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ORIGINAL ARTICLE

Various Drying Techniques for Conversion of Extracted Chicken Feather Keratin Solution to Powder

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ABSTRACT – Keratin powder is produced from the drying of keratin solution extracted from chicken feathers. Powdered form of keratin protein eases the storage and transport of keratin and can be further developed into nutrient supplements. The objective of this research is to convert liquid keratin obtained from chicken feathers into powder through different methods and also to identify the effects of various drying methods on the keratin sample. Liquid keratin was converted into solid particles through spray drying, freeze drying and vacuum-oven drying where the products were visually observed and analysed using FTIR and SEM to determine the effects of the drying methods on the keratin sample. The SEM results show that the product of spray drying produced smaller spherical particles with diameter ~3µm-17µm while freeze drying and vacuum-oven drying produced coarse, flaky irregular-shaped particles with diameter ~70µm-470µm and ~100µm-530µm respectively. FTIR spectroscopy shows that the keratin samples remained their characteristics as a true protein including spray drying when encapsulated with Arabic gum even at high temperatures up to 110°C. Conclusively, spray drying should be considered for future development of keratin as a nutrient supplement while freeze drying and vacuum-oven drying for storage and transport of keratin.

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INTRODUCTION

Keratin protein is a fibrous protein that is the primary constituent of feathers, nails, hair, and wool and is used in several different applications. It also ranks third as the most plentiful polymer found in the environment, following cellulose and chi-tin [1]. It has unique properties that make it highly biodegradable and biocompatible and non-toxic. Keratin is known to have cross-links of cysteine disulfide bonds due to the inter- and intra-chain causing it to have greater stability and less solubility. Typically, keratin is extracted by breaking the disulfide bridges using oxidation or reducing the free sulfhydryl groups. However, ionic, hydrophobic and hydrogen, bonds contribute to the chicken keratin stability and properties [2].

In addition to reducing waste in the environment, there are many benefits and diverse applications keratin has to offer making it a versatile material. However, safer, cleaner and more commercially effective extraction, transportation and packaging for industrial purposes also need to be studied in order to utilize keratin on a large scale. Therefore, this study aims to obtain keratin solution from chicken feathers through sodium hydroxide (NaOH) method and convert into powder to identify the effects of the various drying methods which are freeze drying, vacuum oven drying, and spray drying on keratin solution obtained from chicken feathers through visual observation, Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) analysis.

RELATED WORK

Various methods and sources of extraction were developed and studied to extract keratin protein to be used mainly for cosmetic and pharmaceutical products. One of the sources of extraction is bird feathers, or to be more specific, chicken feathers due to the fact that bird feathers contain approximately 90% keratin. In the poultry industry, waste originating from the feathers of chicken amounts to approximately 4 million tons a year around the world and is considered to be an alarming issue in many nations [3]. The worldwide annual feather amounts up to about 8x105 tones and a huge amount of these are deposited in landfills, followed by incineration and composting. On top of that, it has been found that the primary way of disposing feathers is by incineration which ultimately consumes a large amount of energy and releases high quantities of carbon dioxide, impacting the environment negatively thus making it an undesirable solution [4].

There have been various products formulated with keratin protein as the active ingredient. In the cosmetics, biomedical and health industries, countless products such as anti-aging creams, shampoos and hair treatment oils along with various types of creams have been developed for the local and international markets. Additionally, bioactive keratin, which is essentially a form of solubilized keratin have been found to offer vast benefits in the health, pharmaceutical and beauty industries when ingested orally regardless of the source it originated from [5].

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However, with chicken feathers being a suitable source to extract keratin, recent studies have shown to head towards this direction. There have been experiments to extract keratin from chicken feathers and it is shown to produce total mass of protein up to 53% [6]. This is an essential factor as increase in the concentration of protein can contribute to the lessening of degradation of protein during drying processes [7]. In another study, chicken feathers were treated with 2.5% NaOH during the process of extracting the keratin, demonstrated the improvement of extraction effectiveness which in turn increases the yield to 94% [6].

Furthermore, most of the methods of extraction of keratin is in liquid form and can be further processed into products such as shampoos and anti-aging cream for hair care, skin care and treatment. Moreover, the keratin protein can also be processed into pharmaceutical products to be used as a nutrient supplement and has shown to improve hair appearance, hair strength. Also, in the growth phase, it is also found to increase in hair follicles and reduce hair fall. On top of that, for the regrowing and regenerating of the tissue that has been found to be defective, keratin-based biomaterials can also offer a biocompatible matrix [8].

