PROTEIN DENATURATION IN PILOT SCALE SPRAY DRYER

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ABSTRACT

The aim of this project is to investigate the protein denaturation in a pilot scale spray dryer. The occurrence of protein denaturation is due to external stresses, such as heat. Thus, this project describes a pilot process for obtaining protein isolates from whey protein in powder form via spray drying process with improved water solubility and technofunctional properties as well as reduced thermal damage. The experiment, including varying the inlet air temperature at 100°C and 160°C as well as utilising the microencapsulation technique using a starch derived polysaccharide (or known as maltodextrin) to minimize the protein denaturation. The Reversed phase HPLC (RP-HPLC) technique has been applied to measure the denaturation of proteins in the samples which are with maltodextrin and without maltodextrin resulting from the spray drying of milk solution at inlet temperature of 100°C and 160°C. The combination of maltodextrin (MD) and fresh milk (FM) is at standard ratio of FM/MD (9: 1) in order to produce spherical and smooth powder. Apart from the RP-HPLC method, the SDS-PAGE method also used to analyze the protein content. The morphology of the milk powders was examined using a Scanning Electron Microscopy (SEM) while the particle *size of milk* products can be easily assessed using Mastersizer with model Scirocco 2000. To determine the portion of whey protein in the spray dried milk samples, the Total Solid Content method was employed. Besides, sterilization tests such as detection of *Salmonella sp.*, and detection of *Escherichia coli sp.* along with microbial tests which is Standard Plate Count Method also applied. The spray-drying process was particularly critical for inducing some thermal damage but it is hypothesized that the crust formation which resulting in high particle temperatures while still maintaining a wet core, is likely to lead to high levels of denaturation up to 55% at high temperature (160 °C). Stability of α-lactalbumin proved higher as compare with β-lactoglobulin. The study reveals that low outlet gas temperatures along with microencapsulation are required to avoid excessive denaturation of protein which can reduce the protein denaturation until 16%. Up to 93.2% reduction of the microorganisms also proved that spray drying is another form of sterilization.

ABSTRAK

Tujuan projek ini adalah untuk menyiasat penyahaslian protein dalam pengering semburan skala perintis. Terhasilnya penyahaslian protein adalah disebabkan oleh pengaruh luaran, seperti haba. Oleh hal yang demikian, projek ini menggambarkan proses perintis untuk mendapatkan pengasingan protein daripada dadih protein dalam bentuk serbuk melalui proses pengeringan semburan dengan kelarutan air yang telah dipertingkatkan serta sifat *technofunctional* dan juga mengurangkan kerosakan akibat terma. Eksperimen ini, termasuk mempelbagaikan suhu udara masuk seperti pada 100 °C dan 160 °C serta memanfaatkan pemikrokapsulan dengan menggunakan polisakarida terbitan kanji (atau dikenali sebagai *maltodekstrin*) untuk meminimumkan penyahaslian protein. Teknik *Reversed phase HPLC (RP-HPLC)* telah dilaksanakan bagi mengukur kehilangan kelarutan *α-lactalbumin* dan *β-lactoglobulin* dalam sampel yang bersama dengan *maltodekstrin* serta tanpa *maltodekstrin* yang dihasilkan daripada pengeringan semburan larutan susu pada serokan suhu 100 °C dan 160 °C. Kombinasi *maltodekstrin* (MD) dan susu segar (FM) adalah pada nisbah standard FM / MD (9: 1) untuk menghasilkan serbuk yang bulat dan halus. Selain daripada kaedah *RP-HPLC*, kaedah *SDS-PAGE* juga digunakan untuk menganalisis kandungan protein. Morfologi daripada sebuk susu adalah diperiksa menggunakan *Scanning Electron Microscopy* (SEM) manakala saiz zarah produk susu boleh ditaksif dengan mudah dengan menggunakan *Mastersizer* model *Scirocco* 2000. Untuk menentukan bahagian dadih protein dalam sampel susu yang telah dikering semburan, kaedah *Total Solid Content* telah digunapakai. Selain itu, ujian pensterilan seperti pengesanan *Salmonella sp.* dan pengesanan *Escherichia coli sp.* bersama dengan ujian mikrob, di mana kaedah *Standard Plate Count* juga diterapkan. Proses pengeringan semburan ini penting terutamanya dalam mendorong beberapa kerosakan akibat terma tetapi hipotesis menunjukan bahawa pembentukan kerak yang mengakibatkan zarah pada suhu yang tinggi sementara mengekalkan teras yang lembap, bermungkinan membawa kepada penyahaslian berperingkat tinggi sehingga 55% pada suhu yang tinggi (160 °C). Kestabilan *α-lactalbumin* terbukti lebih tinggi berbanding dengan *β-lactoglobulin*. Penyelidikan ini mendedahkan bahawa suhu yang rendah berserta dengan pemikrokapsulan adalah diperlukan untuk mengelakkan penyahaslian protein yang melampau dengan mengurangkan penyahaslian protein hingga 16%. Pencapaian 93.2% daripada pengurangan mikroorganisme juga membuktikan bahawa pengeringan semburan merupakan bentuk pensterilan yang berlainan.

