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# Effects of spray drying chicken feather keratin with Arabic gum encapsulation for nutrient supplementation

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**Abstract**

Keratin has always been a topic of interest as a novel protein source in human nutrition, consistent with waste-to-wealth initiatives. Hydrolyzed chicken feather keratin is spray-dried to facilitate storage, transportation, and development into dietary supplements. High-heat drying, however, may affect the quality and function of this bioactive compound. The objective of this research is, thus, to determine the suitable spray-drying parameters involving inlet temperature (70 – 190 °C), feed flow rate (3 – 9 ml/min), and concentration of Arabic gum (AG) (0 – 5%w/v) for the encapsulation of hydrolyzed chicken feather keratin as a nutrient supplement. The effects of the spray-drying on powder yield, moisture content, flowability, and total protein content were investigated. Fourier Transform Infrared Spectroscopy (FTIR) determined the presence of functional groups, while Scanning Electron Microscope (SEM) investigated the powder morphology. High Performance Liquid Chromatography (HPLC) was also conducted to obtain amino acid profiling of the powder. Then, capsule quality control tests were done to ensure compliance with industrial standards. Good powder quality was obtained from the parameters of inlet temperature 190 ± 5°C, feed flow rate of 3 ml/min and AG concentration of 2.50% since it showed high yield of 81.88%, moisture content of 3.74% (<5%), flowability with AOR 43.70° (<50°), high (minimal lost) total protein content of 0.7168 g protein/g solid. The keratin functional groups remain distinguishable through FTIR with AG encapsulation. SEM showed spherical and intact structures, which suggests high integrity of encapsulating materials. Amino acid profiling confirmed the presence of essential and non-essential amino acids, which are important as building blocks of proteins in the body. The product met all capsule quality control standards according to National Pharmaceutical Regulatory Agency of Malaysia (NPRA) and United States Pharmacopeia (USP). This result showed that spray-drying hydrolyzed keratin in the presence of AG would retain its function and powder quality.

**Keywords:** Arabic gum encapsulation, Chicken feather keratin, Keratin characterization, Nutrient Supplement, Spray drying

## 1. Introduction

The worldwide annual feather amounts to about $8 \times 10^5$ tonnes and a huge amount of these are deposited in landfills, followed by incineration and composting [1]. The insignificant portion of feathers that are being recycled and an absence of environmental-friendly disposal methods might be the motivating force for research on this topic. Since about 90% of keratin is found in chicken feathers, it would be an apt waste to wealth initiative to extract and turn chicken feather waste into useful products [2].

Keratin is a complex biopolymer, comprising of 19 amino acids that are connected by peptide bonds in ladder-like polypeptide chains. The molecular chain could either consist of a tight packing of alpha (α) helix or beta (β) sheet structure. This provides for a stable and sound structure of the keratin [3]. It is a natural protein with approximately (7–13%) content of cysteine residues, an amount that is considered high and is widely found in hooves, horns, hair, wool and nails. The high cysteine content that can support healthy epithelia, glutathione synthesis, antioxidant functions, and skeletal muscle functions [4, 5]. It has been noted in previous studies that the molecular weight of extracted feather keratin (β-keratin) can be found to be < 10kDa and around the ~60kDa region [6, 7]. While raw feather keratin usually has a mixture of higher molecular weight of 130kDa – 250kDa and lower molecular weight closer to 10.5kDa, hydrolysed or extracted keratin can result in low molecular weight peptides <10kDa [8]. Thus, it was found that keratin in these ranges is suitable for further processing applications such as in the polymer, biomedical and nutraceutical industries [3, 9].

Amino acids which are the building blocks of protein (including keratin), can either be provided naturally (11 types) or obtained through diet and supplementation (9 types). Adequate amounts of amino acids coupled with exercise prevents muscle atrophy, metabolic ailments and muscle dysfunction [10]. Individuals suffering from conditions such as sarcopenia (muscoskeletal), alopecia (hair), and onychoschizia (nails) have shown improvement with the supplementation of amino acids [11-13]. Nutrient supplements can be delivered in various ways such as injections, pulmonary, infusions and oral consumptions. Capsules, taken orally, is a simple and direct method of consumption [14]. Thus, the first step would be to convert hydrolyzed chicken feather keratin into powder form through a most common drying method used in industries which is spray drying.

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Spray drying atomizes a solution of a desired product into droplets which is then evaporated into powder (solid) by hot air at a suitable temperature and pressure. The physicochemical properties of products that have been spray dried primarily depend on factors such as concentration and types of carrier agent, inlet temperature and feed flow rate. Consequently, these parameters affect the physical properties of the spray dried product including product yield, total protein content, moisture content and flowability besides other contributing factors such as hygroscopicity, solubility, color and particle size [15]. These factors also play a role for industrial applications where product cost, equipment (and setup), manufacturing time, storage and transportation affect the marketability of products. For example, freeze-drying is regarded as a method that minimizes protein degradation, but is more costly and time-consuming than spray drying. Also, when selecting suitable parameters, higher processing temperatures would result in higher production costs [16].

Though studies on spray drying have been done on various types of proteins such as whey, fish and soybean proteins and even keratin extracted from wool sources [5, 17-22], the main concern is the preservation of the products' bioactivity due to the high temperatures used [23]. It was reported that at temperatures above 70°C, protein loses part of its chemical and biological activity, and the yield reduced [7]. To overcome this problem, encapsulation with materials, such as Arabic gum (AG), maltodextrin and chitosan, are common. Another study demonstrated that adding AG boosted resistance of polyphenols and anthocyanins to heating, which was likely a result of the gum carrier characteristics' creation of a suitable matrix structure and formation of a barrier between the phenolics and heat. In the study stated above, AG worked better as a coating material than maltodextrin at protecting against damage [24]. Moreover, AG has been found to be a highly soluble material, with good film creating and emulsifications properties which not only successfully encapsulate phenolic compounds, but displayed high encapsulation yield (efficiency) of more than 80% for total compounds, and 74.66 to 87.29% for individual anthocyanins. This is a good indication that spray drying is a convenient and appropriate technique for encapsulation with AG [25].

Generally, the operating parameters during spray drying is critical to obtaining high-quality products and should be carefully selected [15, 22, 26]. However, very few studies have been done on bioactive chicken feather keratin which may be structurally, chemically and physically different from other sources of proteins. By undergoing drying processes, the final product may differ in terms of yield, flowability, solubility, moisture content as well as qualitative properties such as amino acid content. For example, keratin derived from sheep wool may have different impurities, properties and behaviour than keratin derived from bird feathers. Hence, it is important to explore the effects of industrial manufacturing processes such as spray drying on chicken feather keratin as it may have different results from other keratin or even protein sources [27]. Also, in order to comply to strict regulations within the pharmaceutical and nutraceutical industry, it is important for nutrient supplements to be subjected to quality control studies before being introduced into the market.

Therefore, in this study, the effects of operating parameters relating to feed flow rate and temperature were observed. Moreover, an encapsulation agent was used to mitigate the effects of high temperatures during the spray drying process in order to obtain a good quality powder. Besides that, capsule quality control testing was carried out to ensure the product meets the specified standards as provided in the guidelines of United State Pharmacopeia (USP) and National Pharmaceutical Regulatory Agency (NPRA).

## 2. Materials and methods

Hydrolyzed keratin solution (molecular weight <10kDa & ~60kDa) (refer to supplementary materials) was obtained from Keraglow Sdn Bhd. Arabic gum (AG) blocks (food grade) was obtained from Kurma Madinah Kuantan. Hard gelatin capsules were obtained from Nashmir Capsule Sdn Bhd. Ultrapure water was obtained from a high-quality ultrapure water purification system (Milli-Q Advantage A10, EMD Millipore, Germany). Other chemicals used in the study was Bovine serum albumin (BSA) (Sigma-Aldrich, New Zealand), hydrochloric acid (HCl) (R&M Chemicals, Selangor, Malaysia). All chemicals used in this study were of analytical grade unless otherwise stated.

