UNIVERSITI PUTRA MALAYSIA

FORMULATION AND PRODUCTION OF HIGH PROTEIN AND HIGH CALORIES (HPHC) FOOD PRODUCT FOR CANCER PATIENTS

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ABSTRACT

Abstract of thesis presented to Faculty of Engineering in fulfilment of the requirement for the Bachelor of Process and Food Engineering.

PRODUCTION AND OPTIMIZATION OF HIGH PROTEIN HIGH CALORIES (HPHC) FOOD PRODUCT FOR CANCER PATIENTS

By MUHAMMAD YUZRUL HAFIZI BIN KAMARUL BAHARIN SEPTEMBER 2020

Supervisor : ASSOC. PROF. DR. AZHARI SAMSU BAHARUDDIN

Faculty : Faculty of Engineering

Vegetable-based food products is one of the most sought after food product in the market nowadays. It can be an excellent protein source and also source of other essential nutrients like minerals and vitamins, with also an additional energy booster. These are the factors that rendering vegetable-based food products to be a potential production for a high protein high calorie (HPHC) food product that will benefits for cancer patients. The aim of the present study was carried out to find the best and optimize medium formulation for a food product that contains high protein high calories (energy) by using vegetables-based food products. Sugar content (sucrose from range 10.0g to 20.0g), amount of soy protein isolate (SPI) (within 10.0g till 20.0g), and the amount of polydextrose (PD) (from 3.0g to 9.0g) were selected as process parameters to determine the medium formulation by using response surface methodology (RSM). The medium formulations that are obtained from RSM have been used to analyze, and its properties are observes. The pH that are analyses from the sample solutions produce a slightly acidic properties (within pH 6.26 to pH 6.49). The solubility index for the sample solution was observed to have high solubility indexes (from 88.8% till 95.9%). The °Brix value proven that some of the sample solutions from the medium formulations able to produce high calorie food product due to °Brix obtained (from range between 7°Brix till 11.3°Brix). Based from the three analysis of the vegetable-based high protein high calorie media formulations, the optimize medium formulation is produce by using Design Expert software. The optimize formulation have acceptable pH value for consumer preference (pH 6.39), high solubility index that proven the food product are soluble in solvent (91.6%), and with the °Brix value of (9.70°Brix). Therefore, vegetable-based products can be used to produce high protein high calorie (HPHC) food product that have benefits for critically-ill patients especially those that have and suffers from cancers.

Key words: Vegetable-based product, High protein high calorie (HPHC), Medium formulation

ABSTRAK

Abstrak tesis yang dikemukan kepada Fakulti Kejuruteraan sebagai memenuhi keperluan untuk Bacelor Kejuruteraan Proses dan Makanan

PENGHASILAN DAN PENGOPTIMUMAN PRODUK MAKANAN YANG TINGGI PROTIN TINGGI KALORI (TPTK) BAGI PESAKIT KANSER

By MUHAMMAD YUZRUL HAFIZI BIN KAMARUL BAHARIN SEPTEMBER 2020

Penyelia : ASSOC. PROF. DR. AZHARI SAMSU BAHARUDDIN

Fakulti : Fakulti Kejuruteraan

Produk makanan berasaskan sayuran adalah produk makanan yang terlaris di pasaran buat masa kini. Ia boleh menjadi sumber protin yang terbaik dan juga sumber kepada nutrien penting seperti vitamin dan mineral, dengan tambahan penggalakkan tenaga. Ini adalah faktor kepada pemilihan produk makanan berasaskan sayuran berpotensi sebagai produk makanan tinggi protin tinggi kalori (TPTK) yang memberi faedah kepada pesakit kanser. Tujuan pembelajaran ini dilaksanakan adalah bagi mencari rumusan sederhana yang paling terbaik dan optimum bagi produk makanan yang mengandungi tinggi protin tinggi kalori (tenaga) dengan menggunakan produk berasaskan sayuran. Kandungan gula (sukrosa dalam lingkungan 10.0g sehingga 20.0g), kandungan protin asingan soya (dari 10.0g sehingga 20.0g), dan polidekstrose (dari 3.0g sehingga 9.0g) dipilih menjadi parameter proses bagi menentukan rumusahn sederhana dengan menggunakan metadologi permukaan tindak balas (MPTB). Rumusan sederhana yang diperoleh dari MPTB telah dianalisa, dan ciri-cirinya telah diperhatikan Nilai pH menunjuk larutan sampel mempunyai ciri berasid setelah dianalisa (dari pH 6.26 sehingga pH 6.49). Index kelarutan menunjukkan larutan sample mempunyai kadar larutan yang tinggi (dari 88.8% sehingga 95.9%). Nilai °Brix membuktikan larutan sample mampu menghasilkan produk makanan yang tinggi kalori (7°Brix sehingga 11.3°Brix). Berdasarkan tiga analisa sebelum ini, rumusan sederhana yang telah dioptimum mampu dihasilkan menggunakan perisian Design Expert dan telah diterima kerana mempunyai nilai pH yang bersesuaian dengan pilihan pengguna (pH 6.39), tinggi kadar larutan (91.6%), dan nilai °Brix yang diterima (9.70°Brix). Oleh itu, produk berasaskan sayuramn boleh diterima pakai bagi penghasilan produk makanan tinggi protin tinggi kalori (TPTK) yang berkhasiat bagi pesakit yang kritikal terutama sekali pesakit kanser.

Kata kunci : Produk berasaskan sayuran, Tinggi protin tinggi kalori (TPTK), Rumusan sederhana

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LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percentage
g	Gram
g/L	Gram per liter
h	Hour
s	Seconds
HPHC	High Protein High Calorie
SPI	Soy-Protein Isolate
PD	Polydextrose
RSM	Response Surface Methodology

CHAPTER 1

INTRODUCTION

1.1 Background

In an article written by Jessie Szalay (2015), she wrote that protein is considered to be essential macronutrients especially for the one that targeted to build muscle and gaining weight. Protein is commonly found in animal products such as in chicken, cows, fish and others, though can also present in other sources, such as in nuts and legumes. She also wrote that according to the National Institute of Health (NIH), protein is the building blocks of muscle mass and muscle mass is a product from the breakdown proteins which can be link directly back to the amount of amino acids. Next, in her article also states that when protein is broken down in the body it will helps to fuel muscle mass, which helps metabolism and a lot of research has shown that protein also helps the immune system to stay strong and helps the consumer stay full and also gave satiety effects.

In another article written down also by Jessie Slazay (2015), calorie is a unit of energy or heat that could come from a variety of sources, such as coal or gas. In terms of nutritional wise, all types of food-whether they are fats, protein, carbohydrates or sugars-are important sources of calories, which people need in order to live and functioning well. In her article, a registered nutritionist dietitian states that every cell in our bod-require energy to function in its optimal state which can also leads to negative consequences, whether its losing lean muscle mass, not being able to concentrate or even not having the energy we need on a day-to-day basis. The general guidelines of calories requirements for various ages and activity levels that is provided by the National Institute of Health (NIH) states a middle-age moderately active female should consume 2000 calories per day while a moderately active male should consume 2400 to 2600 calories per day.

Next, according to a website article by WebMD states that there are more than 100 types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, and others. The symptoms for these cancers are varies and depended on the type of the cancer itself. In this article also mention that cancer patient is someone who suffers from an uncontrolled developing of cancer cells which will then crowd out healthy cells, and eventually draws nutrients from the body tissues. Thus, early treatments need to be done as soon as one is diagnosed to be having cancers.

In regards to cancer patients, they will need to undergo specific treatments which depends on the stages of their cancer and these treatments have their own side effects such as constantly feeling nausea, always vomiting, lack of appetite and others. Cancer patients who suffers from lack of appetite such as lung and mouth cancer patients need to undergo strict diet and thus changing their nutrients daily intake. Based on an article written by Stanford Medicine Cancer Institute, they suggested that cancer patients specifically should have smaller meal in their daily intake but must be taken regularly throughout the day before and especially after treatments. Lungs and mouth cancer patients specifically tends to lose their appetite easily which is caused from have the side effects of the whether the cancer itself or the treatments such as mouth sores, constantly vomiting and also feeling nausea. Plus, when a cancer patient started to lose their appetite, the cancer patient will start to lose weight rapidly which will then can lead to lack of energy especially after chemotherapy sessions.

In this study, vegetable-based food products are the ideal food products to be used as medium in producing a high protein high calories food. Therefore, this study is required to develop a medium formulation to produce a high protein high calories food products that are beneficial to be consumed by cancer patients which are extracted from the vegetable-based food products.