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LIST OF ABBREVIATIONS/TERMINOLOGY/ SYMBOL

°C	=	Degree Celsius
α-lac	=	α-lactalbumin
β-lg.	=	β -lactoglobulin
BSA	=	Bovine Serum Albumin
CFU/ml	=	Colony forming Unit (Microbial test)
CMP	=	Caseinomacropeptide
ECSA	=	Electron Spectroscopy for Chemical Analysis
e.g.	=	for example
et al.	=	and others
hr	=	hour
IgG	=	Immunoglobulin G
i.e.	=	that is
LAWN	=	Result not clear (Microbial test)
LP	=	Lupin Protein
MCA	=	MacConkey Agar (Sterility test)
mAU.ml	=	Unit for protein area (HPLC analysis)
Pa	=	Pascal (pressure)
RP-HPLC	=	Reversed phase HPLC
SDS-PAGE	=	Sodium Dodecyl Sulfate - Polyacrylamide gel electrophoresis
SEM	=	Scanning Electron Microscopy
WPC	=	Whey Protein Concentrate
TNTC	=	Too many spots to be counted (Microbial test)
XLDA	=	Xylose, Lysine, Deoxychocolate Agar (Sterility test)

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CHAPTER 1: INTRODUCTION

1.1 Problem Statement

One of the problems associate with the spray drying process is that it is very hard to predict the quality of the product. The quality of product that describe here include the parameters such as moisture content, thermal degradation, aroma retention, shape and size of the particles, stickiness and so on. Firstly, recognised that a quality parameter in the end product is the result of what a particle experiences (temperatures and humidities) in the spray dryer and how it reacts to that (evaporation rate, thermal degradation rate).

The combination of factors that address the environment of a particle (air temperature, air humidity) is called the equipment model, while the set of factors that address the responses of a particle is called the feedstock model. Thus, the equipment model comprises the influence of the spray drying process on the quality of the product. In most cases, spray drying operates at a very high temperature up to 170°C which cause protein denaturation happened.

Denatured protein has little or no nutritional value. For example in 2004, up to 50 babies die due to malnutrition in China when the substandard baby milk which contained only 6% of the protein needed for a growing infant was consumed. Another incident of melamine issue (protein pretense) due to tainted baby formula kills three, sickens 6,000-plus children in China may also be prevented. The formula used the chemical (melamine) to give the product artificially high protein content.

Even though there are a huge number of studies on spray drying, unfortunately relatively few work has been reported on the effects of spray drying operating conditions on denaturation of proteins.

1.2 Objectives

- To establish a more favorable method for preserving protein content in milk during the spray drying operation.
- To develop a reliable analytical method for quantifying the protein content in milk powder.

1.3 Scopes

(i.) To perform a spray drying of milk:
- with and without microencapsulation
- at various operating condition

(ii.) To analyze the morphology, particle size distribution and protein content of milk powder.

1.4 Rationale & Significance

Spray-drying is one of the well established methods for producing dry powders in which an atomised spray is contacted with hot gas which is used as the drying medium. Evaporation takes place to yield dried particles, which are subsequently separated from the gas stream by a variety of methods (Masters, 1991).

Currently, spray drying is the preferred method for producing whey proteins in powder form (Anandharamakrishnan et al., 2007). Normally, it comes at the end-point of the processing line, as it is an important step to control the final product quality. It has some advantages such as, rapid drying rates, a wide range of operating temperatures and short residence times.

Proteins are known to suffer from denaturation process when exposed to high temperature over long period. Anandharamakrishnan et al. (2007) for instance, reported an increase rate in protein denaturation in a tall pilot scale spray dryer when the operating temperature increases. Protein denaturation during spray drying also reported by many authors (e.g. Matzinos and Hall, 1993; Mumenthaler et al., 1994; Prinn et al., 2002; Anandharamakrishnan et al., 2007).