Chicken feather keratin solution with the solid content of 11.0% was prepared at a fixed amount of 250 ml and added into the dissolved AG (3.75 g solid) solution to a final concentration of 1.25% AG (w/v). The mixture is then stirred at 600 rpm for another 1 hour to ensure complete mixing. The process was repeated to produce 2.5% (w/v) and 5.0% (w/v) AG in solution. A control was then prepared with 300 ml of keratin solution without any addition of AG [20, 22].

Three different variations of parameters temperature (70 - 190°C), flowrate (3 - 9 ml/min) and concentration of AG (0 – 5 %w/v) were prepared. The solutions were then spray dried in a spray dryer (Model SS-07A, LabPlant, United Kingdom) with 0.5 mm diameter spray nozzle, under a spraying pressure of 1.5 ± 1 bar and hot air co-current downward flow [28]. The experiment was run one-factor-at-a-time. For every condition, three independent spray drying experiments were performed.

### *2.1 Yield of spray-dried product*

The mass of product after spray-drying was measured and the yield of the spray-dried powder is calculated using Eq. 1 [29].

$$Yield = \frac{Mass\ of\ solid\ collected\ after\ spray\ drying}{Mass\ of\ solid\ in\ the\ solution} \times 100\% \quad \text{Eq. 1}$$

### *2.2 Total protein content*

Total protein content (TPC) is determined by reading at 280 nm wavelength using UV-Visible spectrophotometer (AquaMate Model AQ8100, Thermo Scientific Orion, United States). A standard curve was obtained by preparing Bovine Serum Albumin (BSA) in solution at various concentrations [30, 31]. For every condition, three independent readings were obtained. The values were then compared with a standard curve obtained from absorbance values of BSA.

*2.3 Moisture content*

The moisture content of the spray-dried powder was determined using the standard Association of Official Analytical Chemists (AOAC) method. The moisture content of spray-dried powder on a wet basis was calculated using Eq. 2 [32]:

$$Moisture\ content\ \% = \frac{(\text{Weight of sample} - \text{Weight of dried sample})}{\text{Weight of sample}} \quad \text{Eq. 2}$$

*2.4 Flowability of powder*

A manual powder flow tester (EFT-01, Electrolab, Mumbai, India) was used to measure the angle of repose (AOR) with fixed funnel method. The height of the heap formed was measured and recorded. The AOR was calculated using the Eq. 3 [33].

$$\tan(AOR) = \frac{height}{0.5\ base} \quad \text{Eq. 3}$$

*2.5 FTIR analysis*

Fourier Transform Infrared Spectroscopy (FTIR) (Nicolet iS5, Thermo Fisher Scientific, USA) was used to analyse the functional groups contained in keratin. The spectra were collected in triplicate in the region between 4000 $cm^{-1}$ and 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ and 32 accumulations with Omnic™ v7.0 software. A previous air-background correction was considered.

*2.6 SEM analysis and size distribution of particles*

The surface state, morphology and structure of the spray dried powders were determined by acceleration voltage of 3 – 5 kV using scanning electron microscopy (JEOL, JSM-IT200, Japan) at the magnification of 2.0 – 14 k. Particles size was measured by analysing SEM images using the image processing software Digimizer and plotted with Python 3.10 [34, 35].

*2.7 Amino acid profiling*

The composition of amino acids in the sample were done using High-performance liquid chromatography (HPLC) (Waters, Alliance e2695, USA) with a fluorescence detector. Mobile phase A used AcccQ Tag Eluent A in 1:10 ratio with deionised water. Mobile phase B is acetonitrile while mobile C is water. Column temperature was 36 ± 1°C with flow rate of 1ml/min and injection volume of 10 µl. Reference standards consist of 16 amino acids at 2.5 mM certified concentration, Cystine at 1.25 mM certified concentration, L – Hydroxyproline at ≥ 99%, 2.5 mM (MW = 131.13 g/mol) and L-2-Aminobutyric acid at ≥ 99%, 2.5 mM (MW = 103.12g/mol) as internal standard. The values of amino acids are obtained directly from result output of the HPLC run based on the internal standard and single point calibration.

*2.8 Thermogravimetric Analysis (TGA)*

TGA analysis was carried out using thermogravimetric analyser (TA instruments, Q500, USA) under nitrogen atmosphere. The phase change temperature was measured with 30 - 900 °C range at heating rate of 10 °C/min. Approximately 3 mg samples were put on to aluminum crucible and the data was analysed.

*2.9 Capsule quality control testing*

The spray dried chicken feather keratin powder was used to fill capsules size 0 using a manual capsule filling machine. The selection of keratin powder used to fill the capsules was based on the results of the chicken feather keratin powder characterization which takes into account the parameters of yield, moisture content (< 5%), flowability (angle of repose < 50°) and total protein content [15, 36].

Lead (Pb), arsenic (As) and cadmium (Cd) detection was done using American Public Health Association (APHA) 3010 method. For mercury samples, a Direct Mercury Analyzer (Milestone, DMA-80TRICELL, Italy) was used to heat about 0.1 - 0.2g of sample until 800°C to release mercury vapor for detection. The Total Aerobic Microbial Count (TAMC), Total Yeast & Mold Count (TYMC), *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* were tested with reference to the method in Appendix XVI B, British Pharmacopeia (BP) 2009 Harmonised Method. Weight variation testing was done according to the guidelines provided in USP NF 2091. Three independent readings were obtained. Disintegration testing (Distek, 3100, USA) was done using method referenced from USP <701> that has been harmonized with the corresponding texts of the European Pharmacopoeia and the Japanese Pharmacopoeia [37]. Dissolution testing used the paddle method in USP <711> using a dissolution tester (Delta, DL-80, India) [38, 39]. Then, total protein content is determined by reading at 280 nm wavelength using UV-Visible spectrophotometer (AquaMate Model AQ8100, Thermo Scientific Orion, United States). The amount of dissolved active ingredient (Q) is expressed as a percentage of the total content of the dosage unit (one capsule) using Eq. 4:

$$Q = \frac{Dissolved\ active\ ingredient}{Total\ content\ of\ dosage\ unit} \times 100\% \quad \text{Eq. 4}$$

*2.10 Statistical analysis*

Each experiment was repeated in triplicates and results were expressed as mean value ± standard deviation. One-way Analysis of Variance (ANOVA) was performed using data analysis tools in Microsoft Excel 365 to compare means with a confidence interval of 95%.

## 3. Results and discussion

### *3.1 Physical properties of spray-dried powder*

For inlet temperature of 70 ± 5°C, the weight of powder was determined by calculating the difference in weight of collection chamber before and after drying. The flowability of product at this temperature setting was unable to be determined due to the stickiness of the sample.

