to the other types of gums as a higher concentration (40-50%) of gum Arabic does not produce too high viscosity and can create a perfect stability [12, 13]. Gum Arabic is also a good emulsifying agent because of its protective colloid ability [13]. There has been many research regarding the extraction and active compound from *C.nutans* essential oils but there no significant literature has been reported on about the microencapsulation of *C.nutans* with gelatinized sago starch and the influence of different type wall materials on the encapsulation efficiency of this *C.nutans* essential oil. This work aims at to determine the characterization of microparticles of *C.nutans* with combination of gelatinized sago starch and other wall materials ratio in terms of encapsulation efficiency, particle size, moisture content and its morphology.

Material and Method

Materials

Sago starch was purchased from local suppliers. *Clinacanthus nutans* leaves were collected in Meru, Klang, Malaysia and gum arabic was supplied by Sigma-Aldrich. All chemicals were used as received without further purification.

Plant Material Preparation

The leaves of *C.nutans* were washed several times to remove any unwanted residuals. Before the extraction process, the leaves were dried using an oven (Memmert, Germany) for 1 day at the temperature of 45°C to remove moisture. The drying leaf was pulverized into fine powder using mechanical blender (Retsch GmbH, Germany) and stored in an air-tight container for further used.

Preparation and Purification of Extracted Oil from C.nutans Leaves

The dried and ground leaves of *C.nutans* were extracted by using soxhlet extraction for about 6 hours. Dried fine powder of *C.nutans* was placed in a thimble which was then set in an extraction chamber which is suspended above a flask containing ethanol as the solvent. The flask was heated at 80°C and the solvent evaporated and moved up into the condenser. The evaporated solvent was converted into liquids that trickle into the extraction chamber that contained the sample. At the end of the extraction process, the flask containing the solvent and samples were removed. The pooled ethanol solvent and extracted *C.nutans* were evaporated to dryness and concentrated by using a rotary evaporator (RotavacVario Control) at a temperature of 35°C and 90RPM. The solvent was efficiently removed from the samples through an evaporation process. The purified *C.nutans* oil was obtained.

Preparation of Extracted Oil-Encapsulated with Gelatinized Sago Starch/Gum Arabic

Sago starch (300mg dry solids) was dissolved in 30mL distilled water, 1% (w/v). Subsequently, the dry solids were blended with pre-heated extracted oil (60mg) at 50°C. The mixture of starch and extracted oil with the ratio 5:1 (mg: mg) basis was vigorously stirred in water bath for a duration 3h and at a temperature of 80°C. While at the same condition, the mixture of sago starch, gum arabic and extracted oil (SS: GA: EO) with different wall materials at ratios of 2:3:1, 3:2:1, 1:2:1 and 2:1:1 were prepared as the next samples. Then, the mixture was slowly cooled down at room temperature with continuous stirring using shaker for about 8h, and then centrifuged to obtain supernatant (5000g, 30min, 20°C). Then, an ultrasonic homogenizer (Alpha Zulu Technology, Malaysia) was carried out to obtain homogeneous suspension of the dispersion (frequency 42kHz and power 185W) for about 10min. The suspensions were later placed in refrigerator about 24h prior to the freeze drying process.

Proximate Analysis for Encapsulated C.nutans Microparticles

FTIR analyses (model: Perkin Elmer, 2000) for *C.nutans* microparticles (gelatinized sago starch or combination of gelatinized sago starch and gum arabic) for the purpose to obtain an infrared spectrum of absorption or emission of a *C.nutans* microparticles samples. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. FTIR spectra in mid IR range 4000-400cm⁻¹ and IR spectra were recorded by using Jasco FT/IR-460 plus Fourier Transform Infrared Spectrophotometer. Using FTIR fingerprint profiles, sample of extracted oil were analyzed. The total soluble phenolic content in the extract was determined by using Folin-Ciocalteu reagent and gallic acid (also known as 3, 4, 5-trihydroxybenzoic acid) as a standard according to the method by Scalbert & Williamson [14]. A solution of 2mg/mL of extracts and a solution of 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0mg/mL of gallic acid in methanol were prepared. 20µL of extract and each concentration of gallic acid solution were pipetted in separate test tubes followed by the addition of 1.58mL of distilled deionized water and 100µL of Folin – Ciocalteu reagent. Subsequently, the solution in the test tubes was mixed thoroughly. After 8min, 300µL of 20% Na₂CO₃ solution was added. The mixture was then allowed to stand for 2 hours with intermittent shaking. The absorbance of solution was measured at 765nm. All sample determinations were carried out in triplicate. Thermal transition analysis was performed by using a differential scanning calorimeter (DSC) (Seiko DSC 6100, Chiba, Japan) for the purpose to exhibit the DSC thermograms of freeze-dried powders of the *C.nutans* microparticles (gelatinized sago starch or combination of gelatinized sago starch and gum Arabic). Sample solids of 0.9mg were mixed with distilled water (1.8mg) in aluminum DSC pan and the mixture was equilibrated at 4°C for 2h prior to