This work attempt to establish a more favorable method for preserving protein content in milk during the spray drying by controlling the spray drying operating parameters and also by employing the microencapsulation technique.

Finding from this work is useful for production of high quality milk powder. A novel RP-HPLC method has been developed for protein analysis as the purpose of further milk formulation's development in Malaysia.

1.5 Thesis Outline

The outline of this thesis is presented as a schematic form as displayed in Figure 1.1, and the brief description of each individual chapters is showed at the remainder of this chapter.

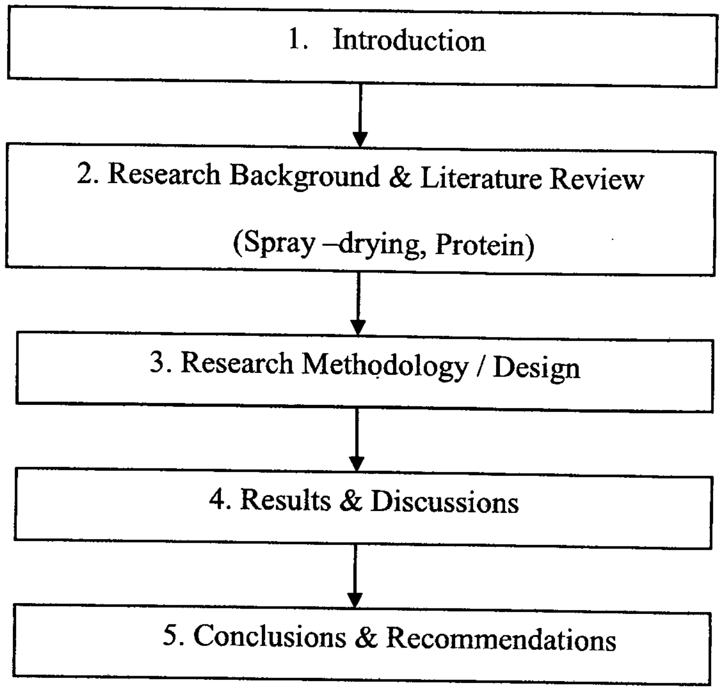

Figure 1-1: Road map for the thesis

Chapter 2 describes literature review and research background of spray drying operation including the related fields of drying and atmospheric drying. It also involves proteins' properties along with technological challenges involved in the spray dried of whey protein.

Chapter 3 is a detailed description of research methodology or design for this research . It is mentioning about the various materials used and the methods of characterising the samples and standards. Furthermore, it includes product quality analysis techniques which are denaturation measurements by reversed phase HPLC (RP-HPLC/ RPC) methods. Particulate characterisation tests include total solid content, particle sizing, morphology (SEM) and microbial tests.

Chapter 4 is about the results and discussions of this research. The detailed report on experimental result along with the discussion of spray-drying milk powder is displayed at this chapter. The effects of process variables (outlet temperatures and employment of microencapsulation) on protein denaturation and also the effects on particle size, morphology, solid content and sterility is being discussed.

Chapter 5 gives the conclusion of this research where the contribution of main findings and also the recommendations for future work.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Spray drying is the technology most widely used in the liquid technology shaping and in the drying industry. The drying technology is most suitable for producing solid powder or particle products from liquid materials, such as solution, emulsion, suspension and pump able paste states, For this reason, when the particle size and distribution of the final products, residual water contents, mass density and the particle shape must meet the precise standard, spray drying is one of the most desired technologies. The most important responsibility for an operator of a spray drier is to maintain constant moisture content of the powder. This is required to meet legal standards and for maintaining a uniform quality.

Average operating conditions for spray drying will vary somewhat depending upon the dryer system used and must be adjusted to produce the desired uniform moisture content. It is important to understand how the final moisture content can be controlled by changing the conditions. But first, it should be noted that the final moisture content is controlled by the relative humidity of the outlet air. If that value is too high, then the powder particles will absorb moisture rather than give moisture away.

The primary conditions which may be controlled directly by the operator are inlet temperature (setting of thermostat), flow rate of liquid feed (pump speed and pump pressure), air flow rate (fan speed and position of baffles), particle size (adjustment of atomizer). Among other operating conditions, outlet temperature and relative humidity of the outlet air are particularly important and need careful attention.