There are certain benefits in the application of keratin protein as a nutrient supplement. It has been observed that chicken feather keratin micro particles presented antioxidant and anticancer activities [9]. In 2014, a double-blind, randomized, placebo-controlled study established that solubilized keratin as a supplement showed positive effects in improving various aspects on hair and nails compared to a placebo among the participants of the study. The effects on hair were measured in terms of hair growth, hair loss, amino acid composition, hair luster and hair strength, while the effect on nails were measured and determined in terms of nail strength and physical appearance of nails [5].

In addition, there is currently rather few studies on the use of keratin, particularly from feathers as nutrient supplements or cosmetics. Although keratin from various sources have been known to have positive effects on the skin and hair of humans, keratin extracted from poultry feathers have yet to be commercially extracted and used as an active ingredient. Among the many benefits include the ability of keratin peptides to improve hair moisture on top of being able to deliver shine and smoothness to the hair. Also, keratin has a hydrating result on the skin and are often incorporated in skin and hair moisturizers [10]. Besides that, hydrolyzed keratins have been found to be used in the concentration of 0.2% in mascaras. Other than that, about 0.028% of keratin is have been included in bath soaps and even detergents. Moreover, keratin plays a significant role in delivering a drug system. Solid keratin is covered with the nonwoven film, which are then incorporated the active pharmaceutical agents thus forming a drug delivery system [11].

However, there are also certain setbacks in the research of converting raw materials which are keratin-based into a soluble form of keratin. This is apparently due to the fact that keratin is highly resistant to numerous enzymes and chemical reagents. The crosslinked network found in the structure of keratin can be a contributor to this condition as it is packed with many disulphide and hydrogen bonds [4]. The importance of keratin lies in its solubility and bioactivity are essential in the process of keratin being an active ingredient in pharmaceutical and beauty products which generally involves the keratin protein playing a necessary role as a nutrient in improving and replenishing various aspects of the body [5].

Methods of Drying

Freeze Drying

Freeze-drying which is also known as lyophilization acts to remove water from a frozen sample through the process of sublimation and desorption. The removal of water is essential in the presence of moisture, disulphide interchange or other reactions might lead to proteins which are freeze-dried to experience inactivation [12]. A typical process of freeze-drying generally comes in three stages, namely freezing, primary drying and secondary drying. The longest stage of freeze drying is the primary drying stage and it is crucial to optimize this stage as it has a great impact on the economics of the process. Correspondingly, other factors such as using surfactants or increasing the protein concentration might aid in the reduction of the degradation of protein at the ice-aqueous interface [7].

Furthermore, carrying out freezing at a slow rate have shown to result in fewer and larger protein domains in the sample as compared to freezing at a fast rate. Therefore, at low rates of cooling, protein aggregation is favoured as the surface stress on proteins during freezing would be minimized [13]. However, depending on the situation, certain issues may arise during the process of lyophilisation due to the procedures involved during freeze-drying. These include possible instabilities in proteins, long processing durations which typically lasts for 3-5 days, on top of costly set up and maintenance of lyophilisation plants [14].

Spray Drying

Spray drying is one of the most commonly used methods that delivers swift evaporation of water and have been commonly used on drying of protein components such as milk powder and whey protein. This particular method maintains the low temperature in elements when undergoing microencapsulation techniques. Through intensive homogenization, the bioactive compound would be encapsulated by mixing it in a suspension containing the wall material prior to the spray drying process [15].

Nevertheless, one of the aspects of concern in the dried powder would be flowability. An appropriate description of powder flowability is suggested as “the ability of a powder to flow under set environmental or processing conditions”. Certain literature sources have demonstrated that the ability of a powder to flow is ultimately determined by the degree to which interparticle interactions occur. For example, van der Waals forces, liquid or solid bridging and electrostatic [16]. These are greatly affected by a range of reasons including:

- Composition of powder bulk which include moisture contents, fat and protein

- Powder physical properties for example: shape and distribution, particle size, and bulk density
- Processing and environmental surroundings such as relative humidity, pressure and temperature

However, the effect of spray drying on protein solubility is very complex and may be dependent on a variation of factors such as, other than the sample itself, the extent of Maillard reaction (MR), degree of protein cross-linking and the occurrence of protein denaturation. In the studies that have been carried out, there did not seem to be any significant structural deviations and protein denaturation observed. Similarly, the extent of MR did not seem to be high since no observable cross-linking and denaturation was present. This finding minimizes the possible negative effects of this applied drying process on the solubility of proteins [17]. In any case, previously published findings established that a certain degree of MR may improve protein solubility as well as thermal stability [18].

Vacuum-oven Drying

Vacuum oven drying involves the use of heat to remove moisture from a sample through evaporation. It can be said that during the process, high-energy water molecules diffuse to the surface and evaporate due to low pressure [19]. This method has been used widely in the food industry. Moreover, this method is suitable for drying materials sensitive to heat or oxygen such as microorganisms and enzymes. This is due to the benefit of removing moisture at low temperatures and generally minimizing the likelihood of oxidation reactions [20]. There are also very few studies on the optimum parameters during drying processes such as freeze-drying (lyophilisation) to overcome physical and/or chemical instabilities and prevent denaturation of protein structures throughout the process [21].