analysis. Scanning was performed at 20 to 250°C at a heating rate of 5°C/min. The empty pan was used as the reference.

Particle Size and Morphology

The mean particle size of microencapsulation was determined by laser light scattering instrument, Mastersizer S (Malvern Instruments, Malvern, UK) with 633nm wavelength. The particle size was expressed as D (4, 3) representing the volume weight mean diameter. The morphology of the freeze-dried microencapsulation was studied by Scanning Electron Microscopy (SEM) (HITACHI TM3000 Tabletop Scanning Electron Microscope, Hitachi High Technologies America, Inc). The frozen dried microencapsulation was placed on a glass plate and dried at room temperature. The dried microencapsulation was then coated with gold metal under vacuum and will be examined for SEM images with magnifications.

Results and Discussion

Fourier Transform Infrared (FTIR) Characterisation

Structural characterization by FT-IR spectroscopy is shown in Figures 1 to 4. Figure 1 and figure 2 showed the result of *C.nutans* encapsulated with sago starch before and after freeze drying and figures 3 and 4 was the result of the wall ratio of *C.nutans* encapsulated with sago starch and gum arabic. Spectra reveal that gum Arabic (GA) showed a broad band at 3750cm⁻¹ and 3310cm⁻¹ due to stretching vibration of O-H that show characteristic of the glucosidic ring. The polymers also showed the characteristic band of C=C stretch, amide NH bend, NO₂ from both aliphatic and aromatic galactoproteins and amino acids around 1603cm⁻¹. The absorption at 1542 and 1411cm⁻¹ was due to C=O symmetric stretching and -OH bending. The distinct band at 1014cm⁻¹ represents alkene C-H bend from polysaccharides for gum arabic.

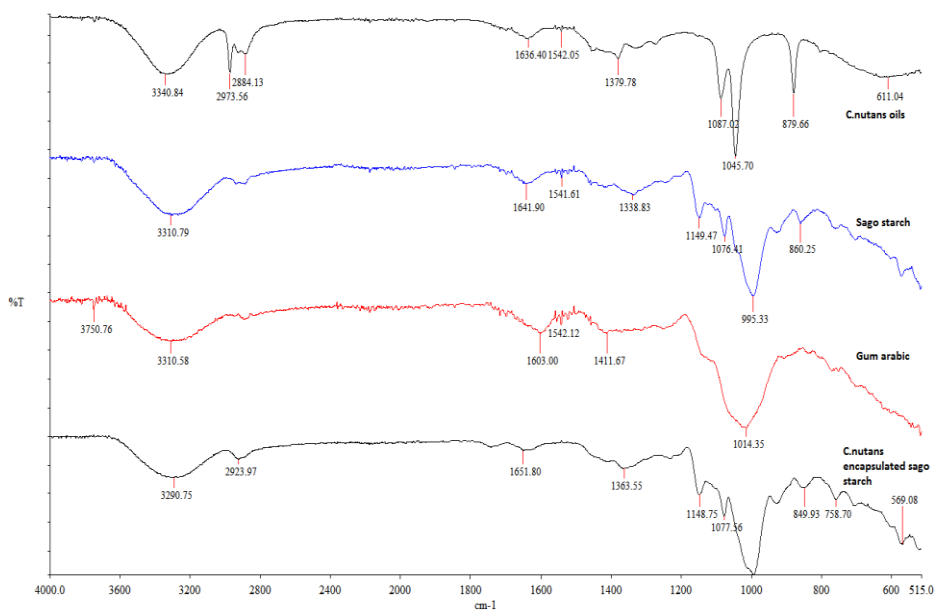


Fig. 1. FT-IR spectra of *C.nutans* oils, sago starch, gum Arabic and *C.nutans* encapsulated with sago starch before freeze drying process