However these can only be indirectly controlled by adjusting the primary conditions. For outlet temperature, the condition is dependent upon liquid feed intake. If the feed intake is increased, the outlet temperature will drop. If the intake is reduced, the outlet temperature will increase and approach the inlet temperature. The outlet temperature will also be affected by the air flow rate. For a constant inlet temperature and constant feed intake, an increase in the air flow will raise the outlet temperature.

2.1.1 Literature review

Spray drying has become the most important method for dehydration in the Western world. Drying is the oldest method of preserving food. In ancient times, man froze seal meat on ice in frozen climates and in tropical climates he dried food under the sun. The first artificial drying of food was recorded in the 18^{th} century (Anandharamakrishnan et al., 2008). A British patent on drying of vegetables was issued in 1840 and dried vegetables produced in Canada were shipped to South Africa for the British forces in the Boer War in 1899 – 1902 (Vanarsdel and Copley, 1963).

However, the technology has been expanded to cover a large food group which now is being successfully sprayed dried. Relatively high temperatures are needed for spray drying operations. Nevertheless, heat damage to products is generally only slight, because of an evaporative cooling effect during the critical drying period and because the subsequent time of exposure to high temperatures of the dry material may be very short. The typical surface temperature of a particle during the constant drying zone is 45-50 °C. For this reason, it is possible to spray dry some bacterial suspensions without destruction of the organisms.

2.1.2 Overview of literature review

The physical properties of the products are intimately associated with the powder structure which is generated during spray drying. It is possible to control many of the factors which influence powder structure in order to obtain the desired properties (Richert, 1974). The advanced techniques measurements have been used for this purpose; however those advanced measurements have limitation because, measurements of air flow, temperature, particle size and humidity within the drying chamber are very difficult and expensive in large scale spray dryers. (Anandharamakrishnan et al. (2007). Several researchers (e.g. Kelly et al., 1998; David et al., 2004; Paul et al, 2004; Alessandra et al., 2005; Rong Reynolds, 2005; Sodini et al.,2006; Anandharamakrishnan et al., 2008) had carried out detail measurement of analysis on protein content for products obtained from spray dryer chamber using High Performance Liquid Chromatography.

The table of the comparison of journals for denaturation of protein by using Spray –drying with the focus of their findings is shown in Table 2.1.

Table 2-1: Published Journal of Denaturation protein by using Spray –drying and their finding

Author	Protein Type	Temperature	Analytical Methods	Remarks
Eskew et al. (1957)	Whey proteins in fresh pasteurized milk.	73 , 100 , and 190 ° F.	Ultracentrifugal tests	The value of foam drying as a means for improving the dispersibility of other dry fat-containing milks is discussed.
Richert (1974)	Whey proteins in dairy products	•Precipitation temperature (30 to 35 °C) •Heating temperature (44 to 46 °C)	• Precipitation • Ultrafiltration • Gel filtration • Electrodialysis	Production rates increase with temperature due to greater ion mobilities, but this, again, is limited by the temperature sensitivity of the membranes and whey proteins.
Modler and Emmons (1976)	Serum proteins in cheese whey	•Heating of the HCl (acidified whey was at 90 °C ± 2 °C) •Cooling of temperature from 50 °C to 63°C (measured at 25 °C)	• Micro-Kjeldahl procedure (Nitrogen determination) • Mojonnier procedure(Fat content determination) • Vacuum-oven method (moisture determination) • Mojonnier procedure (solids content of liquid samples determination)	• Protein recoveries in the range of 35% to 53% (No iron added) are low and of questionable value to potential whey processors. • Low protein recovery was accounted for on the basis of incomplete protein denaturation/aggregation during heating in an acidic environment (7, 8,10, 17). • In view of the unique functional properties of WPC prepared by this process, additional studies will evaluate alternative means of recovering whey proteins.
Matthews (1983)	Whey proteins in base milk products (cheese and casein)	At least 90°C for maximum yields	• Ultrafiltration • Gel Filtration	Yields are normally low, particularly for highly purified products. The total capital cost is high for most of these processes, regardless of the cost of the protein recovery step itself, because of costs of ancillary plant.
Burke et al. (1984)	Darbepoetin alfa (NESP, Aranesp®).	Focus on amount recovered by denaturing instead of temperature.	• Size-Exclusion Chromatography • Western blot	Spray drying, conducted at pilot scale with commercial equipment, is comparable to spray-freeze drying for encapsulation of darbepoetin alfa.