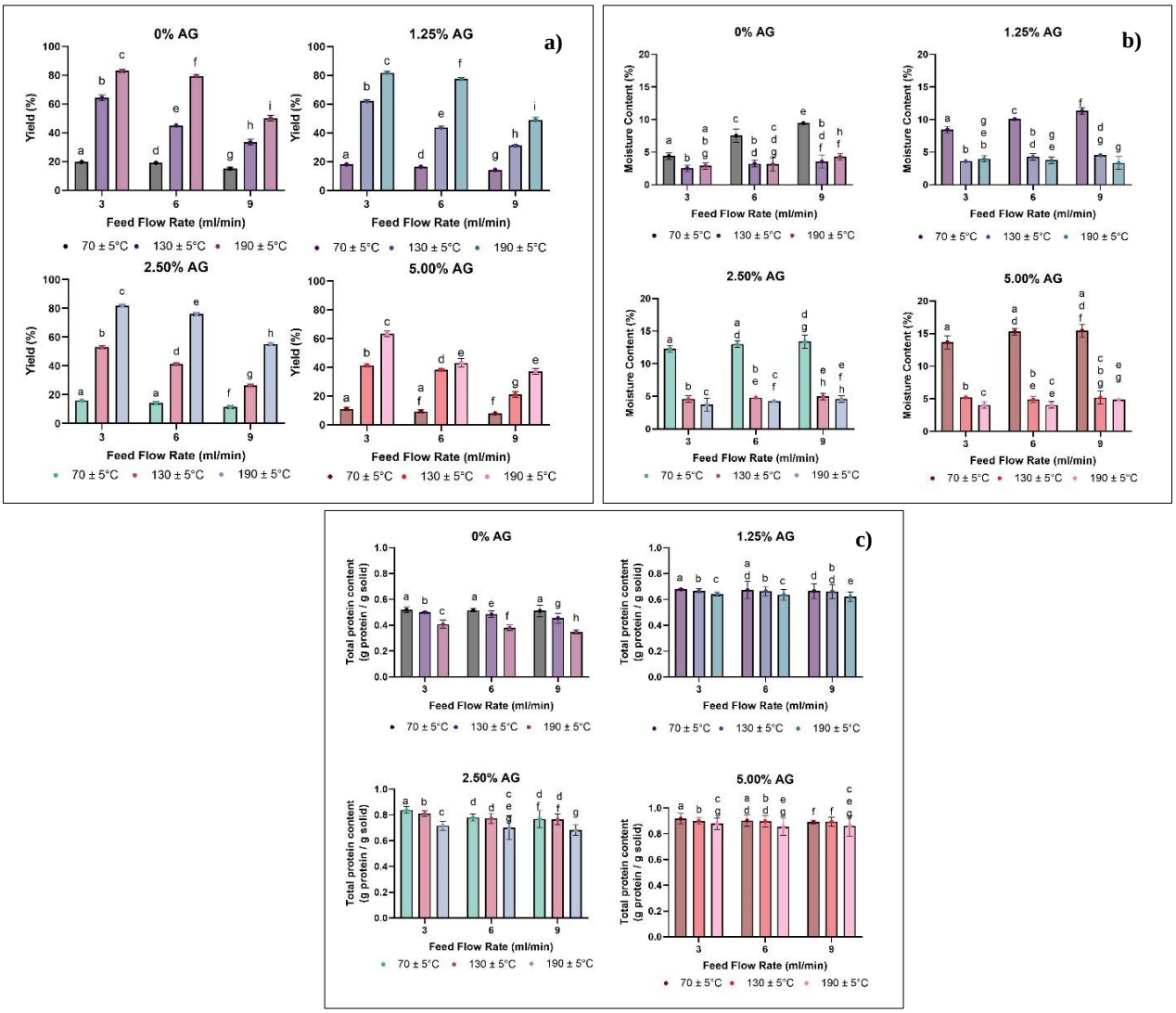


**Figure 1** a) Yield, b) Moisture content and c) Total protein content of the powders (%) at various temperature (°C), flow rate (ml/min) and concentration of Arabic gum (%w/v). Different letters indicate significant differences ($p \leq 0.05$). The data was the average of triplicate ± SD.

#### *3.1.1 Yield of spray-dried powder*

The spray drying setting with a product recovery rate over 50% indicates good performance, resulting in lower production costs and increased efficiency [22]. The drying yield of the products are summarized in Figure 1(a) where an inlet temperature of 190 ± 5°C, the highest yield of 83.02% could be obtained without addition of AG when the feed flowrate was set at 3 ml/min. The lowest yield of 8.05% was obtained at the lowest feed temperature of 70 ± 5°C, highest concentration of AG (5%) and highest feed flow rate of 9 ml/min.

Generally, a higher inlet temperature (190°C ± 5°C) of spray drying encourage rapid evaporation, increasing drying rate and causing the reduction in adhesion of droplets on the wall of the drying chamber [23]. Also, the trend shows slightly lower yields were obtained at higher AG concentrations. Despite being a good encapsulating agent, forming a barrier to protect heat sensitive materials, it has a nature of high hygroscopicity and stickiness as the concentration increases, resulting in a decrease of powder yield [24]. When the feed flow rate is low at 3ml/min, the heat transfer and mass transfer were more effective, resulting in higher yields in the collected product. Arumugham et al. [29] showed in spray drying date fruit extract with maltodextrin and AG that the maximum powder yield was achieved at the feed flow rate range of 3 – 4 ml/min similar with findings in this study. Thus, the results show that lower feed flow rate and AG concentration with higher inlet temperature would result in a better yield.

*3.1.2 Total Protein Content (TPC)*

The protein content in hydrolyzed keratin before spray drying is 0.9404g protein/g solid. The results in Figure 1(b) show that the higher the concentration of AG, the higher the total protein content found in the product. There is only approximately 2.5% lost in protein content when 5% AG was used to encapsulate the keratin during spray drying at inlet temperature of 70 ± 5°C and 3 ml/min feed flowrate. With lower concentrations of AG (e.g., 1.25%), the protein loss can go up to 27.8% even though the inlet temperature and feed flowrate was the same. AG showed stabilizing ability and had an emulsifying effect on the encapsulation procedure, forming a strong protective matrix around the core material, which resulted in higher encapsulation efficiency values with increasing AG concentration [40, 41].

Previous findings show that protein denatures and loses some of its chemical and biological activity reducing yield at temperatures higher than 70 ℃ [19, 33]. The results were in line with various studies using AG as one of the encapsulating agents. For example, microencapsulated particles of chokeberry extract were found to exhibit high encapsulation efficiency ranging from 74.7% to 87.3% at 5.0% Arabic gum, inlet temperature of 130°C and feed flow rate at 8 ml/min [24].

*3.1.3 Moisture content*

Moisture content is a significant indicator in microcapsules on the storage of microcapsules and spray dried powders should ideally be lower than 5% to allow for long-term product storage [15]. In Figure 1(c), the moisture content obtained for the product of spray drying at 190 ± 5°C shows the lowest values ranging from 2.91% to 4.86% which are considered as good even for food grade products. Temperature setting at 130 ± 5°C showed a slightly higher moisture content ranging from 2.54% to 5.23%. Temperature setting at 70 ± 5°C showed the range of 4.41% to 15.45% which is the highest moisture content and majority is > 5%. Therefore, increasing the inlet air temperature resulted in acceleration of the moisture evaporation rate and a lower moisture content in the product [42].

Besides that, upon the increase in concentration of AG, the moisture content increases slightly. A similar finding was noted in another study of encapsulation using the combination of maltodextrin with AG at various proportions, where higher moisture content was observed when the proportion of AG increased. Since AG is known for having a more hydrophilic groups, such as hydroxyl groups in its structure, it can bind to more water molecules which increases moisture content [43]. The trend for results of moisture content is in line with the results of yield obtained for the product where lower moisture content results in higher yield largely due to less adhesion to wall of drying chamber [23].

*3.1.4 Flowability of powder*

Table 1 and Table 2 shows the flowability of powder by angle of repose (AOR) at different AG concentration obtained at 190 ± 5°C and 130 ± 5°C with different feed flow rate setting. Good powder flowability (AOR < 50°) is important for control of powder behaviour during handling, storage, and processing such as prevention buildup on conveyors. The AOR was calculated to classify the flowability of powders using classification of flow properties of solids based on AOR known as Carr's index [44]. Generally, the higher the inlet temperature and the lower the flowrate setting, the better the flowability of the powders obtained. Only two results achieved "Excellent" classification but none of the results were in the "Very poor" or "Very, very poor" classification.