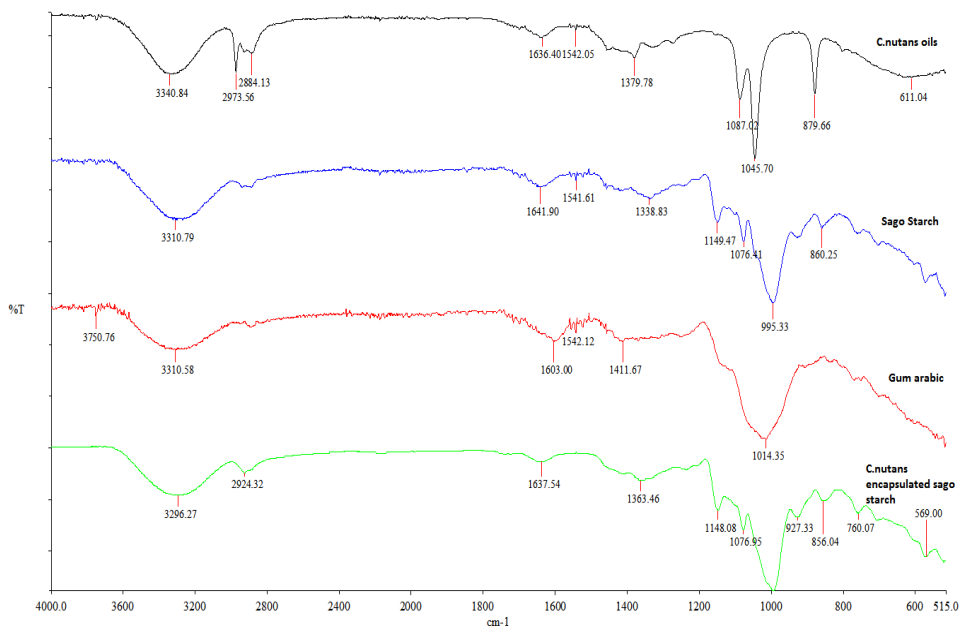


Fig. 2. FT-IR spectra of *C.nutans* oils, sago starch, gum Arabic and *C.nutans* encapsulated with sago starch after freeze drying process

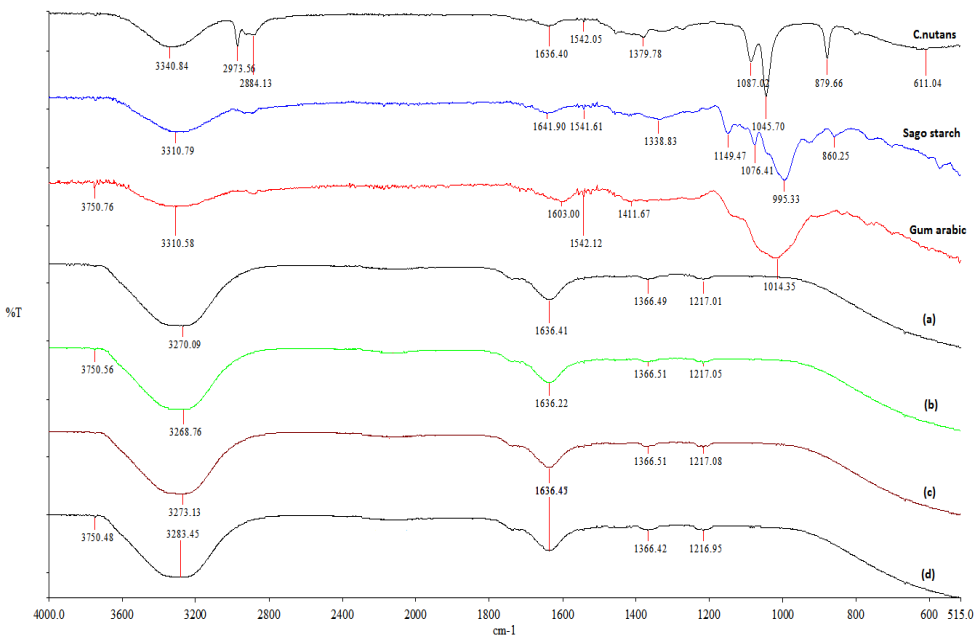


Fig. 3. FT-IR spectra of *C.nutans* oils, sago starch, gum Arabic and *C.nutans* encapsulated with different wall ratios (GA: Sago Starch) (a) 1:2; (b) 2:1; (c) 2:3; (d) 3:2 before freeze drying process