Table 2-1: Published Journal of Denaturation protein by using Spray –drying and their finding (Cont'd)

Author	Protein Type	Temperature	Analytical Methods	Remarks
Herrera et al. (1989)	*Spirulina* biomass	Temperature not specified in mentioning.	• Activated charcoal adsorption, ultrafiltration and spray drying (for high quality colourant grade phycocyanin) • Activated charcoal adsorption, ammonium sulphate precipitation, dialysis&chromatography (for preparing reagent grade phycocyanin)	Phycocyanin fraction provide a food colourant and biomarkers, and a protein-rich leftover useful as aquaculture feed.
Kelly et al. (1998)	Whey proteins in skim milk powder	• ***preheat temperatures:*** 75°Cx15 s; 97.5°C x 15 s; and 120°C x 2 min • ***inlet air temperatures:*** 170°, 187.5° and 205°C	• Reversed phase HPLC • Infra-red spectroscopy • Enzymatic assay.	• Succeeded in correlating the effects of process parameters of this technically advanced pilot plant (Tall-form drier) with the physicochemical properties of powders containing varying fat (20-80%) and protein contents. • The free fat content of powders may now be controlled much more precisely using an appropriate combination of total fat, atomiser nozzle selection and post-drying blending.
Brent et al. (1999)	•Pure Proteins Bovine Serum albumin (BSA) •β-lactoglobulin	• Air-dried, at 78 or 88 °C	• Ultrafiltration • Gel filtration	• Spray-drying whey protein concentrate (WPC) without sugars resulted in a dramatic decrease in the foam stability, whereas drying in the presence of sugars gave better retention of the original foaming properties
Keogh et al. (2000)	Milk protein hydrolysates	• Not specified (more emphasize on free fat content in ranging from 2	• Microencapsulated for fat powder performance	• Microencapsulated fat powders were developed and optimised to suit various baking applications. • Free fat was found to have the greatest effect on the • Properties of the bread.

Table 2-1: Published Journal of Denaturation protein by using Spray –drying and their finding (Cont'd)

Author	Protein Type	Temperature	Analytical Methods	Remarks
Maa Y-F et al. (2000)	Protein/Peptide-based drug formulations	Not specified (more emphasize on summarize of methods available for protein powder preparation)	• Lyophilization, spray drying, pulverization, and precipitation (common methods) • Supercritical fluid precipitation, spray-freeze drying, fluidized-bed spray coating and emulsion precipitation	• To evaluate each method from a more practical sense in terms of process versatility and scalability • To examining the individual process effect on protein stability that is always the focus of formulation scientists
Konrad et al. (2000)	*β*-lactoglobulin (*β*-lg)	More focus on characterization of physicochemical properties instead of temperature.	• Electrophoresis • Fast protein liquid chromatography (FPLC)	• The isolated *β*-lg retained a high degree of purity and native properties
Remondetto et al. (2001)	Soybean proteins	• 65°C • 80°C • 95°C	• Isoelectric precipitation • SAADH 205 model Westfalia centrifuge (Westfalia, Oelde, Germany) • Basket Type Centrifuge	The hydration properties of soybean isolates obtained at pilot plant scale and different processing variables showed good correlation between one another and also with the degree of denaturation.
Anna et al. (2001)	whey protein isolate (WPC-80)	• No pre-heating • 60 °C - 90 °C	• ESCA • SEM	The heat-treatments of WPC affect the functional properties of the spray dried powders
Remondetto et al. (2002)	Protein from Soy	Temperature range of 67—75° C	Surface Response Methodology	Existence of a critical temperature above which the rupture of disulphide bonds would increase the molecular flexibility

Table 2-1: Published Journal of Denaturation protein by using Spray –drying and their finding (Cont'd)