MATERIALS AND METHODS

Materials and Equipment

Liquid keratin extracted from chicken feathers was used in this study. The extraction of the liquid keratin was done using method of using NaOH as reducing agent in a sterile and hygienic laboratory environment [4]. Ammonium sulfate was used as a precipitating agent and hydrochloric acid, HCl was used to neutralize the keratin solution [9]. Arabic gum was used to encapsulate keratin protein for drying at high temperatures. Chicken feathers were collected from chicken processing plants in Jaya Gading, Kuantan, Malaysia and cleaned. The equipment used includes lab scale Christ ALPHA 2-4 LSC freeze dryer, LabPlant SS-07A spray dryer and Memmert UF55 oven dryer. For analysis of product, all SEM analysis samples were coated using Q300T D Plus Sputter Coater and images were taken with Hitachi TM3030 Tabletop Microscope while FTIR results were obtained using Thermo Fisher Scientific Nicolet™ iS™ 5 FTIR Spectrometer.

Methodology

Figure 1 shows the process flowchart of the experiment from extraction of keratin of chicken feathers, the conversion of liquid to solid keratin and then the methods of analysis.

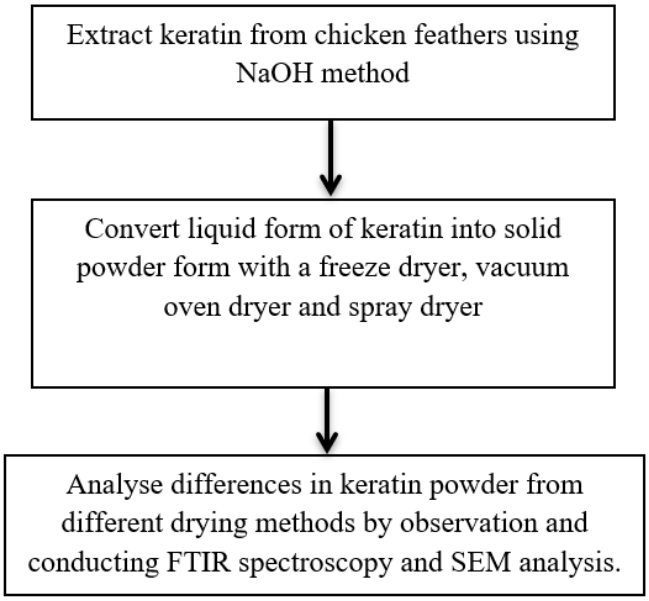

Figure 1. Process flowchart of experiment

Extraction of Keratin Solution

Preliminary treatment of the feathers

The collection of chicken feathers was obtained from a nearby poultry establishment which processed chicken and discarded the feathers. The feathers were soaked in ether for a period of 24 hours. The main objective of this step is to hygienically process the feathers to free it from stains, oil and grease before continuing to the next process. The chicken feathers were then thoroughly cleaned with soap water and left to dry under the sun. The dry feathers were then collected and set aside in a plastic bag before sealing it to prevent contamination of the already clean feathers during storage [9].

Dissolving of chicken feathers

100g of the cleaned chicken feathers were weighed and placed in a glass beaker. 2L of 1M NaOH solution was prepared by dissolving 80g of NaOH pellets in 2L of distilled water and added into the beaker containing the chicken feathers. The solution was heated and kept constant at 60°C and stirred continuously at 300rpm for about 6 hours until the chicken feathers have dissolved. The solution then underwent a filtration and centrifuge process at 10,000 rpm for a period of 5 mins. The supernatant liquid was then collected and followed by a filtration process using filter paper to ensure it is free of any large and unwanted particles.

Preparation of ammonium sulfate solution

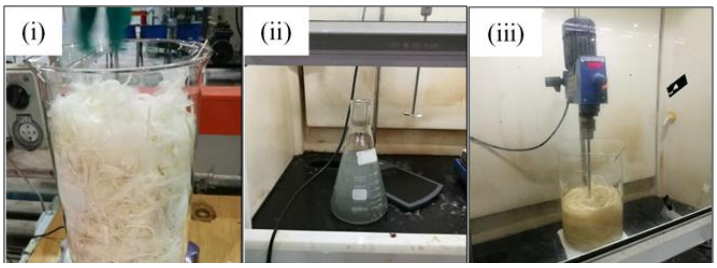

Figure 2. i) Weighing of cleaned chicken feathers, ii) Preparation of 1M NaOH solution, iii) Stirring of chicken feathers in NaOH solution

700g of ammonium sulfate was dissolved in 1L deionized water. The mixture was stirred to ensure all the ammonium sulfate particles had been dissolved. The solution was then submitted to filtration to ensure it was free from unwanted particles.