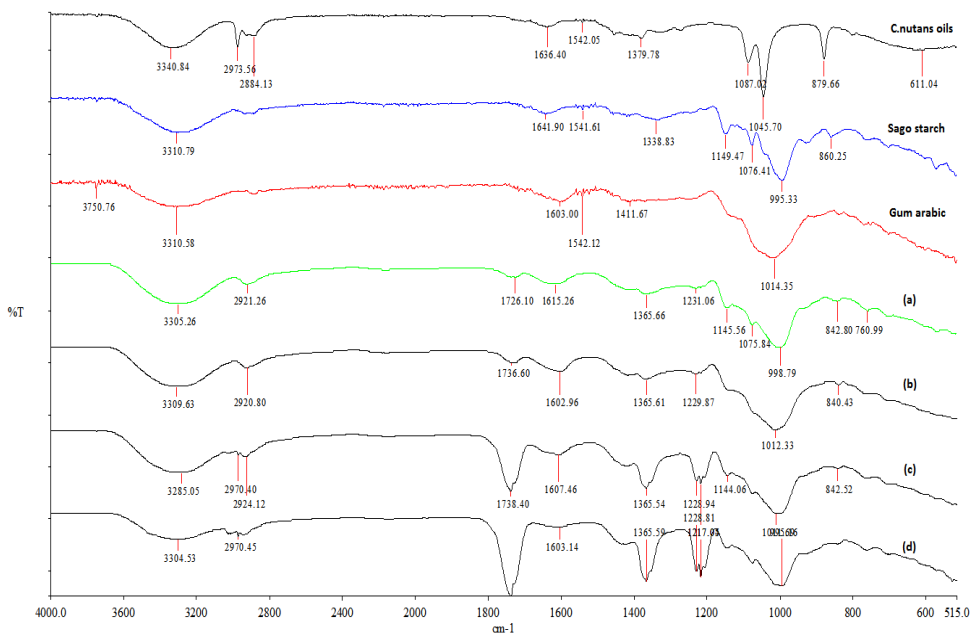


Fig. 4. FT-IR spectra of *C.nutans* oils, sago starch, gum Arabic and *C.nutans* encapsulated with different wall ratios (GA : Sago Starch) (a) 1:2; (b) 2:1; (c) 2:3; (d) 3:2 after freeze drying process.

The *C.nutans* (CN) essential oils spectrum showed a range of band between 4000-2000cm⁻¹ are typically due to the functional groups such as -OH, C=O, N-H, CH₃. A broad band at 3340cm⁻¹ corresponding to O-H stretching vibration from alcohol; a band at 2973cm⁻¹, due to stretching vibration of the methyl groups (-CH₃); and at 2284cm⁻¹ owing to methylene groups (-CH₂-). The regions 2000-500cm⁻¹ are referred to as the fingerprint region, which is highly specific for each taxon. The spectra band at 1542 and 1636cm⁻¹ indicates the amides groups. The peak at 1379 cm⁻¹ denotes lignin. The peaks at 1087 and 1045cm⁻¹ represent carbohydrates such as starch. The band below 1000cm⁻¹ is shared by two accessions. Sago starch spectrum shows the broad band at 3310cm⁻¹ was attributed to O-H bond stretching. The bands between 1641 and 1541cm⁻¹ were assigned to water (H₂O) bending vibrations. The peak at 1338cm⁻¹ was attributed to C-O-H stretching. The spectra band at 1149cm⁻¹ due to C-O bond stretching. The bands at 1076cm⁻¹ was associated with the ordered and amorphous structures of starch; at 995 cm⁻¹ owing to D-glucopyranosyl ring vibration; and at 860cm⁻¹ stretching of C-H absorbance of D-glucopyranosyl stretching.