1.2 Problem Statement

i) To our knowledge, there are no specific prepared food products that contain both high protein and high calories that are available in the market.

ii) Most of high protein food products comes from animal-based such as cow's milk, chicken meat and etc.

iii) Carbohydrates with high GI are easily absorbed and metabolized to produce high energy but will also increase blood sugar level.

iv) Cancer patients tend to develop mouth sores due to the side effects of lack of appetite and having eating and drinking disorder.

1.3 Objectives

The purposes of this study are:

i) To develop formulation of food products that contained high protein and also high calories (energy) that suitable and healthy for cancer patients

ii) To further understand the properties of the developed HPHC food product for consumer consumptions

1.4 Scope of the Study

Based on the introduction and the problem statements as written above, the importance of this research can be seen and are understandable. For an example, due to the after effect that came from having cancer or the treatments, cancer patients tend to have lack of appetite and thus need a special meal that will help them have more appetite and ease them to digest the meal. Based on our knowledge, there are no specific prepared food products that contain both high protein and high calories that specifically targeted cancer patients. Thus, it is important for this research to be conduct in order for a prepared food product that are able to be consume by cancer patients can be produce or at least a continuation of this study will bear the fruits in the end.

Besides that, this study also will first be conduct through a set of questionnaires and surveys from volunteers that has medical background such as doctors, nurses, post-graduate students and even bachelor students. In this set of questionnaires, the volunteers need to answer based on their knowledge in medical field or dietary which

can also be used in any other researches in the future. The stability of the food product that will be produce will also play major roles in the production of the food product which are a very tough job and are very important if the product is a successful and mass production need to be done.

For this research, the specific cancer patients that this food product will be aiming at is the one who suffers from mouth and lung cancer patients due to the commonly having the side effects from cancer itself or the treatment such as mouth sore, vomiting, nausea and others. Plus, this side effects will also cause in rapidly losing of weight and constantly feeling hungry due to lack of food intake that was cause by lacking in appetite. Thus, for now this research are aiming for this both cancer patients and also this research have also the capabilities to be continued so that other types of cancer from the cancer patients can be treated or at least ease their burden and sufferings.

CHAPTER 2

LITERATURE REVIEW

2.1 Protein

2.1.1 Protein from Legumes or Nuts

Next, there are many healthy beans and legumes that are good for critically ill patients which have a number of health benefits, including reducing cholesterol, decreasing blood sugar levels and increasing healthy gut bacteria. For examples, chickpeas are a great source of fiber and protein which contains roughly 14.5g for each cup (164g) and chickpeas can help reduce weight, risk factors for heart diseases and potentially even the risk of cancer (Robertson, R., 2017). Thus, by adding beans and legumes with soups, stews, and salads will add more flavors to the foods and thus will also might improve the appetite for someone who has lack of appetite. Besides that, analysis that was done on the eastern and western daily diets might suggested that a positive implication on overall health are bound to occur when balancing the protein contribution between plant and animal (T. M. Campbell II, 2006)

2.1.2 Consumption of High Protein Diets for Cancer Patients

Firstly, protein is made up of smaller units which known as amino acids and has been categorized to have 22 types of amino acids. There are 9 types of amino acids is considered as "essential", which mean that this type of amino acids need to be consumed because out body cannot produce these amino acids. For examples, vegetable proteins do not able to provide the adequate amounts of every essential amino acid but can be combined with other plant sources in order to produce or make a complete protein (Spritzler, F., 2017). Beans, legumes, grains, soy, nuts and seeds are examples of high-protein plant foods and soy/soybeans are considered a whole source of protein which means that this protein can provide the body with all the essential amino acids is needs (Petre, A., 2016).

According to the nutritional claim done by the European Commission in their website, they state that for a food to be claim as high protein food, the total energy value of the food that must be provided by protein must at least be at 20%. There are many beneficial aspects practicing high protein diets especially for cancer patients such helps the patients to stay full for a much more period of time. There are also a test that is conducted on ten healthy, normal weight, female volunteers which 5 of them are asked to consume a high-protein, low-fat diet while the other half are asked to take a high-carbohydrate, low-fat diet and both of the meal needed to be taken during their breakfast and the results shows that the volunteers who take a high-protein meal showed improved in their appetite, satiety and diet quality and thus able to stay full until dinner time compared to the volunteers who take the high-carbohydrates meal (Johnstan, C. S. *et al.*, 2002).

Research has been done which the result shows that by using an approximate equation, cancer patients' loss around 5.3kg of muscle (McCurdy, B. *et. al.*, 2019). This is probably due to the after effect of the cancer treatments which sometimes interfere with the patient's ability to eat or drink and thus also affected their ability to maintain their healthy weight. Thus, it is very important for cancer patients to have a constantly consumption of high-protein meal in order to counter the rapidly weight losses from the after effect of their treatments.

2.2 Calories

2.2.1 Calories from Carbohydrates

Carbohydrates are by far the most complex macronutrients compared with protein and fats. This is because each person's body will use the different types of carbohydrates (such as fiber, starch and sugar) in very different ways from each other's. Plus, carbohydrates also are used by body as a quick source of energy, particularly for the brain, liver and muscles. Besides fiber which our body can't digest, all carbohydrates provide 4 calories per gram which might be varies depending on the degrees of carbohydrate quality (Penner, E., 2016). The breakdown of carbohydrates

in our body systems will produce sugars called glucose which will provides energy for all of your cells. Sugars is a great way to provides instant calories and can also acts energy booster but by taking them excessively, glucose can lead to weight gain and accumulation of the less harmful subcutaneous fat and overdosing fructose in daily diet can overwhelm the liver and can cause fatty liver disease, insulin resistance and more.

In a nutshell, there are three main types of carbohydrates which are sugar that are the simplest form of carbohydrates and can be found in fruits, milk and milk products. There are also starch which considered to be complex carbohydrate can be found naturally vegetables, grains, and cooked dry beans and peas and then there is also fiber which are also a complex carbohydrate and can be naturally be found the same as starch.

2.2.2 Calories from Fats

According to the same article, besides form being a potent and flavorful source of energy, fats are slow to be digested, deliver important fat-soluble vitamins to the body, and also able to provides important building blocks for every one of our cells. Besides that, in terms of calorie wise; all dietary fats are stated to provide about 9 calories per gram of food which also varies depending on the quality of the fats. For example, polyunsaturated omega-3 fats, which are naturally found in foods like wild salmon and flaxseed, have protective, anti-inflammatory properties, whereas artificial trans-fats have been linked towards heart diseases. Based on an article written by the Cleveland Clinic on their website, the total fat intake that should be taken on average diet that was recommended by the Institute of Medicine and the American Heart Association are 25-35 percent of calories which about 80 g of fat or less a day if you eat 2000 calories in a day and this will be differs according to the person needs or occupation.

2.2.3 Consumption of High Calories Food Products for Cancer Patients

According to the article written on a website by Stanford Health Care (2019), they state that there are some cancers that can affected the metabolic processes which can cause a situation known as hyper-metabolism which affects how the body uses proteins, fats, and carbohydrates. Plus, with hyper-metabolism you may need to increase your calorie and protein intake. For a rough estimate of calorie needs, plan on consuming 25 to 35 calories for each kilogram of weight and thus high calories of food is needed to supply this much of calories. Besides that, according the American Cancer Society (2019), the changes in the patients will naturally occurred during cancer treatment in order to help build up their strength and withstand the effects of the cancer and its treatment. Thus, they also state that patients will also starts to eat things that aren't normally recommended when you are in good health. For instance, the patients might need high-fat, high-calorie foods to keep their weight in-check, providing strength and energy, lower the risk of any infection and also helps to heal and recover faster.

2.3 Soy Protein Isolate

2.3.1 Application of Soy Protein Isolate

Soybean protein isolate or soy protein isolate is considered to be an important protein that are plant-based. As a kind of food additive, soy protein isolate is widely used in all kinds of food, for example baking, dairy, meat and other food (Sun D., *et. al.*, 2016). The protein solutions can play roles in the function of protein, thus the soluble of SPI was naturally used in most food processing

2.3.2 Soy Protein Isolates in Beverages

Nowadays, soy proteins isolate can be seen listed on the labels of many types of beverages which depending on the region, soy protein isolate was included from 9% up to 53% of the beverages (P. V. Paulsen, 2009). Plus, some of these beverages contain varies of protein sources and soy protein isolate are the chosen one as the most economical alternatives when compared with the other high-quality proteins and some of the products as emphasis the inclusion of soy protein and the benefits that it's promote in terms of health on their labels. Based on figure 2.1 (K. Liu, 2007), the number of beverages that was launched with content of protein ingredients including soy protein ingredients between the date of January 2001 and October 2007 are displayed. Plus, beverages that contained soy are found to be more appeal nowadays by most of the consumers especially the ones that are health-conscious and people that are not too keen with dairy products (G. Post, 2005).