Protein in feather precipitation

The feather solution that was filtered and collected previously was transferred to a beaker and continuously stirred. The addition of the solution containing ammonium sulfate solution was slowly added drop by drop. The ultimate ratio of feather filtrate solution and ammonium sulfate solution added is 1:1. The mixture was then subjected to a centrifuge at the speed of 10,000 rpm for a duration of 5 mins and the particles which were solids were collected. The supernatant liquids were separately obtained and step 2 and 3 were repeated with it [6].

Protein in feather purification

The collected solid particles were washed by adding them into 100ml deionized water and stirring. The resulting solution was then centrifuged at 10,000rpm for 5mins and the solids were gathered carefully. The collected solid particles were added to 100ml of 2M NaOH solution then stirred until dissolved. The solution was then submitted to centrifuging again at the speed of 10,000rpm for a duration 5mins and all the liquid material collected were stored while the solids were discarded. The steps of precipitation, washing and dissolution were repeated 3 times[6]. 1M hydrochloric acid (HCl) was slowly added to the keratin solution obtained to achieve a neutral pH of 7.

Preparation of keratin solution with Arabic gum

Arabic gum was crushed into powder form to make it easier to dilute in water. 1g of Arabic gum were added a beaker containing 10ml of distilled water each [22]. Then each beaker was heated to 60°C stirred continuously for 10min until the Arabic gum was fully dissolved. The Arabic gum solution was then added to keratin solution at ratio of 1:1.

Drying Methods

Freeze dryer, vacuum oven dryer and spray dryer are used to convert the keratin solution into a solid powder form.

Freeze dryer

Freeze-drying process comes in three stages; freezing, main drying and final drying (see Figure 3). First, the sample of keratin solution was put into centrifuge tubes with maximum volume of 10ml. The tops of the tubes were sealed with film and small holes were created for the moisture to be released during the drying process. The tubes containing the

sample were then placed in the freezer overnight to ensure the sample is completely frozen. Then, the sample was placed on the tray in the freeze dryer to undergo freeze drying process. The drying was conducted over a period of two days to ensure complete drying of the sample. Table 1 shows the parameters for freeze drying of sample.

Table 1. Parameters for freeze drying of sample

Process	Temperature (°C)	Pressure (mbar)
Freezing	-80	N/A
Main Drying	-60	-0.011
Final Drying	-80	-0.0011

Figure 3. Christ ALPHA 2-4 LSC freeze dryer

Vacuum-oven dryer

The keratin in the form of solution was poured in a petri dish and placed on the tray of the vacuum oven dryer (see Figure 4). The oven door was tightly closed and connected to a vacuum pump to reduce pressure to -0.011bar and temperature of 60°C. Drying was conducted for a period of 24 hours. In order to ensure complete drying, the sample was placed in an oven for another 24 hours. Product was collected from trays when the sample has completely dried.

Figure 4. Memmert UF55 oven dryer

Spray dryer

The sample of keratin solution was fed to the spray dryer (see Figure 5), which is then sprayed through a nozzle in to a chamber where hot gas entering at inlet temperatures 120°C and 190°C and outlet temperature 60°C and 110°C respectively acts as a heater to dry the liquid. The product will then be collected at the exit as a powder.

Figure 5: LabPlant SS-07A spray dryer

Analysis of keratin powder after drying

A series of analyses which are physical observation, FTIR spectroscopy and SEM analysis was conducted to determine the differences between the drying methods used in the drying of keratin solution. FTIR spectroscopy is used to conclude the existence of appropriate functional groups that should be in the keratin powder after drying as compared to keratin solution before drying and SEM analysis is done to study the morphology of keratin protein.

For the SEM analysis that was carried out, the sample was first coated with a metallic substance (Gold, Au). This is done because in order for the images in SEM analysis to be visible, the material should be solid, dry and electrically conductive. Under these conditions, electron signals are emitted and detected through electron-beam interactions with specimens. Hence, it is possible for the images to be interpreted [23].

EXPERIMENTAL RESULTS

Analysis of Keratin Powder after Drying
Product of Freeze Drying

The physical appearance of the keratin powder in Figure 6 after freeze drying appeared to be “flaky” with rough edges and course when observed. The colour of the keratin powder was light milky yellow. The keratin sample after freeze drying formed several big lumps that had to be manually crushed to resemble a powder form. It had a brittle texture and easily broke apart when crushed. However, the keratin sample with added Arabic gum could not be crushed as the texture of the keratin sample was rubbery to the touch and was soft instead of brittle. Under room temperature conditions, both products remained stable and showed no physical changes.