**Table 1** Flowability of the products at 190 ± 5° C with various flow rate and AG concentration

| **190 ± 5°C** | **3ml/min** | | **6ml/min** | | **9ml/min** | |
|---|---|---|---|---|---|---|
| **AG Concentration** | **AOR (°)** | **Expected Flow** | **AOR (°)** | **Expected Flow** | **AOR (°)** | **Expected Flow** |
| **5.00** | 47.67 | Poor-must agitate, vibrate | 49.02 | Poor-must agitate, vibrate | 50.04 | Poor-must agitate, vibrate |
| **2.50** | 43.70 | Poor-must agitate, vibrate | 43.25 | Passable-may hang up | 45.34 | Poor-must agitate, vibrate |
| **1.25** | 34.85 | Passable-may hang up | 37.72 | Fair-no aid needed | 40.13 | Passable-may hang up |
| **0** | 27.07 | Excellent | 28.89 | Excellent | 31.03 | Good |

**Table 2** Flowability of the products at 130 ± 5°C with various flow rate and AG concentration

| **130 ± 5°C** | **3ml/min** | | **6ml/min** | | **9ml/min** | |
|---|---|---|---|---|---|---|
| **AG Concentration** | **AOR (°)** | **Expected Flow** | **AOR (°)** | **Expected Flow** | **AOR (°)** | **Expected Flow** |
| **5.00** | 48.80 | Poor-must agitate, vibrate | 50.33 | Poor-must agitate, vibrate | 52.62 | Poor-must agitate, vibrate |
| **2.50** | 46.63 | Poor-must agitate, vibrate | 47.59 | Poor-must agitate, vibrate | 49.76 | Poor-must agitate, vibrate |
| **1.25** | 38.01 | Fair-no aid needed | 46.20 | Poor-must agitate, vibrate | 44.81 | Passable-may hang up |
| **0** | 32.38 | Good | 33.84 | Good | 38.24 | Fair-no aid needed |

Also, AOR of particles increased with increasing concentration of encapsulating agents. The effect of the AG on AOR can be attributed to the increased moisture and friction between particles, in addition to increased adhesion and cohesiveness [36]. The classification of flow parameters, appears to be compatible with the majority of the pharmaceutical literature [45]. The obtained AOR (25–30° for "Excellent" and 31-35° for "Good") of spray dried powders shows a good and ideal flowability of powders which is between 40° and 50° [33].

*3.2 FTIR results*

Based on Figure 2, there was no significant difference observed between the setting of 3 ml/min and 9 ml/min at the same temperature. It can be observed that at inlet temperature of 70 ± 5°C, all concentration of AG prepared showed absorption bands for amide A (N-H) ranging from 3300 – 3400 $cm^{-1}$ and the stretching or bending of C=O for amide I at 1600 – 1700 $cm^{-1}$. However, at 1240 – 1450 $cm^{-1}$ bands which shows C–N stretching corresponded to amide III (high content of β-sheet structures) can only be observed in 1.25% AG, 2.5% AG and 5.0% AG and is totally missing in 0% AG. Furthermore, in 0% AG and 1.25% AG, the N–H deformation and C-N stretching at 1560 $cm^{-1}$ was absent which corresponds to amide II (1500-1600 $cm^{-1}$) [46].

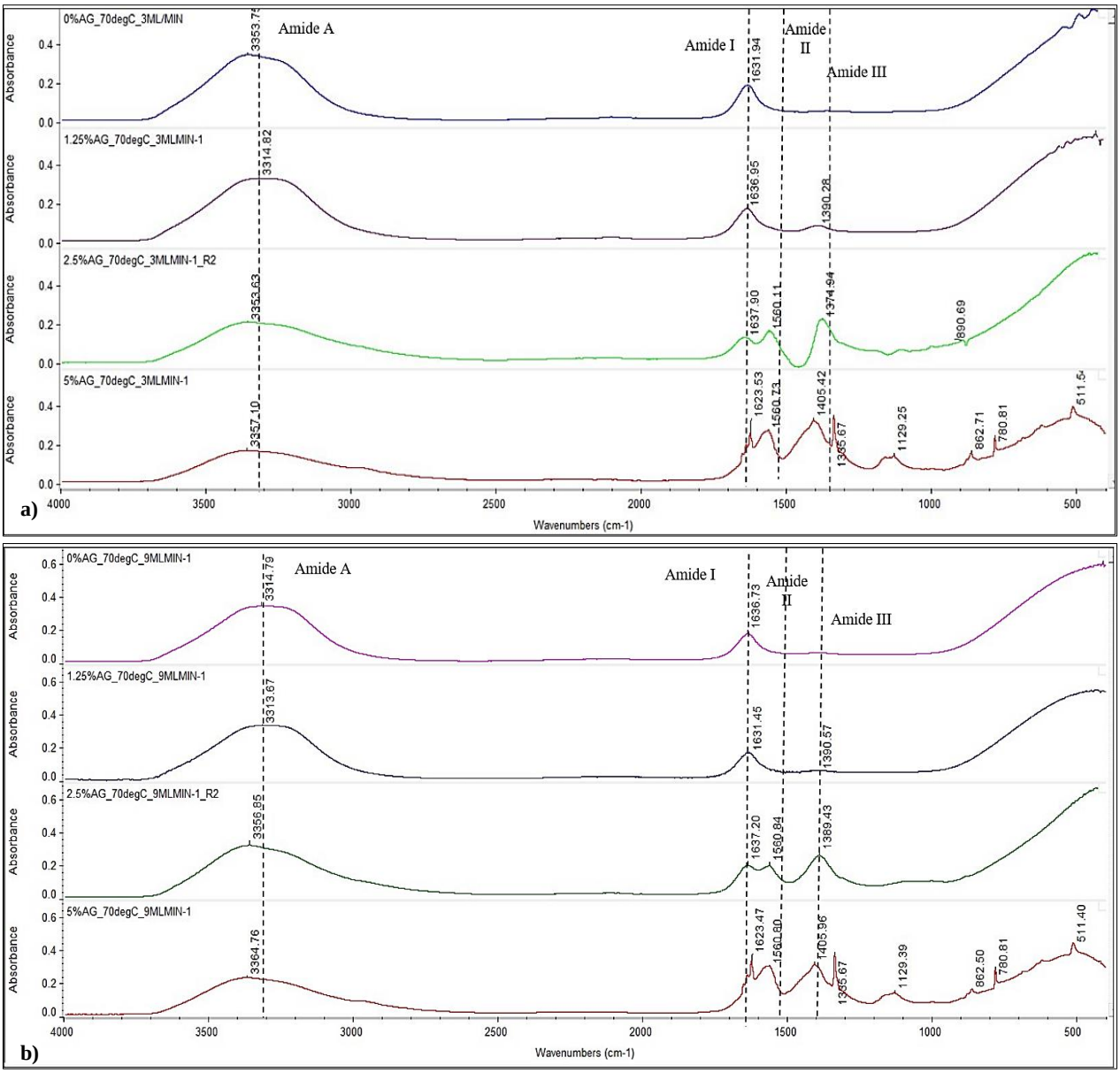


**Figure 2** (a) FTIR Spectrum for 70 ± 5°C, 3 ml/min at Arabic gum concentration of 0%, 1.25%, 2.50% and 5.00%; (b) FTIR Spectrum for 70 ± 5°C, 9 ml/min at Arabic gum concentration of 0%, 1.25%, 2.50% and 5.00%

For the inlet temperature of 190 ± 5°C shown in Figure 3, absorption bands for amide A (N – H) ranging from 3300–3400 $cm^{-1}$ was not distinguishable in 0% AG sample. However, some weak peaks at ~2815 $cm^{-1}$ to ~2942 $cm^{-1}$ could be contributed by the formation of carboxylic acid groups (2800–3500 $cm^{-1}$) which can be mainly $CH_3$ and $CH_2$ groups in some amino acids (alanine, valine, leucine, isoleucine, threonine) [47]. According to Liya and Umesh [48], the formation of carboxylic acid groups indicated degradation of chicken feathers to a certain extent. For 5% AG at 190 ± 5°C at 3 ml/min and 9 ml/min, a sharp peak at ~2923 $cm^{-1}$ in all the spectra corresponds to the symmetric or asymmetric stretching vibration of aliphatic amino acids –CH and $–CH_2$ [49].