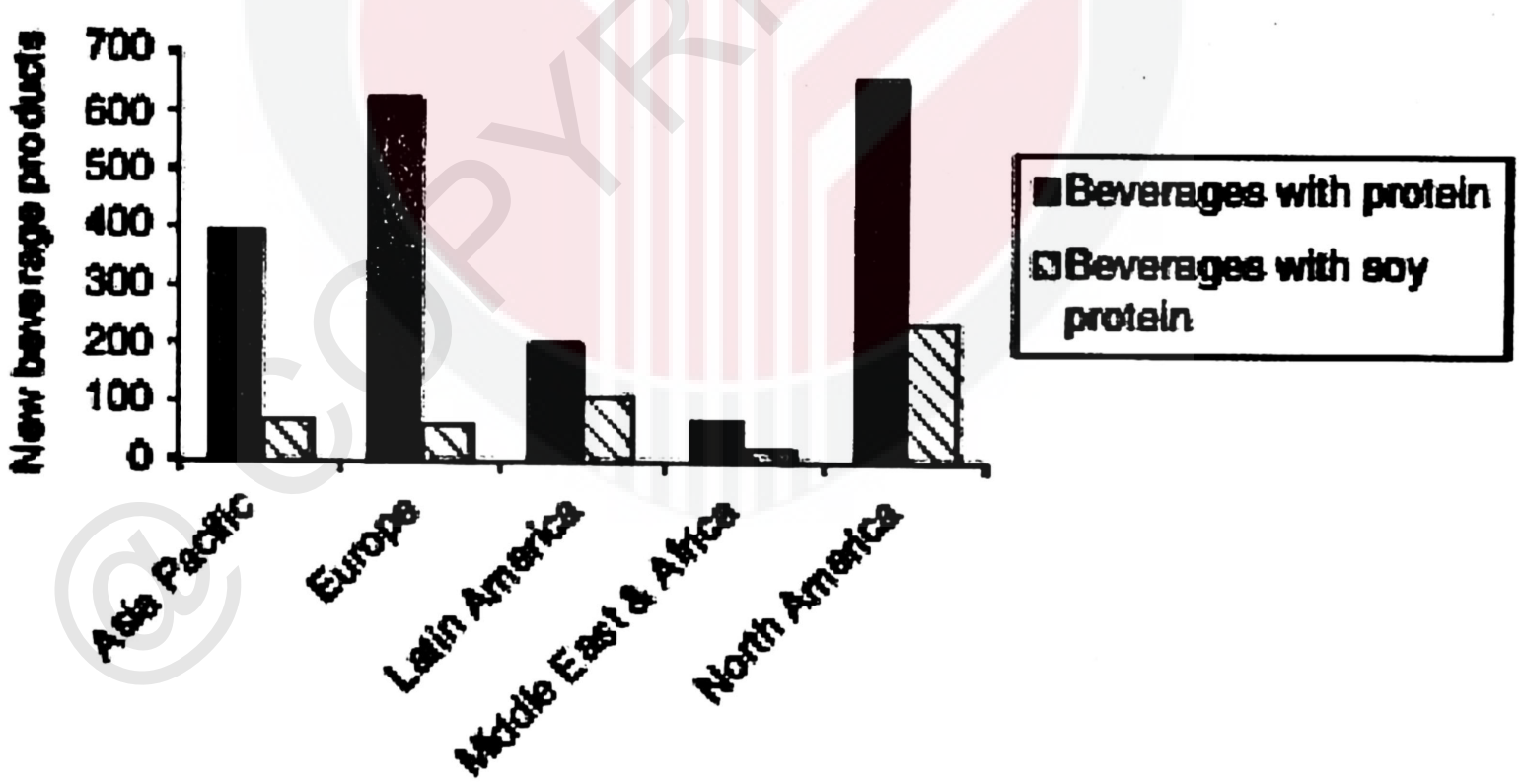

Figure 2.1 Plot of the new beverage that launches with any 'protein' or 'soy protein' in the ingredient label for different world regions. The figure was created by Solac, LLC from data tracked by Mintel GPND, Chicago between January 2001 to October 2007.

2.3.3 Benefits of Soy Proteins on Health

The soybean is known to be a rich source of beneficial nutrients (Golbitz and Jordan, 2006). Besides that, SPI was also associated with multiple benefits for health which includes heart health, cardiovascular health, weight management, menopausal symptom relief, and also the reduction of cancer risk. In multiple of studies, SPI has been shown and found to be able to reduce total and low-density lipoprotein (LDL) cholesterol, while being able to maintained and slightly raised high-density lipoprotein (HDL) cholesterol (P. V. Paulsen, 2009). Soy isoflavones have been hinted and observed to improve the artery health when soy protein are consumed by patients (Steinberg, 2003). Based on an article by Jenkins *et al. (2003)*, the figure 2.2 shows that when a low-saturated fat, and low-cholesterol diet that contained soy protein, plant sterols, oat fiber and nuts are fed to volunteers, the LDL cholesterol decreased by almost 30% and the ratio of LDL:HDL cholesterol decreased by significantly 25%.

In addition, soy protein also helps to reduce hunger in part through lowering a food's glycemic index which is a value that are given to a food that based on its ability to increase or raise the blood glucose in the body. Because of that, by consuming foods that are lower with glycemic index throughout the day could help to control the blood sugar swings, which by right should help an individual to feel more satiated and of course energetic throughout the day.

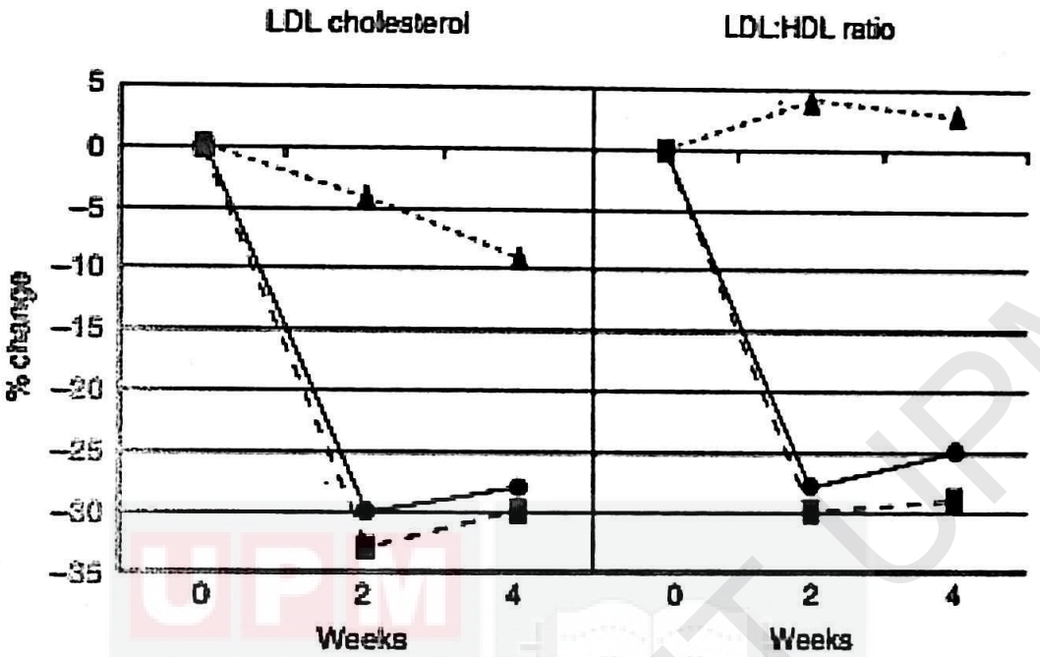

Figure 2.2 The impact of low saturated fat, low cholesterol (portfolio) diet containing SPI, plant sterols, oat fiber, and nuts verses drug therapy on serum cholesterol (Jenkins *et al.,* 2003)

2.4 Polydextrose

2.4.1 Polydextrose as a Functional Ingredient

Polydextrose (PD) is considered to be a low calorie, sugar free, low glycemic carbohydrate that has multiple of functional properties which includes high water solubility, high glass transition temperature, prebiotic properties, good stability at high temperature and have wide range of pH and are also widely known as a soluble dietary fibre (N. Veena, 2016). Plus, PD is also widely used in many food applications such as baked goods, ice cream, beverages, confectionery, chocolate, yoghurt, and salad dressings, among many others with a no maximum established limit that can be placed for PD. In addition, from an article by (Burdock & Flamm, 1999), PD has been agreed and approved as a direct food additive by the US Food and Drug Administration due to the use which are as a nutrient supplement, texturizer, stabilizer or thickener, formulation aid and also humectants. Besides that, PD are also able to give aids to the

blood glucose homeostasis because of its low glycemic index that it exhibit compared to the reference glucose (Foster-Powell *et al.*, 2002) and can also decreases the LDL cholesterol levels and the total cholesterol values that are currently in human blood.