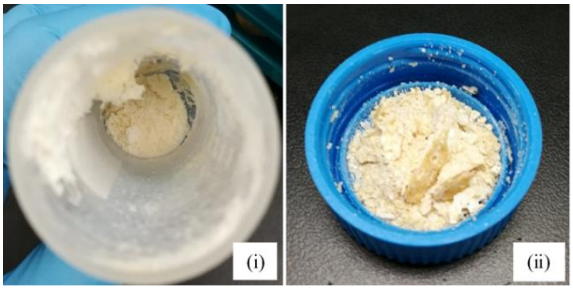

Figure 6. Physical appearance of (i) Pure keratin powder after freeze drying (ii) Keratin-Arabic gum powder after freeze drying

Product of vacuum-oven drying

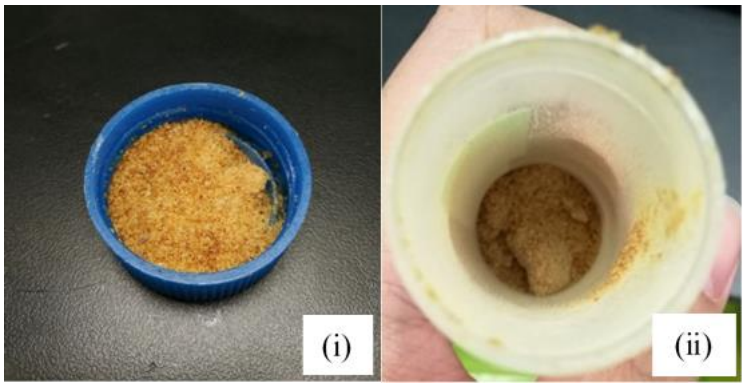

Figure 7. Physical appearance of (i) Pure keratin powder after placing in oven for 24 hours (ii) Keratin-Arabic gum powder after placing in oven for 24 hours

The physical appearance of both pure keratin sample and keratin-Arabic gum sample was observed to resemble a semi-solid state after 24 hours of vacuum oven drying as shown in Figure 7. The texture of the sample was sticky and could not be fully removed from the petri dish. This is due to the remaining moisture content that was still in the sample. After being left in the oven dryer for another 24 hours, the pure keratin powder appeared to have a course and free-flowing, grainy texture. The colour of the pure keratin powder was also a dark shade of brown. On the other hand, the keratin-Arabic gum powder was course but with lumps of powder with a rubbery texture when touched due to the presence of Arabic gum. When stored under room temperature conditions, both products remained stable and in solid form.

Product of freeze drying

As shown in Figure 8, the pure keratin powder after spray drying at 60°C had a darker shade than keratin-Arabic gum powder after spray drying at 60°C. Both the samples had a sticky texture. When subjected to removal from the collection chamber attached to the cyclone, the product of the samples was rubbery and stuck together and did not have a powder-like texture. This is due to the moisture content that was still present in the sample due to low temperature of drying that was carried out to avoid damaging the protein structure in keratin.

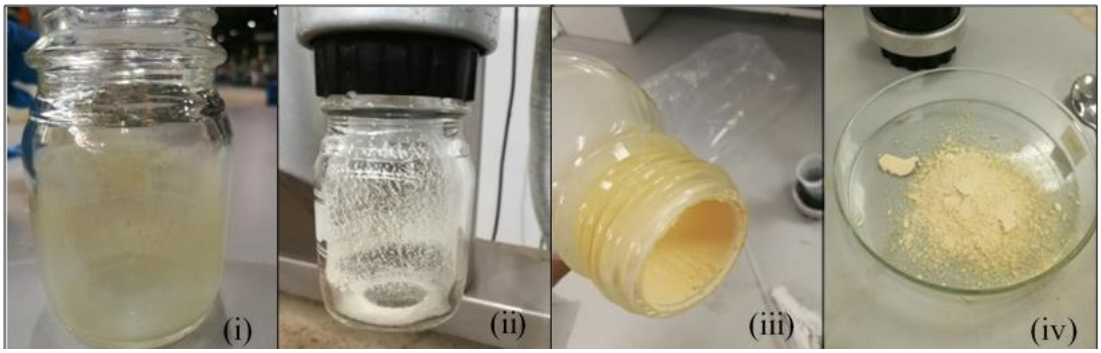

Figure 8. (i) Pure keratin powder after spray drying at 60°C, (ii) Keratin-Arabic gum powder after spray drying at 60°C, (iii) Pure keratin powder after spray drying at 110°C (iv) Keratin-Arabic gum powder after spray drying at 110°C

The product of pure keratin powder and keratin-Arabic gum powder after spray drying at 110°C appeared to be a fine powder with the former having a darker shade than the latter. When removing the sample from the collection chamber attached to the cyclone, the sample was easily removed as a free-flowing powder. This shows that the air was heated to a suitable temperature too allow the evaporation to remove the moisture in the sample to produce a free-flowing powder.