Therefore, while the inlet flow rate during spray has minimal/negligible effects on functional groups according to FTIR results, varied temperatures and AG concentration affects the structure of keratin differently. Encapsulation with AG showed to be necessary in order to preserve the structure and functional groups of the keratin.

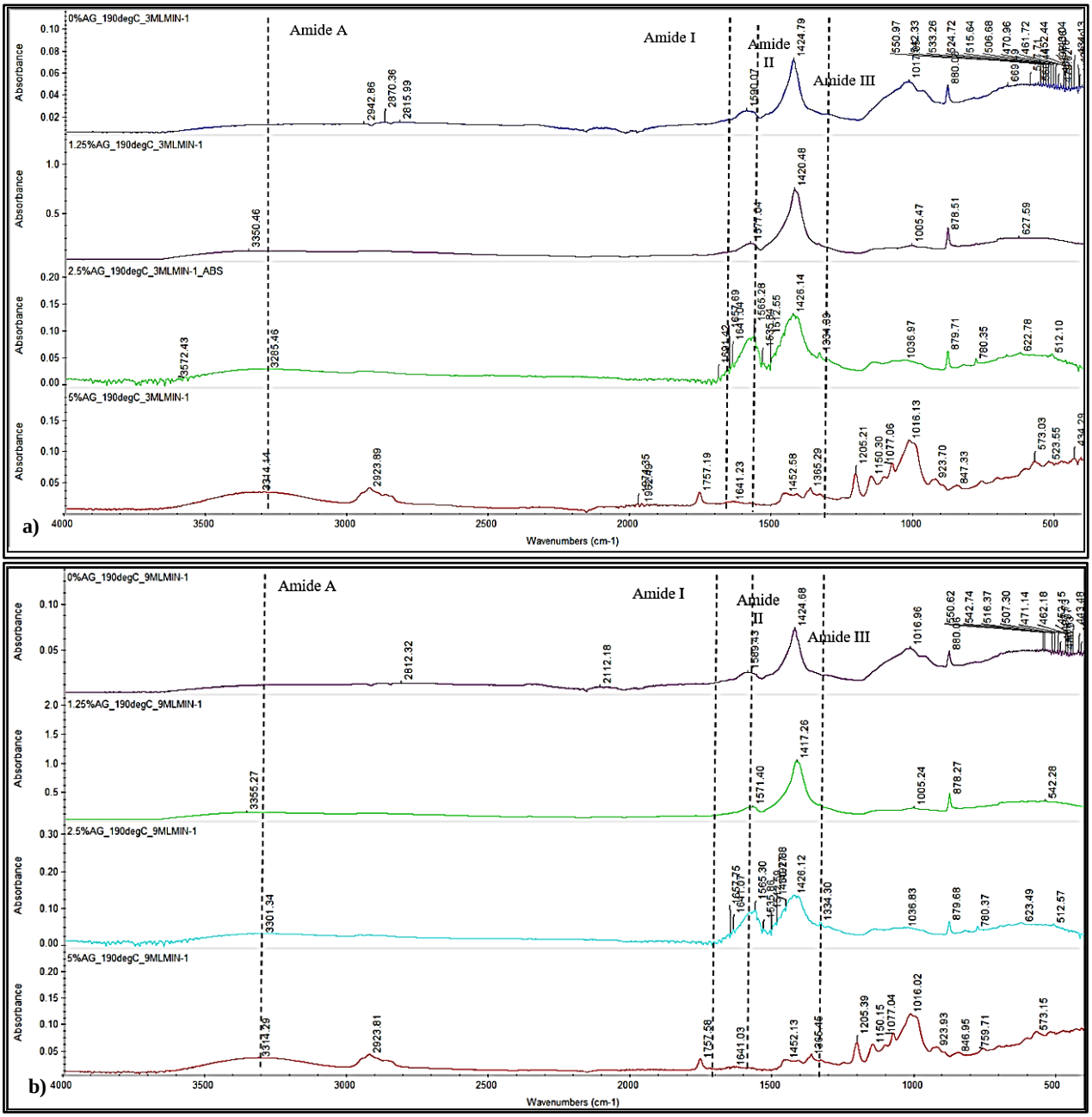


**Figure 3** (a) FTIR Spectrum for 190 ± 5°C, 3 ml/min at Arabic gum concentration of 0%, 1.25%, 2.50% and 5.00%; (b) FTIR Spectrum for 190 ± 5°C, 9 ml/min at Arabic gum concentration of 0%, 1.25%, 2.50% and 5.00%

*3.3 SEM results*

Figure 4 shows the Scanning Electron Microscopy (SEM) images of keratin powder obtained with different concentrations of Arabic gum which shows the morphology of the spray dried keratin powders.

The images generally showed a round shaped particles with wrinkled and concave creases of varying sizes with no significant differences between the general shape of a), b), c) or d). The existence of dents, creases, and depressions at their surfaces is a result of vacuum conditions inside the SEM [50-52]. According to Carpentier et al. [53], these creases could improve the release properties of potential encapsulated microparticles due to a greater surface area. The microparticle surface showed a regular polymeric network, absent of any porous structures suggesting a high integrity of the encapsulating materials [53]. The appearance of these microstructures has been observed by various authors in the preparation of microparticles using a spray-drying process. Encapsulated products can arbitrarily be divided into reservoir type (single shell around active ingredient), matrix type (active ingredient is dispersed over carrier agent) and coated-matrix type (combination of both reservoir and matrix type). As a comparison, without additional coatings, active ingredients in the matrix type may also be present at the surface of the encapsulate as opposed to the reservoir type [54].

Although studies for the interaction of keratin with AG is currently scarce, studies on the association of polysaccharides (AG) with proteins have shown promising results. In a study by Cao et al. [55], polysaccharide-protein granules formed a stable complex resembling matrix type encapsulates and showed improved stability that when heated at a temperature higher than the denaturation temperature of the protein. Thus, the disassociation of unheated particles at different environmental conditions was prevented. Besides that, during the heat treatment, some free proteins in the aqueous state may agglomerate and bind with AG in a loose state. Then, as

the temperature rapidly drops to room temperature, a core-shell structure (coated-matrix) to produce individual particles (powder granules) with the active ingredient within [55]. In addition, Yeop et al. [56] found that a mixture of AG and protein results in a thermal barrier due to the development of surface-active protein. Since polysaccharides (AG) has a specific heat capacity relatively higher than proteins, this aids to preserve the protein from further denaturation suggesting encapsulation of coated-matrix type [56].