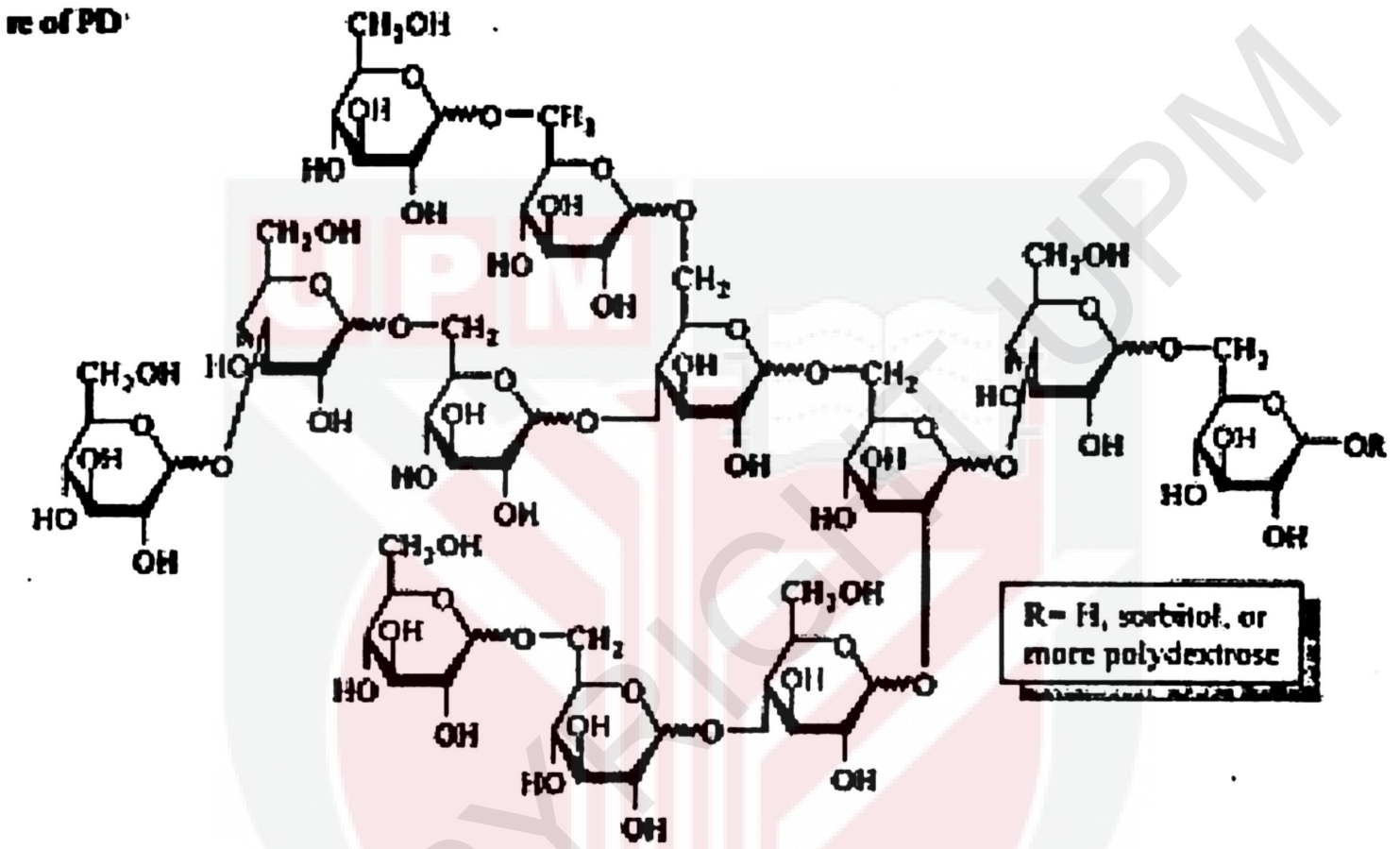

Figure 2.3 The physico-chemical properties of generic PD. Received from (N. Veena, 2016)

2.4.2 Polydextrose (PD) in Beverages and Dairy Drinks

Nowadays, PD would be used for either the replacement of sugar and/or fat in beverages and dairy drinks as low calorie bulking agent in order to improve the creaminess and mouthful. Plus, PD can also be used in variety of beverages which includes carbonated and non-carbonated, concentrated and ready-to-drink, and also hot and cold type beverages. Besides that, PD can also be applied in dairy drinks; neutral or flavored, drinks that are low in pH, pasteurized drinks, or UHT and in many other

clear beverage formats. The benefits for PD in beverages is that the ability to improve the mouth-feel, giving the taste experience of a product that are a much higher fat content; this is particularly became noticeable in the applications of the low-dairy drinks (Anonymous, 1991). After that, PD is also usually added to beverages as the source of dietary fibre as PD itself are very soluble, forming a clear solutions, and are very stable over shelf-life.

2.4.3 Effects of Polydextrose on Health

Polydextrose (PD) has been the subject of multiple studies which are due to its versatility and the multifunctionality. Besides being an excellent and functional ingredients, it has also been approved for use in foods in over more than 60 countries and is one of the most dietary fiber that are recognized in more than 20 countries. Besides that, PD has also been observed to improve physiologic functions without adverse effects when 4g to 12 g of PD are taken daily (J. Zhong, 2000). For an example, studies shows that the overall energy intake for the whole day was able to reduce when taken PD, also in a dose-dependent manner (A. Ibarra, 2015). When included in a midmorning snack, PD are observed to lead a significant reduced in terms of energy intake during the subsequent lunch. Plus, this effect was observed to be dose-dependent; 5% less energy intake with a preload of 6.25 g of PD and can up to 17 % less energy intake when 25 g of PD are taken. The most vital part of this studies is that the reduced in energy intake in the morning did not lead to a compensation of energy intake during the following dinner. This correlates with the nature of PD itself being a dietary fibers which have a tendency to influence the satiation (the feeling of fullness that leads to termination of a meal) and the satiety (the time after a meal until one is hungry again) (J. E. Bludell, 1996)

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Table Sugar (Sucrose) Powder

Powdered sugar is bought at the local supermarket, 99 Speedmart for a price of RM3.00 only. Then, the powdered sugar is ground again using High Speed Blender (HSB) (Xianganbangle) that is stainless steel and food grade. The powdered sugar is ground again in order to produce powder particles that have the same size. The blender is kindly provided by Process and Food Engineering Department Laboratory, Faculty Engineering, Universiti Putra Malaysia

3.1.2 Soy Protein Isolate Powder

Soy protein isolate (SPI) powder that will act as the main protein supply is kindly ordered and prepared by Dr. Mohd Sabri Pak Dek, Co- Supervisor for this project.

3.1.3 Polydextrose Powder

Polydextrose powder are most of the times used mainly for a sugar replacer and dietary fiber in foods (M. T. Flood, *et. al.*, 2004). This material is kindly ordered and prepared by Dr. Mohd Sabri Pak Dek, Co- Supervisor for this project.