FTIR Spectroscopy

Analysis of keratin solution before drying

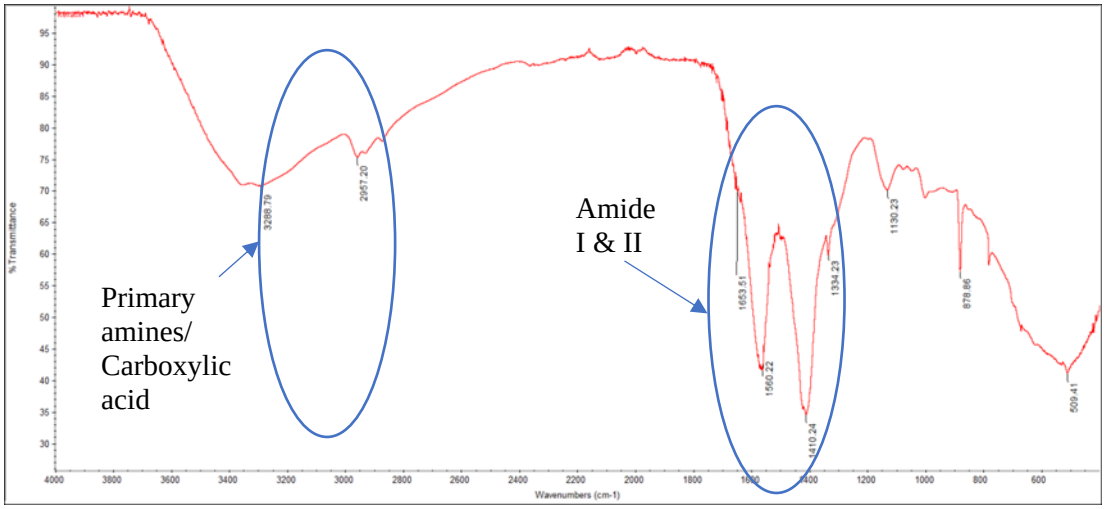

Figure 9. Spectrum wavelength of keratin solution before drying

It can be observed that the spectrum wavelength of the keratin solution displays distinctive bands for α-helix, β-sheet, β-turn, and random coil conformations in the amide I (1700–1600cm^{-1}) and amide II (1560–1500cm^{-1}) regions [24]. It has been observed previously that mainly from in-plane N-H bending while from the C-N stretching vibration, a α-helical conformation has an amide I and II bands between 1657 and 1650cm^{-1} and between 1550 and 1540cm^{-1}, correspondingly. On top of that, the β-sheet has an amide I and II bands between 1635 and 1615cm^{-1} and between 1535 and 1520cm^{-1}, correspondingly [25].

Since it is exclusively localized on the NH group, the N-H stretching vibration (3310–3270cm^{-1}) of peptide bond (−CO-NH-) is considered to be indifferent to the conformation of the polypeptide backbone. However, the strength of the hydrogen bond affects frequency of N-H stretching [26]. The spectrum wavelength of the sample in this case, shows the existence of carboxyl group and amino groups that can be found in amino acids. This confirms it is a true protein [6].

Analysis of keratin solution after drying

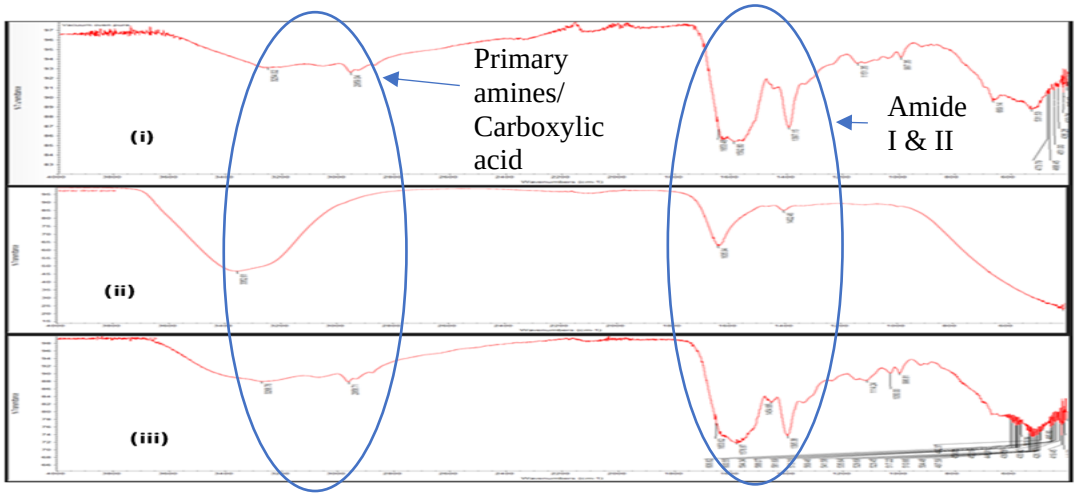

Figure 10. Spectrum wavelength of pure keratin powder after (i) Vacuum oven drying at 60°C (ii) Spray drying at 110°C (iii) Freeze drying −80°C