Author	Protein Type	Temperature	Analytical Methods	Remarks
David et al. (2004)	Soy protein	• Optimized conditions of 45 °C. • Previous conditions of 20 °C.	• Soy protein fractionation method • Reversed phase HPLC	Optimized conditions yielded more β-conglycinin with higher isoflavone and saponin concentrations , but fraction purity was diminished by glycinin contamination
Paul et al. (2004)	Darbepoetin alfa (NESP, Aranesp®)	Comparison of product yielding between spray drying and spray-freeze drying instead of thermal properties.	• Size-Exclusion Chromatography • Western blot	Spray drying, conducted at pilot scale with commercial equipment, is comparable to spray-freeze drying for encapsulation of darbepoetin alfa
Alessandra et al. (2005)	Obtaining protein isolates from white lupin seed (Lupin Protein)	• Ultra filtration with 40 °C (Initial Process) • Ultra filtration at cold temperature = 15 °C (Following Trials)	• Microbiological Analysis. • RP-HPLC (Analysis of Furosine) • Proximate Analysis of Protein Isolates (Analysis Of chemical composition such as dry matter, nitrogen, ash, and oil contents of the recovered Lupin Protein isolates.	• Permitted two added-value food ingredients to be produced from lupin seeds: i. LPI-E, endowed by a valuable emulsifying capacity ii. LPI-F, which may be useful when foam production and stabilization are required. • The effort dedicated to the careful selection of the process parameters, especially during spraydrying, permitted thermal damage to be limited to levels, which is comparable to other food ingredients.
Oldfield et al. (2005)	*β*-lactoglobulin A, *β*-lactoglobulin B, *α*-lactalbumin, bovine serum albumin (BSA), and Immunoglobulin G	• Preheating temperatures (70 °c - 120 °c for 52 s) • Concentrate heating temperatures (65–74 °C) • Temperatures for spray drying (200/101 °C until 160/89 °C) (inlet/outlet temperature)	• IDF standard method (1993a) • Method of Sanderson (1970) • IDF method (1988) • Orion pH meter	• Varying the preheating conditions had a marked effect on the denaturation of whey protein • Varying concentrate heating temperature and inlet/outlet air dryer temperature had a minimal effect on whey protein denaturation

Table 2-1: Published Journal of Denaturation protein by using Spray –drying and their finding (Cont'd)

Author	Protein Type	Temperature	Analytical Methods	Remarks
Michael et al. (2005)	Immunoglobulin G (IgG)	Inlet (130°C) & outlet air temperatures (190 °C)	• Fourier Transformation • Infra-Red Spectroscopy	The addition of trehalose (high glass transition temperature)similarly protects the IgG during spray-drying
Rong Reynolds (2005)	• β-Lactoglobulin-Vitamin A • β-Lactoglobulin-Vitamin D	Incubated at 40 °C for 2 hr.	• HPLC analyses. (Determination of content of vitamins in the powder complexes)	• The addition of lactose into the complexes would yield higher recovery of vitamins from spray drying. • Both lights and acidity lead to the quick degradation of vitamin A in the beverage.
Ashish et al. (2006)	• Whey protein	pH behaviour is in investigating with pH 3.5 (max) & pH 4.5(min) instead of thermal behavior.	• Ultrafiltration • UF-retentate	• UF-retentate showed highest thermal stability under acidic conditions followed by freeze dried WPC and spray dried WPC.
Sodini et al. (2006)	whey buttermilk	pH is specified in this research (4 -6) instead of temperature for whey protein.	• Kjeldahl method • Mojonnier Ether Extraction Method • HPLC	• Whey buttermilk showed stable levels of protein solubility, emulsifying capacity, and viscosity. • Sweet or cultured buttermilks, rich in casein, had lower solubility and emulsifying capacity, and a higher viscosity at acidic pH (pH < 5).
Perla et al. (2007)	Whey proteins: i.) $WP\text{-}P_{85}$ ii.) $WP\text{-}P_{138}$ iii.)WP-L	Two inlet/outlet air temperatures as below : i.) 170/85 °C ii.) 260/138 °C	• Tryptophan Emission Wavelength • Column Foam • Air Injection • Video-Foam Images	• Solution of $WP\text{-}P_{138}$ spray-dried powder, obtained using the highest air temperature conditions, has higher foaming properties than $WP\text{-}P_{85}$ and WP-L solutions. • $WP\text{-}P_{138}$ powder exhibited lower moisture and free lactose contents, a higher particle internal porosity, and a thinner particle wall thickness than the $WP\text{-}P_{85}$ powder.
Burak (2007)	Alginate/protein solutions	• T_{inlet} from 125 °C to 175 °C • T_{outlet} from 63 °C – 81 °C	• Confocal Laser Scanning Microscope • Bradford Modified Method(Coomassie Kit, Pierce, Fisher, Canada)	Particle recovery depended on the inlet temperature of the drying air, whereas the particle size was affected by the feed rate and the alginate concentration of the feed solutions.