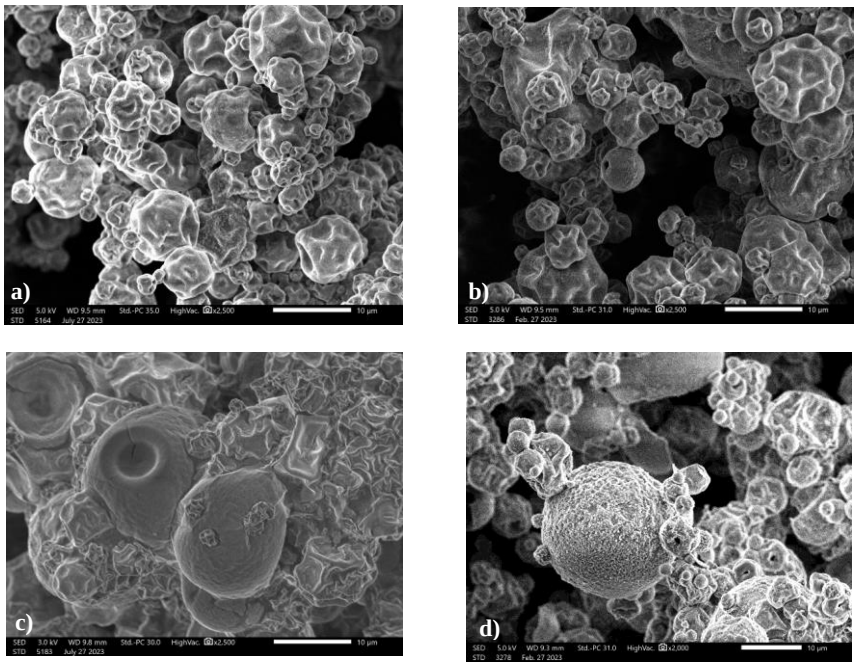


**Figure 4** Scanning Electron Microscopy (SEM) images of keratin powder obtained at 190 ± 5°C and 9 ml/min with different concentrations of Arabic gum, a) 1.25% Arabic gum; b) 2.5% Arabic gum; c) 5.0% Arabic gum; and d) 0% Arabic gum at magnification of 2.5k for a), b) and c) and 2.0k for d)

Figure 5 shows a random single bead of powder. At 4k magnification Figure 5(a), the particle had a ruptured shell possibly due to thermal expansion, though there were no other areas where this type of result was found. Figure 5(b) shows the same bead of powder magnified to 14k magnification supporting the presence of a polysaccharide-protein thermal barrier, though the core structure may have been lost (leaked) during the rupturing of the shell. [55-57].

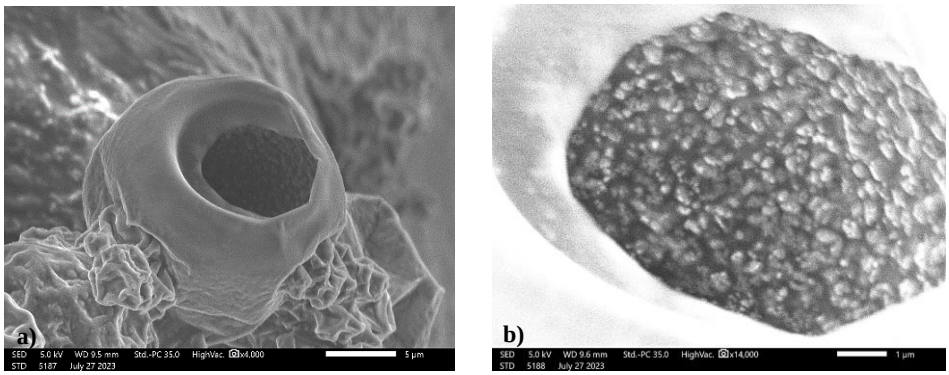


**Figure 5** Single bead of powder after spray drying at 190 ± 5°C and 9 ml/min at Arabic gum concentration of 5.0% with a) 4k magnification and b) 14k magnification

Also, Figure 6 shows the size distribution of the particles having an average of 2.91 ± 1.14μm for 0% AG, 4.01 ± 1.38 μm for 1.25% AG, 4.68 ± 2.59 μm for 2.50% AG and 4.77 ± 2.12 μm for 5.00% AG. The particle size of all the products were found to be similar though an increasing size trend was observed with the increase of AG, confirming that spray drying is an appropriate technique to obtain small particles with uniform size [58]. The gradual increase may be due to the feed solution having higher viscosity. The fluid droplet dimension during atomization changes directly with the liquid viscosity at a constant atomizer speed, resulting in bigger particles, finding is consistent with previous encapsulation studies [15], [35], [59]. Furthermore, preference in size and shape of the particles depends on the intended usage of the product itself. Larger particles offer a more consistent and extended release of the substances they contain, while smaller globules have superior organoleptic properties which is crucial for use in food and medicine applications [58].

Thus, the morphology of the particles were generally spherical, wrinkled and concave creases which are intact, indicating high integrity of encapsulating materials. In general, round-shape particles in smaller size are preferred in nutraceutical and food industries as it contributes to the good flowability of free-flowing powders [60].

*3.4 Amino acid profiling*

Amino acids (AA) are necessary substrates as the building blocks of tissue proteins for the synthesis of hormones, neurotransmitters, muscular growth, and other cellular functions [61]. Therefore, effective encapsulation of amino acids was required in a protein supplement. Figure 7 shows the amino acid composition in the chicken feather keratin powder. Based on the table, essential amino acids (EAA) showing higher percentage namely valine at 10.152%, followed by isoleucine at 6.582% and phenylalanine at 5.741%. Since EAA cannot be synthesized by humans, it is important to obtain them through their diet or as supplements. The amount of histidine was found to be not detected (ND) in the sample and may be due to limitations of the method and equipment for detection since the histidine is present in very small amounts (especially in feather keratin) with previous studies having as low as 0.001% [62].