3.2 Methods

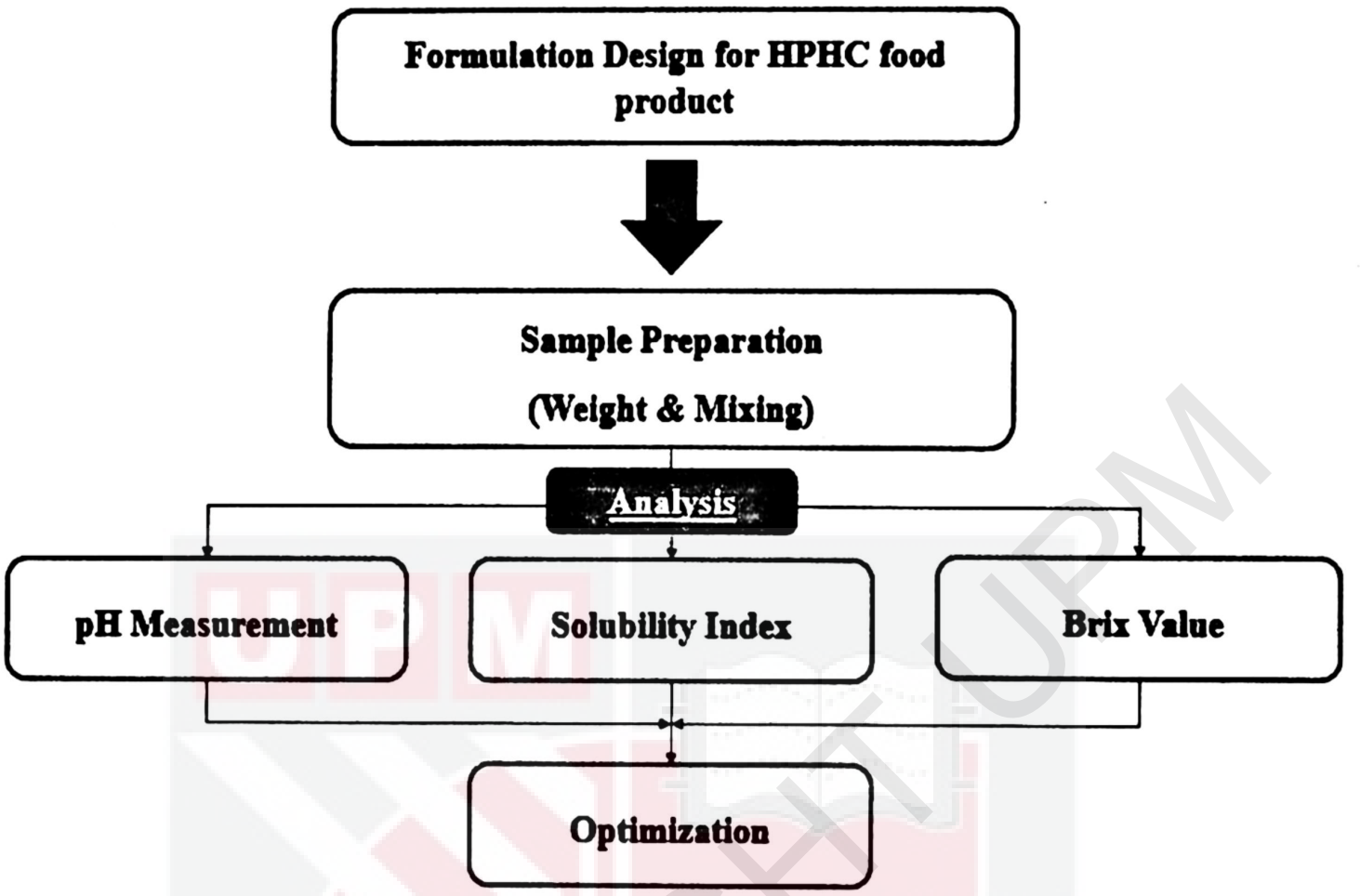

Figure 3.1 Diagrammatic representation of the whole flow process of the experiment in this project.

3.2.1 Formulation and Preparation of Samples

The formulation for the samples was done by using the response surface methodology (RSM) through the statistical computer software, Design Expert. One of the main purposes for using RSM was to formulate sets of random quantity when the levels of the parameters was decided (S. S. Moghaddam *et al.,* 2010). Firstly, the software was run and RSM are selected at the sidebar before the level of parameters was input. Then, the factors was inserted whereby the percentage of sucrose was set as, X_1, the percentage of soy protein isolate, X_2 and the percentage of polydextrose are then set as X_3. Then, the responses was inserts which are the pH values, the solubility indexes and the °Brix and with the help of Dr Sabri, the level for this process parameters was decided. The settings was run and the preparation of the samples was done by referring to the formulation.

Table 3.1 Levels of process parameters used in experiment

Code	Process parameters	Levels		
		-1	0	+1
X_1	Percentage of sucrose	10	15	20
X_2	Percentage of soy protein isolate (SPI)	10	15	20
X_3	Percentage of polydextrose	3	6	9

The samples are prepared by weighing each of the powders to approximately 0.01 g. An electronic weighing scale (TX323L) (SHIMADZU) are used to measure the weight of the desired powder by pouring them onto a folded aluminum foil. The powder are properly poured by using a spatula in order to avoid any spill and have better and accurate measurement. Each powder are weighed and placed inside a zip-lock plastic bag (5cm x 7cm) one by one using different aluminum foils. After that, the zip-lock bag are sealed and shaken moderately for approximately 15 seconds or until the powders are observed to be mix quite properly. The steps are repeated for the next formulation that are stated in RSM.

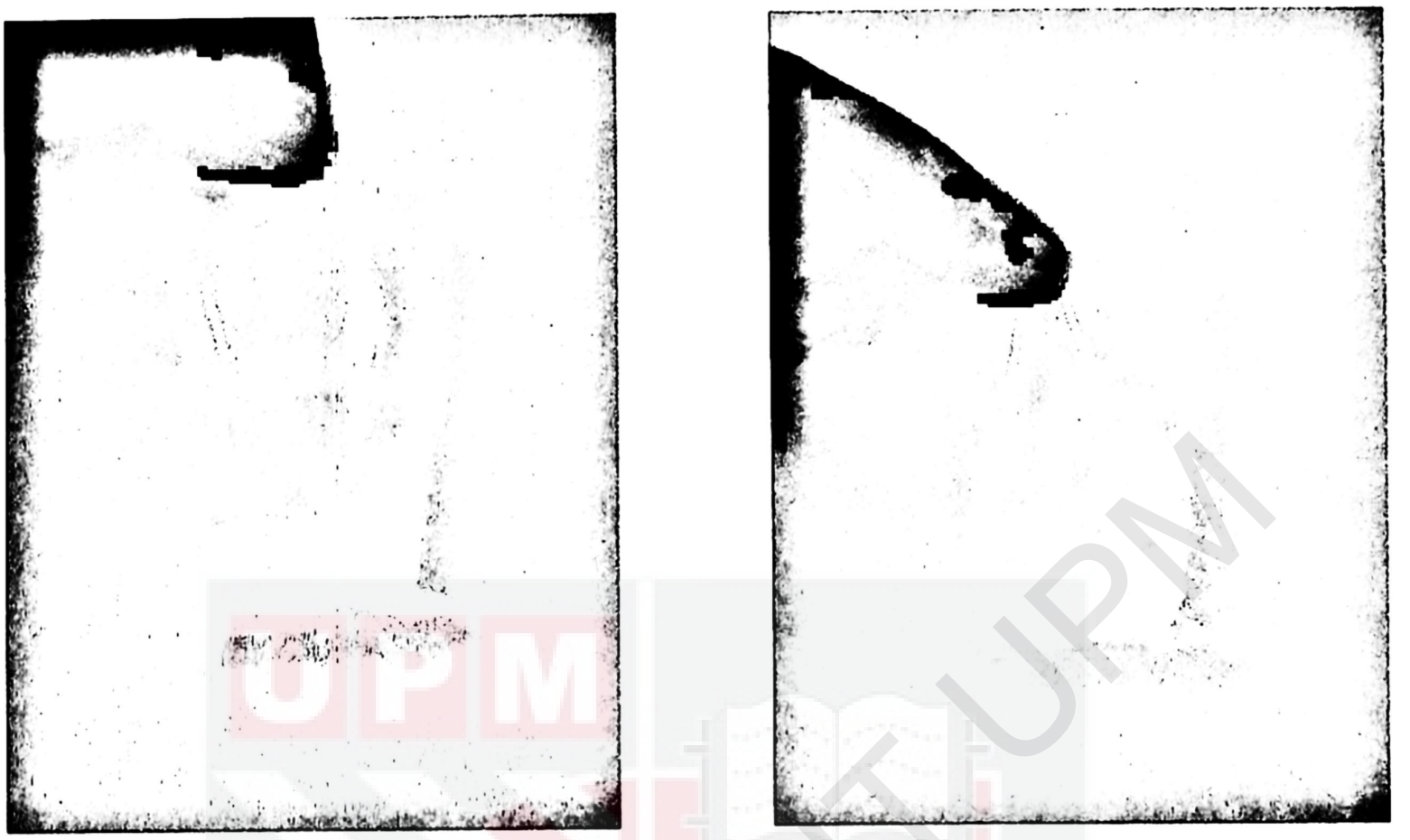

Figure 3.2 The mixture of all the factors or powders inside a zip-lock bag, before (left picture) and after (right picture) the mixing process, respectively.