The presence of the *C.nutans* in microparticles of sago starch before and after freeze drying in figures 1 and 2 can be mainly observed in spectra through appearance of new band at 2924 and 2923cm⁻¹ due to the *C.nutans* essential oils that represents C-H asymmetric or symmetric stretching vibrations which indicate the alkanes (CH₃, CH₂, and CH) which may probably the -CH₂ groups of lipids. The broad absorption peak in the range of 1100-990cm⁻¹ in figures 1 and 2 indicates the C-O stretching from C-O-C and C-O-H in the glycosidic ring of sago starch [15]. The combination of wall materials between sago starch and gum Arabic showing the new spectra was formed. Figure 3 showed the spectra of *C.nutans* encapsulated with different wall materials ratios before freeze drying, while, figure 4 showing FT-IR spectra of *C.nutans* encapsulated with different wall materials after freeze drying. It can be seen in figure 4 that the new spectra at 3750cm⁻¹ was formed when wall materials ratio of gum Arabic at 2:1 and 3:2 due to the %w/w of gum Arabic was higher in the wall material ratio. However, after the freeze drying process, figure 4 the spectra was absences due to stretching vibration of

O-H that show characteristic of the glucosidic ring. Figure 4, the new spectra were observed at 1736-1726cm⁻¹ due to stretching vibration of carbonyl group characteristic of the secondary amides and other compounds containing C=O group. The combination of gum Arabic and sago starch in figure 4 were observe the change (broadening) in bands of the spectrum (in the region 1615-1603cm⁻¹, due to gum Arabic, COO- and water) [16].

Total Phenolic Content

The total phenolic content of *C.nutans* extract and microencapsulation powders are shown in figure 5 and table 1. The results of the total phenolic were presented in mean ±SD mg of Gallic acid equivalent/g of extract. Regarding the total phenolic content, the values were significantly lower for all microencapsulated powders when compared to the original extract (7.5±0.26mg/g). The retention percentage for *C.nutans* encapsulated with sago starch was 37.3% while the retention percentages range from 42.7% to 50.7% for the combination of wall materials (GA:SS). The polyphenols lost during the drying process can result due to several factors. During freeze drying, lyophilisation process and grinding of the microencapsulation powder after freeze drying may lead to degradation compounds, since the powder form may induce the occurrence of oxidation reactions [20].

Table 1. Total phenolic contents of *C.nutans* micro particles after freeze drying expressed in terms of Gallic acid equivalent (mg of GA/g of extract). Results are the mean±SD (n = 3).

Samples	Total Phenolic (mg of GA/g of extract)
Control (<i>C.nutans</i> oils)	7.5 ± 0.26
EO encapsulated with Sago starch	2.8 ± 0.18
GA:SS(3:2)	3.4 ± 0.37
GA:SS(2:3)	3.2 ± 0.21
GA:SS(1:2)	3.3 ± 0.20
GA:SS(2:1)	3.8 ± 0.10

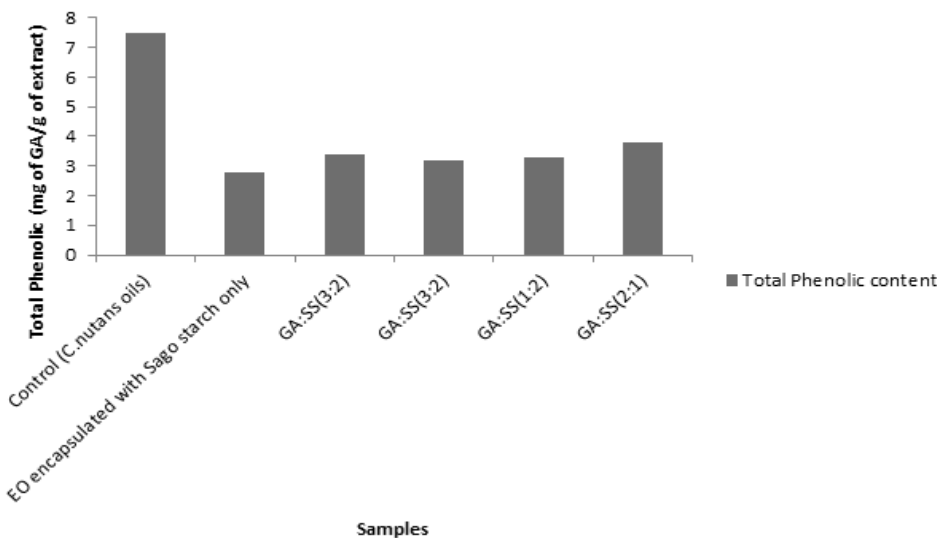


Fig. 5. Total phenolic contents of *C.nutans* micro particles after freeze drying