Based on the spectrum wavelength from FTIR analysis, the pure keratin powder after vacuum drying and freeze drying possess the bands similar to the keratin solution before undergoing drying processes. Conversely, pure keratin powder after spray drying is almost undetectable amide II bands (between 1535 and 1520cm^{-1}) and missing shoulder band for primary amines (between 3300 and 2500cm^{-1}). It was observed that in protein, drying faster at high temperatures, could lead to denaturation. Hence, without encapsulation to preserve the quality of the product, an increase in temperature could adversely affect the product quality [27]. Hence, it is possible that when subjected to high temperatures (110°C) during spray drying process, the keratin sample experienced denaturation.

Analysis of keratin-Arabic gum powder after drying

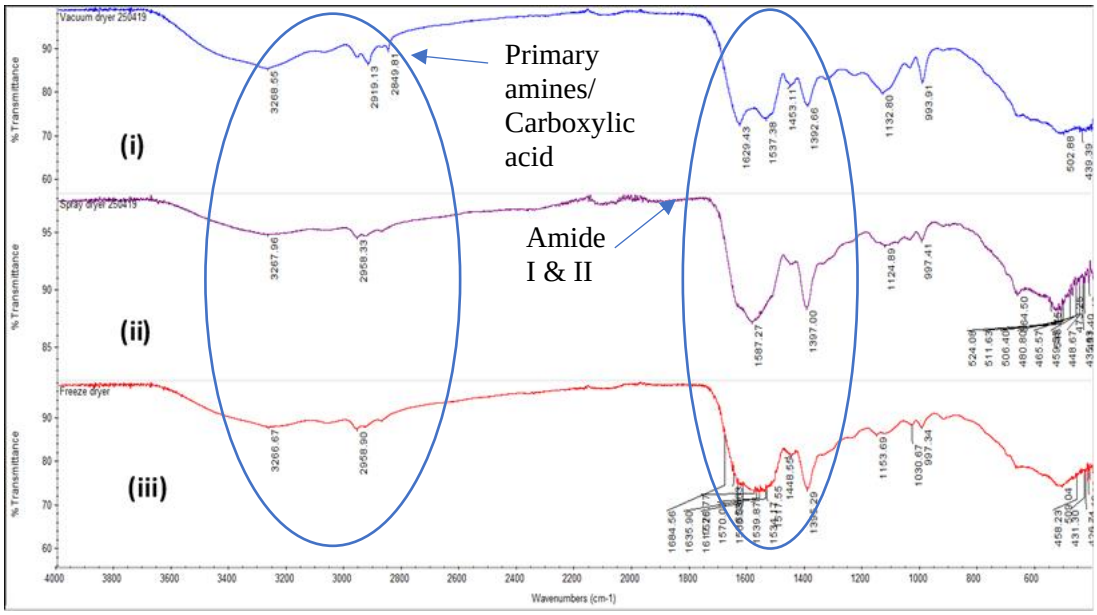

Figure 11. Spectrum wavelength of keratin-Arabic gum powder after (i) Vacuum oven drying at 60°C (ii) Spray drying 110°C (iii) Freeze drying −80°C

After encapsulation of keratin using Arabic gum before undergoing drying process, the spectrum wavelength shows that product of all three drying methods show that spectrum similar to keratin solution before drying. Therefore, denaturation of protein did not occur after encapsulation with Arabic gum especially during spray drying at high temperature of 110°C. All the drying methods enable the keratin powder produced to maintain its characteristics as a true protein [6].