3.2.2 Determination of pH

Based on an article by Walk *et. al.* (2001), the sample was mixed with distilled water at 1:10 ratio. Thus, 10 g of samples was poured inside a 250 ml beaker that were mixed and stirred with 100 ml of distilled water. Next, the mixture was sealed with aluminum foil and placed inside a chiller at 8 □ and left for 24 hours. Before the pH of the mixture was measured using pH meter (Sartorius) Model PB-10, the sample was brought to room temperature (about half an hour). The machine was calibrated first in prior to be used with solution of pH 4 and 7. The pH electrode was rinsed with distilled water before it was placed inside the mixture until constant reading was obtained or for about 3 minutes. The electrode was taken out and rinsed again with distilled water before the pH measurement of other samples.

3.2.3 Determination of Solubility Index

The sample firstly was weighed to 5 g and are directly transferred to a 50 ml round-bottom centrifuge tube that is then added with 35 ml distilled water. After that, before all the samples that is added with distilled water is placed inside a centrifuge machine, all the centrifuge tube is sealed tightly and was shaken gently for about 30 seconds or until the powder is slightly dissolved. Based on the article that is written by J. F. Cone & U. S. Ashworth (1947), the samples was placed inside a centrifuge machine that was set to be running approximately at 900 rpm for about 15 minutes.

After that was done, the supernatant fluid was decanted into a graduated cylinder while the top film of the fluid is retained with the used of spatula. Then, centrifuge tube where the sample is firstly reconstituted is mixed with 40 ml distilled water and stirred with a glass rod for about 10 seconds. Next, the wash water is decanted again while the floating material is maintained using a spatula before the tubes is placed inside an oven (Memmert) overnight which approximately about 12 hours with a temperature of 95 □. Then, the tubes and the dried sediments was weighed and the solubility of the samples is measured using the formula below:

$$[\textit{The weight of tube (g)} + \textit{the weight of dried sediment (g)}] - \textit{The weight of the tube (g)} = \textit{the true weight of the dried sediment (g)}$$

$$100\%\ \textit{Solubility} - \textit{The true weight of dried sediment}\ (\%) = \textit{The sample solubility}\ (\%)$$

3.2.4 Determination of Brix

Brix measurement in this project is done by using a hand refractometer (ATAGO) Model 9523 (made in Japan) that was able to measure up till 90% brix. The hand refractometer was firstly calibrated to zero by dropping a few drops of distilled water onto the view plate. The daylight plate is lowered and the lid is closed to let the water spread throughout the surface prism and the reading were observed. The

refractometer was calibrated by focusing the eyepiece until the multiple lines and numbers were less blurry when using the refractometer under a direct light. The surface of the prism was cleaned with a dry cloth that have soft fibers in order to remove any residue after the refractometer was adjusted to obtain the zero setting for pure water. The method suggested by B. Ruff (2020), the ratio of the sample and the distilled water is 1:10 and thus 5g of the sample was mixed with 50 ml of distilled water in a 100 ml beaker. After that, the mixture was stirred with a magnetic stirrer for 15 seconds or until the sample is dissolved before the readings was taken. An eyedropper was used to pour few drops of the mixture onto the surface of the prism and the brix reading was observed through the viewer (the eyepiece was adjusted when found necessary). The exact spot where the color started to change was kept in mind when looking through the viewer as that where the brix reading should be taken and the steps was repeated for the remaining samples.

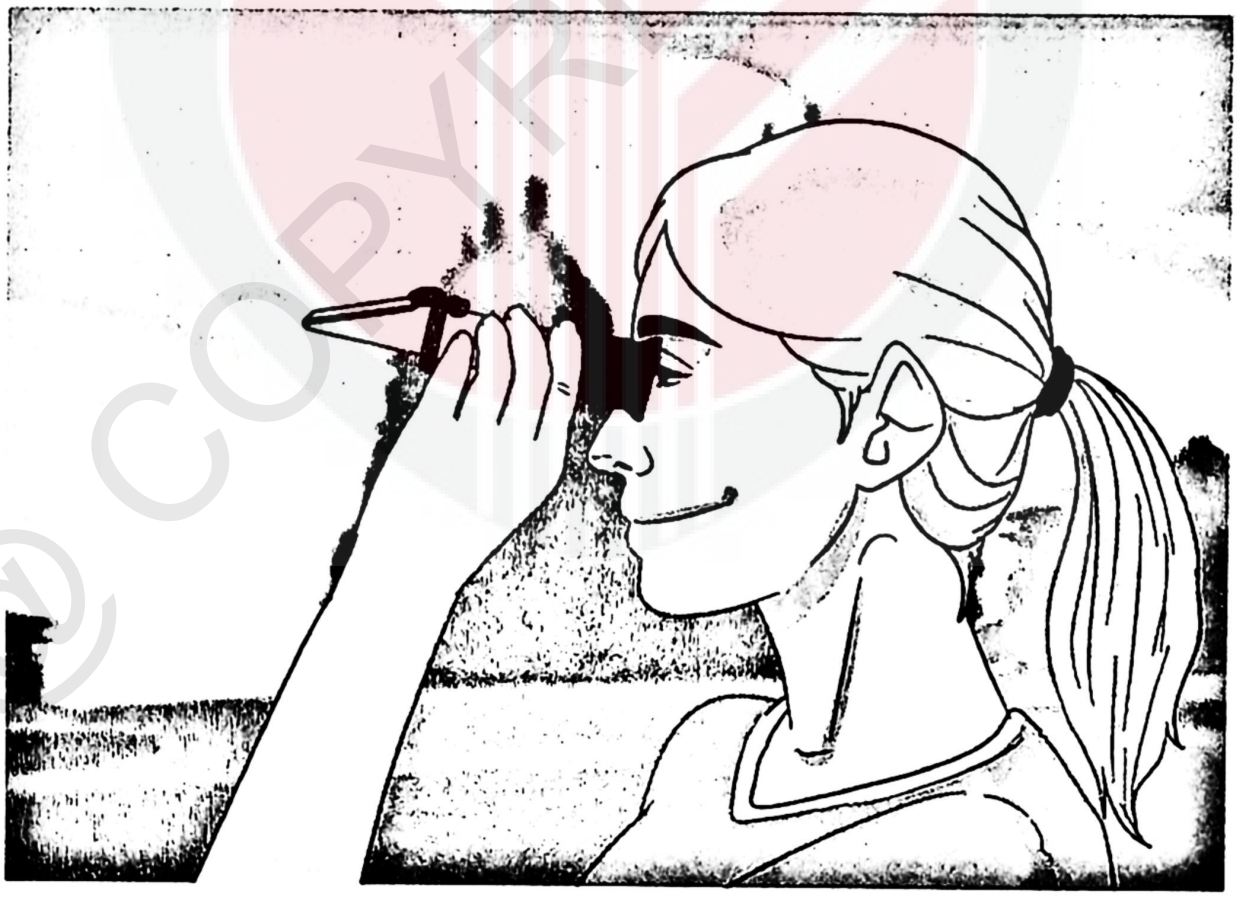

Figure 3.3 Method for measurement reading through hand refractometer. The image was retrieved from wikiHow, (2017).