Differential Scanning Calorimeter (DSC)

The thermal behaviour of the microparticles can be noted in DSC. Figures 6 to 8 showed the DSC thermograms of *C.nutans* extracted oil-encapsulated with gelatinized sago starch only, raw gum arabic and *C.nutans* extracted oil-encapsulate with combination of wall materials ratio (GA:SS). Figure 6 shown an endothermic peak at 72.6°C corresponding to its melting temperature. Based on *Z. Othman et al* [17], the melting temperature for native sago starch is around 73.3°C. For all microcapsules for combination of wall materials, GA:SS (1:2,2:1,3:2 and 2:3), the DSC showed a thermal event (an endothermic peak) corresponding to the melting point of microcapsules which was noted at temperatures between 72.6 °C and 86.8°C. Figure 7 showed the raw gum Arabic endothermic peak around 101.1°C. Based on *R.M. Daoub et al* [18], endothermic peak for native gum Arabic around 100 to 150°C. Figure 8 shows the increases of melting temperature of *C.nutans* extract encapsulated with different wall materials ratio. The increases of melting temperature resulted when adding and combining the different wall materials between sago starch and gum Arabic. Therefore, the melting point was increased by the addition of gum Arabic to improve the thermal stability of the microcapsules. *H.C.F. Carneiro et al* [19], relating to a similar behaviour in samples of flaxseed oil microencapsulated with gum Arabic.