SEM analysis

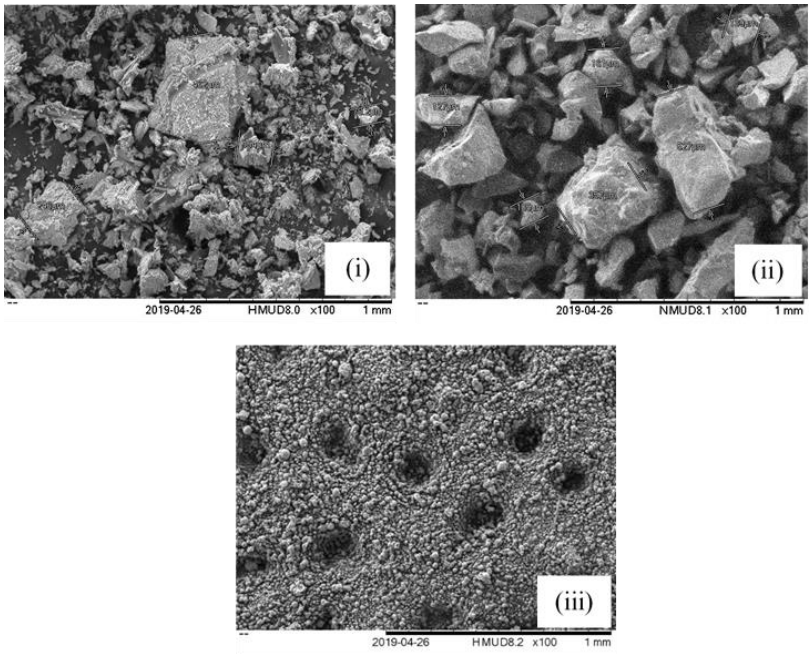

Figure 12. Morphology at 100× magnification of keratin powder using (i) Freeze drying (ii) Vacuum oven drying (iii) Spray drying

The morphology of keratin powder using freeze drying shows that this method produced course and flaky irregular shaped particles. When using vacuum oven drying, the keratin powder also showed irregular shaped particles which showed distinct grainy structures that were visible at 100× magnification. The size of keratin powder particles using freeze drying can be estimated to be ~70µm-470µm in diameter while for vacuum oven drying approximately ~100µm-530µm.

At 100× magnification, the keratin powder using spray drying showed spherical particles that were approximately ranging from ~3µm-17µm in diameter. This shows that spray drying technique produces smaller and more uniformed particles compared to freeze drying and vacuum oven drying. This is similar with findings from another study where their atomized droplets of protein as a result of spray drying were spherical in shape but might change depending on drying conditions and protein formulations. In comparison, the particle diameter of the product of spray drying is relatively smaller compared to that of freeze drying and vacuum oven drying [28].

Studies have shown that a more favourable state a for the enhancement of dissolution rate, enhancement of bioavailability and improvement of intestinal absorption is to have narrow and uniform distribution of particle size. In actual fact, other than the size factor, the particle shape also plays a significant role in affecting drug dissolution. Therefore, another factor that influences stability and dissolution behavior of compounds is crystalline state. Typically, materials smaller in size and lower crystallinity encourage improvement of oral bioavailability and dissolutions rate. Nonetheless, the conservation of original crystalline structure would be beneficial due to its low energy state in terms of long-term stability [29]. Therefore, the small spherical particles obtained using spray drying in this study will have higher dissolution than the flaky irregular shaped particles using freeze and vacuum oven drying and will be more suitable to be developed as a nutrient supplement.

CONCLUSION

Keratin solution was successfully solidified into powder form through different drying methods which were freeze drying, vacuum-oven drying and spray drying. The physical appearances of the products of freeze drying and spray drying were lighter in colour while vacuum oven drying produced darker shades of brown. The product of spray drying displayed powder-like appearance while freeze drying and vacuum oven drying products were flaky and grainy respectively. FTIR spectroscopy results showed that at the keratin solution extracted from chicken feathers before drying is a true protein, which did not change for the product of freeze drying and vacuum-oven drying. On the other hand, when spray drying was carried out on pure keratin at 110°C, analysis shows the sample is almost undetectable amide II bands (between 1535 and 1520cm^{-1}) and missing shoulder band for primary amines (between 3300 and 2500cm^{-1}). After encapsulation with Arabic gum, the product of spray drying even at the temperature of 110°C, the product shows to have retained characteristics of keratin as a protein. The size of particle using SEM analysis showed that the diameter of particles from freeze drying and vacuum oven drying were ~70µm-470µm and ~100µm-530µm respectively while for spray drying it was ~3µm-17µm which is relatively smaller. Also, the product of spray drying was small spherical particles but the product of freeze drying and vacuum drying were flaky irregular shaped particles.

In summary, it is recommended that keratin solution can be converted into powder through different drying methods for various purposes. The keratin powder obtained from the conversion of keratin solution can be further developed to benefit the pharmaceutical industry by producing nutrient supplements. Moreover, keratin solution can also be converted to powder for the purpose of packaging and transport. The method of freeze drying and vacuum oven drying should be considered for this purpose as they are more stable in room temperatures. Further study should be conducted to produce a more stable product through spray drying so that it may be considered for use as a nutrient supplement.

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