3.2.5 Optimization of the Samples

For this purpose, response surface methodology (RSM) was applied in order to optimize the three operating variables for the production of high protein high calorie (HPHC) process including pH of the mixture, solubility indexes and the brix measurement. A statistical computer software, Design Expert (ver. 6. 0. 4) was used to design the experiment and optimize the responses and are quite helpful in order to fully grasped the interactions that is made between different independent variables and of course for the rapid formulation development (A. Hooda *et al.*, 2012). The percentage of sucrose (X_1), the percentage of soy protein isolate (X_2) and the percentage of polydextrose (X_3) were the three independent variables (factors) that were considered in the preparation of the high protein high calorie (HPHC) food product.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Determination of pH

An electronic pH meter was used in the determination of pH that exhibits from each samples. The pH for a specific solutions is important factor in determining the nature of the solutions whether it will be an acidic solution or an alkaline solutions. This properties of a solution are important especially for consumers' preferences, due to some are rather selective when choosing what is good for their body whether for cancer patients or a normal healthy person. Based from figure 4.2, it can be seen that most of the samples that was run through the formulation produced by RSM, exhibited pH that are slightly acidic which are in the range between pH 6.26 (run #17) to pH 6.49 (run #10). This results showed that the content of all samples are made up ingredients that are naturally have acidic pH and this containing acidic ions. Plus, this results can also be interpreted as the samples solutions quite possibly contained more

of hydrogen ions rather than hydroxide ions which cause the solutions to exhibit acidic solutions and have pH that are lower than pH 7 (neutral). Based on the content in each of the samples, the amount sucrose plays the major roles in affecting the pH values. Research that was done by K. L. Macagnan *et al.*, (2017) suggested that the pH when sucrose ions completely dissolved in a pure water solution will produced a sucrose solution that have a pH within the range of pH 5.8 to pH 7.2. In figure 4.1, it can be observed that a clear trend is produced such as when the amount of sucrose increases, the value for pH will decreases instead towards becoming more acidic solutions. For an example, the amount of sucrose from run #17 (20.0%) is higher compared with run #8 (10.0%), thus the results shows that the pH for run #17 (pH 6.26) is lower when comparing with run #8 (pH 6.45).

Besides that, the amount of other factors or ingredients in the samples can also affecting the pH of the sample solutions. Polydextrose (PD) is widely known as a sugar replacement due to its nature being a dietary fibers. Eventhough the amount of PD that mixed with the solutions are less when comparing with the other factors, the PD might still have an effect towards the pH of the solutions. After that, it can also be observed that some of the results showed that the amount of soy protein isolate (SPI) that are added to the mixture can also affecting the pH values of the solution. The same trend as the amount of sucrose can be seen in the SPI whereby as the amount of SPI added into the solution increases, a decrement can be seen in the pH of the solutions. For an example, run #2 and run #8 have different percentage for SPI but shared the same percentage of sucrose and PD; nevertheless both run have different pH values of each of their solutions which shows that the amount of SPI is rather important in this study. According to J. Jiang *et al.* (2009), pH starts to shift when SPI is induced in an alkaline solution which can be identify when the soy protein adopted a molten globule-like conformation. Besides that, the presence of SPI in the samples solution not only affecting the pH values, but also the emulsion to the mixture solutions. An article wrote by H. T. M. Hefnawy & M. F. Ramadan (2011), suggested that SPI exhibit and excellent emulsion properties over the range of pH 3.0 to pH 9.0 which could the help the solution to be more soluble.

Run	Factor 1 A:Sugar %	Factor 2 B:ISP %	Factor 3 C:Poly-Dextros %	Response 1 pH Values pH
1	20.00	10.00	3.00	6.48
2	10.00	20.00	9.00	6.37
3	18.00	12.00	6.00	6.39
4	15.00	15.00	11.10	6.27
5	6.60	15.00	6.00	6.36
6	23.40	15.00	6.00	6.46
7	15.00	6.60	6.00	6.43
8	10.00	10.00	9.00	6.45
9	10.00	10.00	3.00	6.46
10	12.00	18.00	6.00	6.49
11	12.00	15.00	6.00	6.48
12	10.00	20.00	3.00	6.37
13	15.00	18.00	9.00	6.37
14	20.00	10.00	9.00	6.39
15	15.00	15.00	0.95	6.45
16	15.00	12.00	3.00	6.37
17	20.00	20.00	9.00	6.26
18	15.00	15.00	6.00	6.28

Figure 4.1 Results of the pH analysis from each runs. Retrieved from Design Expert software.

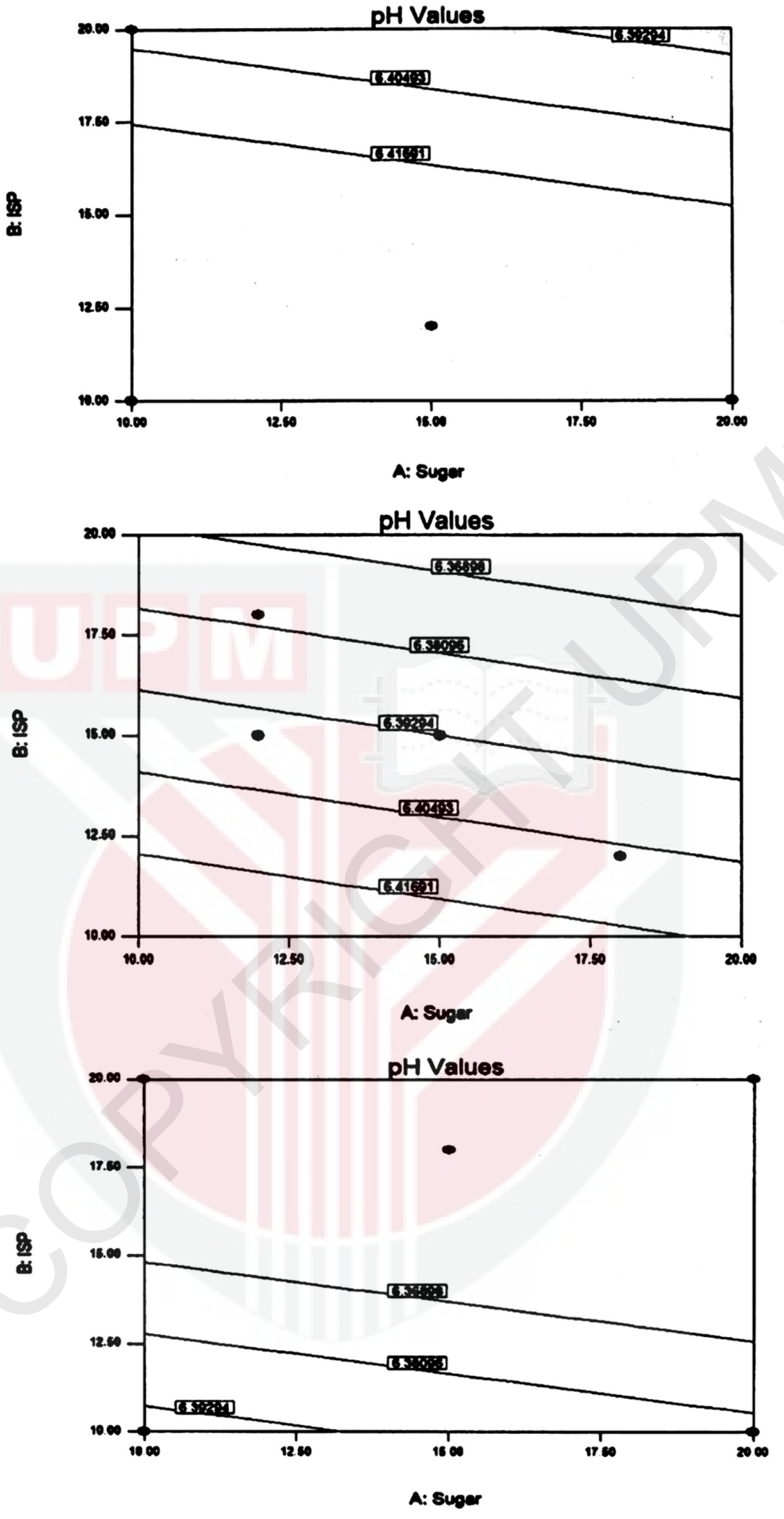

Figure 4.2 Model graphs for the pH analysis of the samples with different range of values for polydextrose; Range 3% of polydextrose (top), 6% polydextrose (middle) and 9% polydextrose (bottom). Retrieved from Design Expert software.

4.2 Determination of Solubility

Solubility of each solutions that derived from the formulation by RSM, are measured by subtracting 100% of solubility with the weight of the dry sediment which has been calculated as the percentage for the insoluble matter (J. F. Cone & U. S. Ashworth, 1947). Besides that, solubility can also be defined as the property of a solute to dissolve in a solvent which are fundamentally depends on the physical and chemical properties of the solute and the solvent itself. The extent of the solubility of the substance that are in a specific solvent is measured as the saturation concentration, whereby adding any more of the substance (solutes) only cause the excess amount of solute to begin precipitate at the bottom of the glass and does not affect the concentration of the solution such as the increase of the solution concentration. Based from figure 4.3, it can be observed that most of the samples display high solubility properties but are not able to achieve 100% solubility. Thus, this shows that there are some of the solutes that became precipitate and the solutes drop at the bottom of the flask.