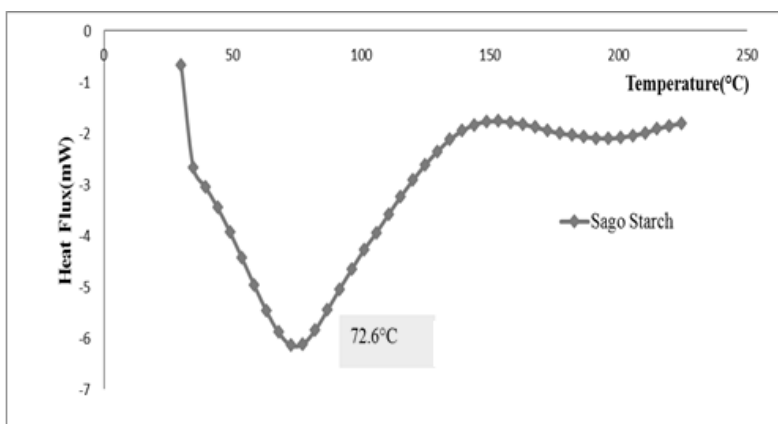


Fig. 6. DSC of *C.nutans* extract encapsulated with sago starch

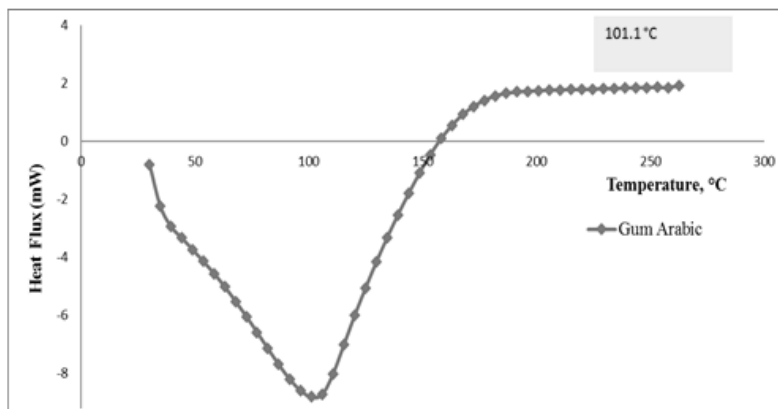


Fig. 7. DSC of raw gum arabic

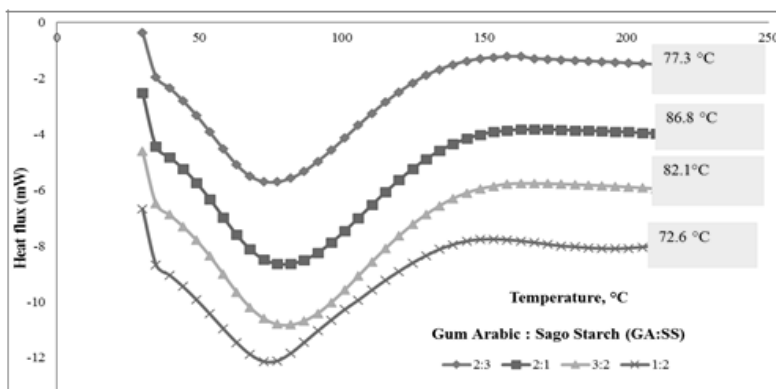


Fig. 8. DSC of combination of wall materials ratios

Size and Morphology of Microparticles

For particle size distribution analysis using a laser light diffraction instrument, both samples, either the combination of wall materials or only using one wall material showed unimodal distribution. Table 2 shows the average size (D_{4,3}) of *C.nutans* extract encapsulated with different wall materials after freeze drying process. Regarding to average particle size, it is observed that for the sample using only gelatinized sago starch as a wall material has higher size (179.98µm) than the one having the combination of core materials between sago starch and gum Arabic (34.16µm). The wider distribution and the higher size in *C.nutans* encapsulated with sago starch depicted that this wall material was less homogeneous. It was also related to the lower stability of the microparticles. Moreover, *C.nutans* encapsulated with sago starch showed higher particle mean diameter due to their high viscosity for the gelatinized sago starch. The atomized droplets size varies directly with viscosity at a constant atomization speed [21]. The higher the viscosity, the larger are the droplet formed during atomization, and therefore, the larger are the powdered particles obtained and decreased encapsulation efficiency of *C.nutans* essential oil microparticles. This is in agreement with the results published by S.A. Hogan et al. [22] for fish oil microencapsulated using carbohydrates and sodium caseinate with different concentration of dextrose equivalence (DE) as wall materials.

Table 2. Average diameter size of the *C.nutans* microencapsulated with sago starch and combination of different wall material ratios (GA:SS) after freeze drying

Samples	Average diameter [D _{4,3}] (µm)
<i>C.nutans</i> encapsulated with Sago starch only	179.98
GA:SS(3:2)	85.87
GA:SS(2:3)	92.02
GA:SS(1:2)	101.07
GA:SS(2:1)	34.16

In Figure 9, when wall material proportion (% w/w) of sago starch was higher than gum Arabic (GA:SS ratio; 1:2 (100mg:200mg); 2:3(140mg:160mg)) the surface of the powder was rougher, had more dented surface and shrinkage. In 4.0k x magnification SEM images shown the presence of crack and dent on the surface of dried powder resulting higher surface oil content at the surface of microcapsules walls. The existence of this oil is undesirable because it will have contact with the surrounding environmental conditions so that it can be damaged. Furthermore, when sago starch (%w/w) is higher than gum Arabic, the particles size appeared to be larger when observed at the same magnification. Higher (%w/w) of gum Arabic provides high emulsion properties. As a result, the combination of *C.nutans* extract with different wall

materials ratios had a smoother surface of microcapsules, higher encapsulation efficiency, low permeability to gases, better protection and retention of *C.nutans* oils compared to the *C.nutans* extract only with gelatinized sago starch as a wall material.