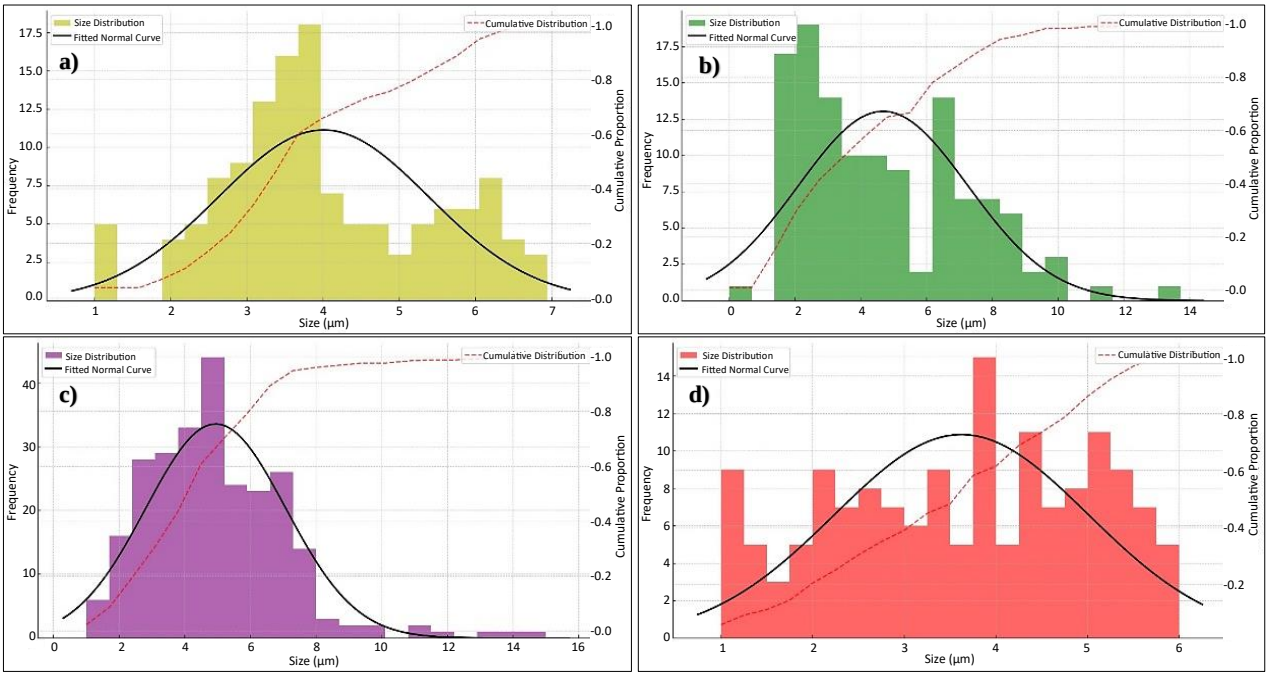


**Figure 6** Size distribution graphs of keratin powder obtained at 190 ± 5°C and 9 ml/min with different concentrations of Arabic gum, a) 1.25% Arabic gum; b) 2.5% Arabic gum; c) 5.0% Arabic gum; and d) 0% Arabic gum

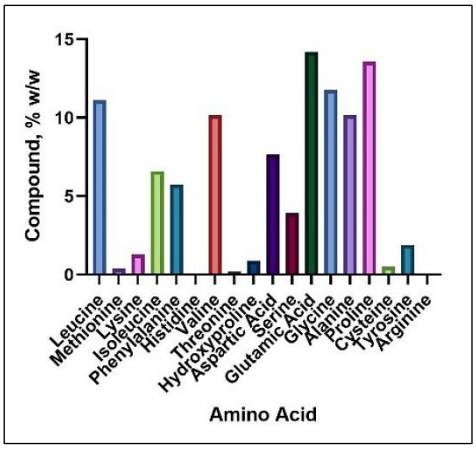


**Figure 7** Amino acid composition in the chicken feather keratin powder analyzed using High Performance Liquid Chromatography (HPLC)

Previous in-vivo studies have shown that leucine and other essential amino acid (mentioned as branched chain amino acids, BCAAs) such as valine, leucine, and isoleucine stimulate protein synthesis and prevents muscle atrophy. In a study involving older sarcopenic adults and post-stroke patients with sarcopenia, leucine-enriched amino acid supplements seem to maintain muscle mass, strength and physical function [11]. On top of that, keratin has been characterized as consisting of a chain of small amino acids where a high percentage of alanine, glycine, serine, cystine and valine can be found in feather keratin consistent with these results [63].

Moreover, non-essential amino acids (NEAA) such as β-alanine supplementation increased physical performance and executive function with exercise in middle-aged individuals. Thus, the high percentage of these amino acids in the supplement would be beneficial for prevention in deterioration of muscle mass besides improving muscle function [64]. Although it is generally accepted that all protein sources have the capacity to muscle protein synthesis, the differences in anabolic response between protein sources may be explained by the digestion and absorption kinetics, which is not within the scope of study, as well as the composition of amino acids [65].

The results show that cysteine content in the sample was only 0.497%. However, it is not unusual for extracted keratin to have lower cysteine content. In fact, protein hydrolysates from wool and leather extracted using alkaline hydrolysis have shown results as low as 1.45 ± 0.03% cysteine content and 0.39 ± 0.03% [66]. Cysteine can experience partial breakdown where disulfide bonds are broken during extraction process to form cysteic acid or oxidation to form cystine. Therefore, it should be noted that there may be other forms of this amino acid present in the form of cystine or cysteic acid which were not detected through this quantitative method even in common practices [67].

Therefore, amino acid profiling of the chicken feather keratin sample showed the presence of NEAA and more importantly, EAA which cannot be synthesized by humans. The benefits of supplements containing amino acids (EAA and NEAA) have been identified in the past to improve physical function, increase muscle mass and muscle strength [11, 64]. This shows the potential for chicken feather keratin to be used as a nutrient supplement.

*3.5 Thermogravimetric Analysis (TGA)*

TGA curves are shown in Figure 8. From TGA curves shown in Figure 8(A), a loss of mass observed below 100 °C can be attributed to water evaporation. Also, temperature ranging from 250 °C to 350 °C, only the sample with 1.25% AG showed a significant reduction in mass at 264.63 °C. This could be attributed to the decomposition of the AG in line with findings from Daoub et al. [68] which identified the thermal decomposition of AG to range from 260.8°C to 339.2°C. A steeper decline in mass can be observed for 0% AG samples compared to 1.25% AG samples for the region between 200 °C to 500 °C which could be due to degradation of protein or the release of sulphur dioxide (between 230 - 250°C) due to the breaking of disulfide bonds at these high temperatures [69, 70].

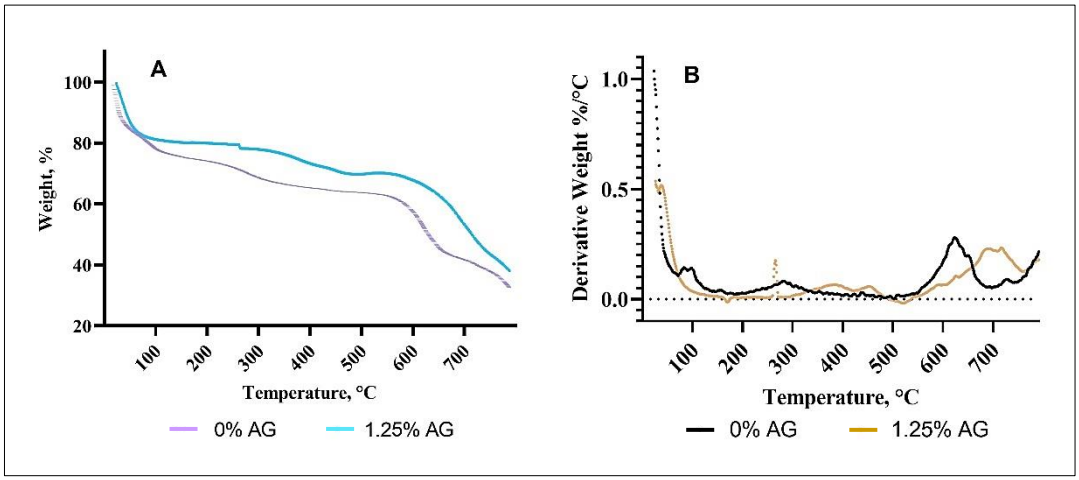


**Figure 8** TGA curves of samples without AG encapsulation (0% AG) and with AG encapsulation at 1.25% AG concentration.

Moreover, from Figure 8(B), the weight loss peak for 0% AG sample was at 606.35°C while for the 1.25% AG sample, it was slightly higher 634.97 °C corresponding with the decomposition temperatures of the samples. Therefore, the decomposition temperature of the sample encapsulated with AG increased by about 20 °C which exhibited a suitable thermal stability [65].

*3.6 Capsule quality control testing*

Several parameters were tested including heavy metals test, disintegration and dissolution test, weight variation test and microbial contamination test in accordance to the National Pharmaceutical Regulatory Agency Malaysia (NPRA) [71].

Heavy metal content and microbial contamination testing is an initial test to help determine the level of contamination in a supplement to meet the safety standards of NPRA. Table 3 shows the results of the heavy metal testing. (Pb), arsenic (As), mercury (Hg) and cadmium (Cd) are the heavy metals commonly found in supplements; thus, it is important to test their possible content. It can be seen that all heavy metals are either not detected or lower than the limit allowed.