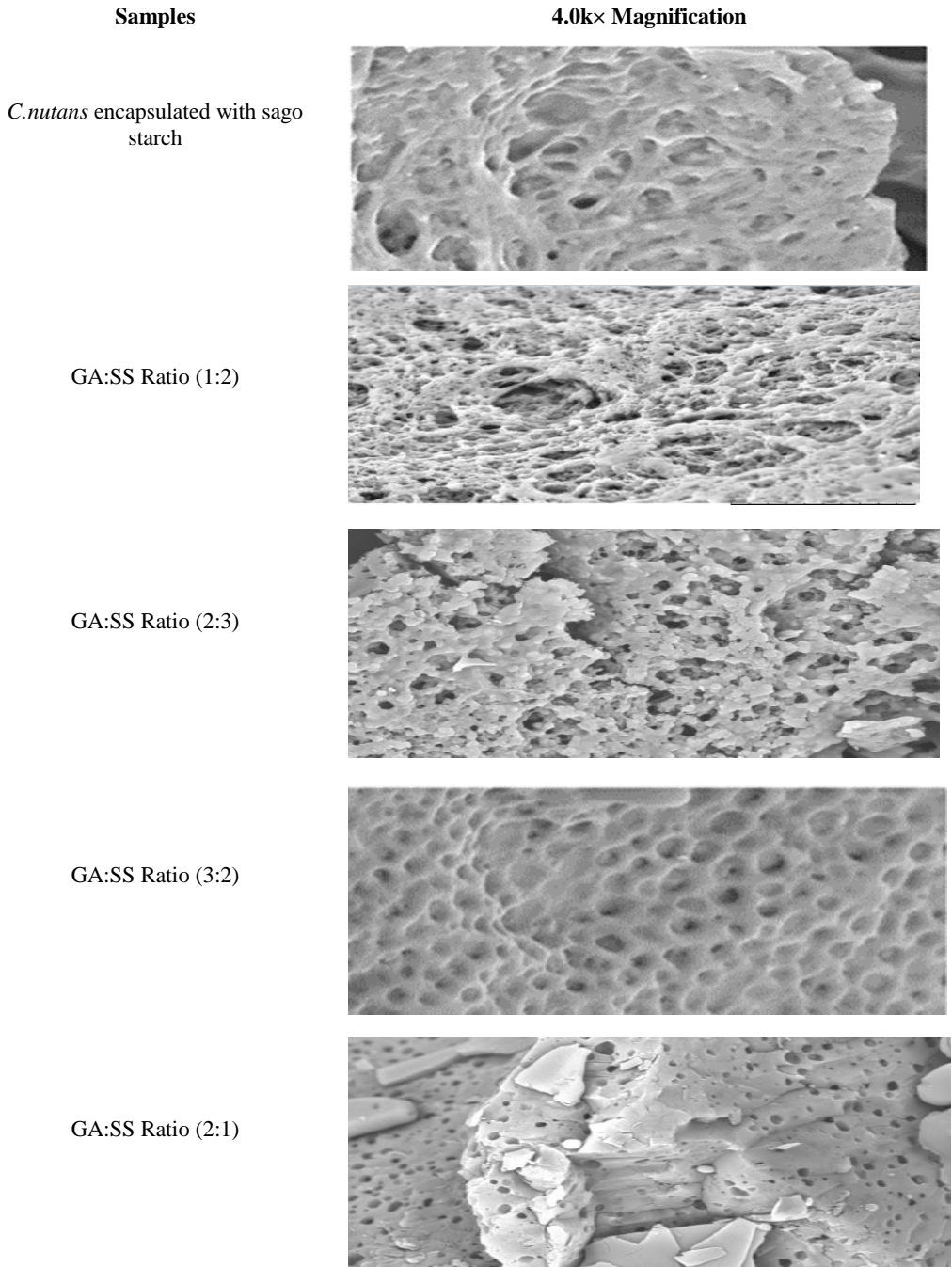


Fig. 9. Porosity of *C.nutans* encapsulated after freeze drying

Conclusion

This study indicates the performance of the *C.nutans* encapsulation with combination of core materials by freeze drying method. FT-IR result shows new appearance band and observed the change (broadening) in bands of spectrum when combination of wall materials (sago starch and gum Arabic). The combination of wall materials ratios make the endothermic of microparticle becomes higher than when only used sago starch as a wall material in DSC analysis. Higher melting point indicated of a strong solvent-solute interaction between wall materials and essential oils. Moreover, the structure of microparticle that only used sago starch as a wall material become rougher, more dented surface and the porosity became higher than those microencapsulated with combination of sago starch and gum Arabic. The combination of core materials had change the structure and surface morphology by decreasing surface dents and increasing smoothness. For particle size analysis, the combination of wall materials showed smaller diameter of particle size distribution (34.16 μm) compared to non-combination of wall material (only sago starch, 179.98 μm). The wider distribution and higher size concluded that this core material was less homogenous. So, it can be concluded that when using the combination of core materials between gum Arabic and sago starch showed the best encapsulation of microparticle of *C.nutans* based on encapsulation efficiency, the morphology of the microcapsule and the particle size distribution. The best encapsulation efficiency was obtained from the combination with different core materials (gum Arabic and gelatinized sago starch) because it can produce microparticle with good emulsion agent, high solubility and low viscosity.

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