**Table 3** Results of the Heavy Metal Testing

| Test | Materials | Conditions | Results |
|---|---|---|---|
| **Limit Test for Heavy Metals** | Lead (Pb) | ≤ 10.0 mg/kg | < 0.1 mg/kg |
| | Arsenic (As) | ≤ 5.0 mg/kg | ND < 0.05 mg/kg |
| | Mercury (Hg) | ≤ 0.5 mg/kg | 0.006922 mg/kg |
| | Cadmium (Cd) | ≤ 0.3 mg/kg | ND < 0.05 mg/kg |
| **Microbial contamination test** | Total Aerobic Microbial Count (TAMC) | ≤ 20,000 CFU/ml | < 10 CFU/ml |
| | Total Yeast & Mold Count (TYMC) | ≤ 200 CFU/ml | < 10 CFU/ml |
| | Absence of *Salmonella* | 10 ml | Absent |
| | Absence of *Escherichia coli* | 1 ml | Absent |
| | Absence of *Staphylococcus aureus* | 1 ml | Absent |

However, a study done on protein powder supplements showed that heavy metals were detected in small amounts and would only be detrimental to the health of the consumer if taken in excess (> 3 recommended servings per day) [72].

For microbial contamination test results in Table 3, the samples were shown to have very low concentrations of microbial contamination and are within the standards of the USP as well as NPRA allowable limits. A contributing factor could also be that the product of spray drying has undergone the process at high temperatures up to 190 ± 5 °C in which many microbial organisms cannot withstand. Since the supplements are to be ingested by consumers, it is important for the microbial contamination to be minimal so as to avoid any harmful side effects [73, 74].

Disintegration testing gives information about the duration required for disintegration of a capsule. Table 4 shows the mean time for disintegration and weight variation of the chicken feather keratin powder capsules. The results show a consistent mean time of disintegration between the capsules of 7.38 – 7.99 minutes. Since hard gelatin capsules were used, guideline from USP Chapter <701> uses water as the immersion medium. This result is consistent with the findings of an in-vivo study done with human subjects for the comparison of hypromellose (carrageenan) capsules and standard gelatin capsules where the latter achieved the time of 7 ± 4 minutes [75]. Besides that, it is by NPRA requirement that the individual weights of capsules are required to be within the limits of 90% and 110% of the average weight.

Due to its simplicity, disintegration tests are generally less time consuming and more cost effective than dissolution tests in the pharmaceutical industries. However, several criteria should be met before such a replacement is allowed to take place because the use of disintegration as a quality control test must be reproducible within the set specifications. Among the criteria include that the product should be rapidly dissolving with dissolution > 80% in 15 minutes at pH 1.2, 4.0, and 6.8 [76, 77].

**Table 4** Results for disintegration and weight variation testing

| Test | Capsules | Conditions | Average disintegration time (min) | USP Criteria |
|---|---|---|---|---|
| **Disintegration** | Set 1 | < 30 min | 7.38 ± 0.92 | Passed |
| | Set 2 | | 7.82 ± 1.12 | |
| | Set 3 | | 7.92 ± 1.02 | |
| | Set 4 | | 7.59 ± 0.44 | |
| | Set 5 | | 7.60 ± 1.40 | |
| | Set 6 | | 7.99 ± 1.07 | |
| **Test** | **Capsules** | **Conditions** | **Average mass difference (%)** | **USP Criteria** |
| **Weight Variation** | Set 1 | 90% < Average mass difference, α < 110% | 94.71< α < 105.23 | Passed |
| | Set 2 | | 98.33 < α < 106.28 | |
| | Set 3 | | 97.60 < α < 106.15 | |

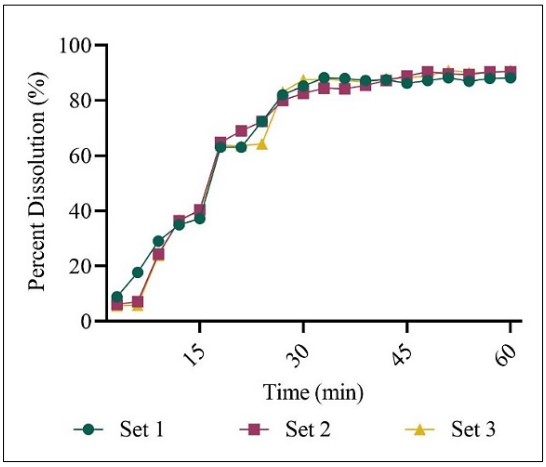


**Figure 9** Dissolution profiles of chicken feather keratin capsules

Since complete disintegration does not certainly imply complete dissolution, it does not provide the drug release kinetics. These missed data can be achieved by the dissolution test [77]. Figure 9 shows the dissolution profiles of chicken feather keratin capsules.

The results of the dissolution tests show that the capsules are within the specified criterion of protein release to reach 80% in 30 minutes [78]. However, since it did not meet the criteria of being rapidly dissolving with dissolution > 80% in 15 minutes, disintegration test should not be used to replace dissolution test as a performance test. In one study of amlodipine besylate, the amount of product that was released from the tablets after 30 minutes varied between 85.57% and 97.46% which is consistent with the findings in this study [79].

In short, it is important to regulate these criteria before a nutrient supplement is allowed to be circulated for human consumption. The chicken feather keratin capsulated within hard gelatin capsules have shown to comply with these standard set by NPRA in terms of heavy metal and microbial contamination limits as well as disintegration testing, dissolution testing and weight variation testing. This shows the potential for the product to be used as a nutrient supplement.

## 4. Conclusion

In conclusion, a good-quality hydrolysed keratin powder with preserved bioactivity could be obtained by the spray drying process. The yield and total protein content from spray drying of AG encapsulated chicken feather keratin is affected by inlet temperature, feed flow rate and AG concentration. Good powder quality was obtained from the parameters of inlet temperature 190 ± 5°C, feed flow rate of 3 ml/min and AG concentration of 2.50%. The product also complies with the standards set by the National Pharmaceutical

Regulatory Agency Malaysia (NPRA). However, the optimum operating parameters depends greatly on the purpose and use of the product which may differ for different industries. Therefore, for future use, the parameters can be suitably chosen to achieve the desired product of choice according to the use and application in especially the pharmaceutical and nutraceutical industries using the findings in this study as a guide. Also, future research directions could evaluate the long-term stability of the dried powders and other aspects that also influence the product of spray drying such as drying velocity, atomizer specification and air humidity.

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## 7. Supplementary Materials

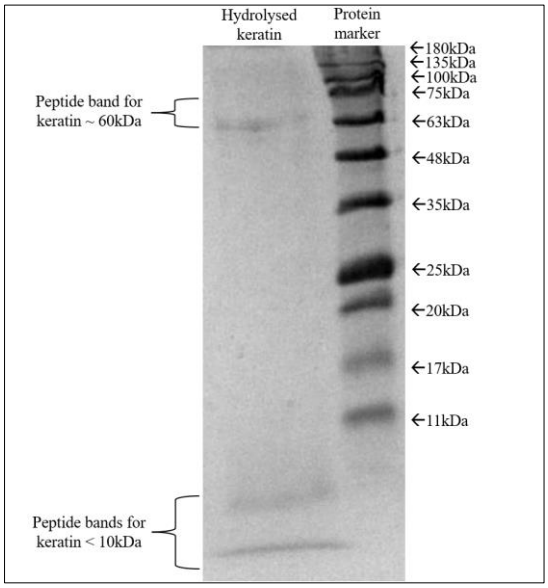


**Figure S10**: SDS-PAGE of hydrolyzed chicken feather keratin (left) with standard protein marker (right)