Besides that, it can be observed that even if large amount of specific content are added to some of the samples, high level of solubility indexes are still able to be achieved. The high level of solubility might also be interpret as the concentration of the solute being lesser when comparing with the concentration of the solvent. According to an article written by N. Kubantseva *et al.* (2004), the results can also be concluded that the concentration and the temperature of the sample ions are the main factors that influencing the solubility of the mixture. Besides that, the high solubility of the samples can also be interpret as the solutes are grounded to very fine particles that it might be easier to dissolved in a solution when mixing the both.

Run	Factor 1 A:Sugar %	Factor 2 B:ISP %	Factor 3 C:Poly-Dextrose %	Response 2 Solubility mg/L
1	20.00	10.00	3.00	90.3
2	10.00	20.00	9.00	89.7
3	18.00	12.00	6.00	91.4
4	15.00	15.00	11.10	91.8
5	6.60	15.00	6.00	90.2
6	23.40	15.00	6.00	93.1
7	15.00	6.60	6.00	84.3
8	10.00	10.00	9.00	92.5
9	10.00	10.00	3.00	95.9
10	12.00	18.00	6.00	88
11	12.00	15.00	6.00	93.4
12	10.00	20.00	3.00	91.3
13	15.00	18.00	9.00	85.4
14	20.00	10.00	9.00	84.8
15	15.00	15.00	0.95	98.7
16	15.00	12.00	3.00	92
17	20.00	20.00	9.00	89.2
18	15.00	15.00	6.00	88.8

Figure 4.3 Results of the solubility analysis from each runs. Retrieved from Design Expert software.

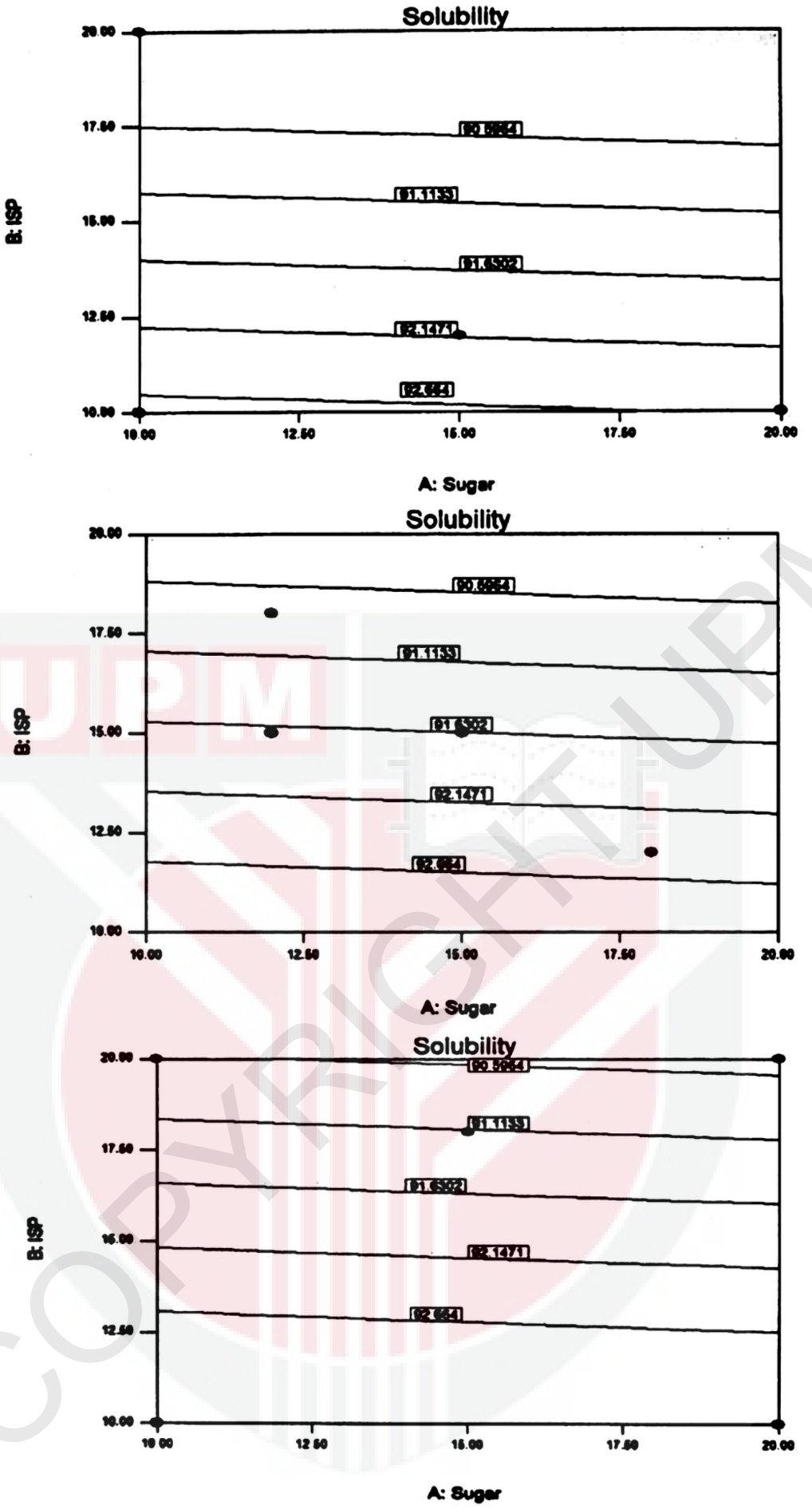

Figure 4.4 Model graphs for the solubility analysis of the samples with different range of values for polydextrose; Range 3% of polydextrose (top), 6% polydextrose (middle) and 9% polydextrose (bottom). Retrieved from Design Expert software.

4.3 Determination of Brix

In the determination of Brix values in this project, a manual, hand-held refractometer was used instead of the digital refractometer in order to reduce and minimize any errors from occurs. Nevertheless, some errors did occurred during taking the measurement such as random. For an example, the results for run #8 might due to some random errors during taking the measurement and this the results for run #8 should have a higher value of Brix when comparing with run #9 due to the more amount of polydextrose (act as sugar replacement) that existed in run #8 when comparing with run #9, however the amount of polydextrose added to the samples might have less or negligible effect towards the °Brix readings even though polydextrose can still be considered as one of the glucose polymer (M. M. R. Do Carmo *et al.*, 2016)

Next, the food and beverages industry applied Brix measurement quite commonly in their production or in researches. Scientifically speaking, the Brix measurement can be defined as the determination of pure sucrose that existed or dissolved in water. Well known food and beverages industry that applied the Brix measurement such as the production of soft drinks, fruit juices, tomato concentrates and etc. Besides that, F. Pistón (2016), stated in his article that Brix are able to shows a relative kind correlation with the sugars content and Brix are a relative good measurement in order to determine the total sugar content in a solution. Based on figure 4.5, a clear ascending trend can be observed quite immediately whereas the relationship between the amount of sugar/sucrose content and the observed Brix values. Plus, the range of the ascending Brix values are between 7°Brix to 12°Brix with the lowest being from run #5 (6.6% sucrose) and the highest being from run #6 (23.4% sucrose) respectively. Besides that, it can also be observed that the amount of SPI that are measured can be considered as insignificant when measuring the Brix for the solution. This is because, the results from run #12 shows a low Brix values when comparing with the other runs even though the SPI content in run #12 is higher when compared with the remaining runs that have a lower SPI content such as run #1. Thus, from this analysis a high sucrose content can be identified which can help in selecting the solution for the optimization to produce a high protein high calorie (HPHC) food products for cancer patients.

Run	Factor 1 A:Sugar %	Factor 2 B:ISP %	Factor 3 C:Poly-Dextros %	Response 3 Brix %
1	20.00	10.00	3.00	11.3
2	10.00	20.00	9.00	8
3	18.00	12.00	6.00	10
4	15.00	15.00	11.10	9.5
5	6.60	15.00	6.00	7
6	23.40	15.00	6.00	12
7	15.00	6.60	6.00	9.8
8	10.00	10.00	9.00	8.5
9	10.00	10.00	3.00	9
10	12.00	18.00	6.00	9.2
11	12.00	15.00	6.00	9
12	10.00	20.00	3.00	8.5
13	15.00	18.00	9.00	10
14	20.00	10.00	9.00	11
15	15.00	15.00	0.95	10.2
16	15.00	12.00	3.00	10
17	20.00	20.00	9.00	11.2
18	15.00	15.00	6.00	9.8

Figure 4.5 Results of the Brix analysis from each runs. Retrieved from Design Expert software.

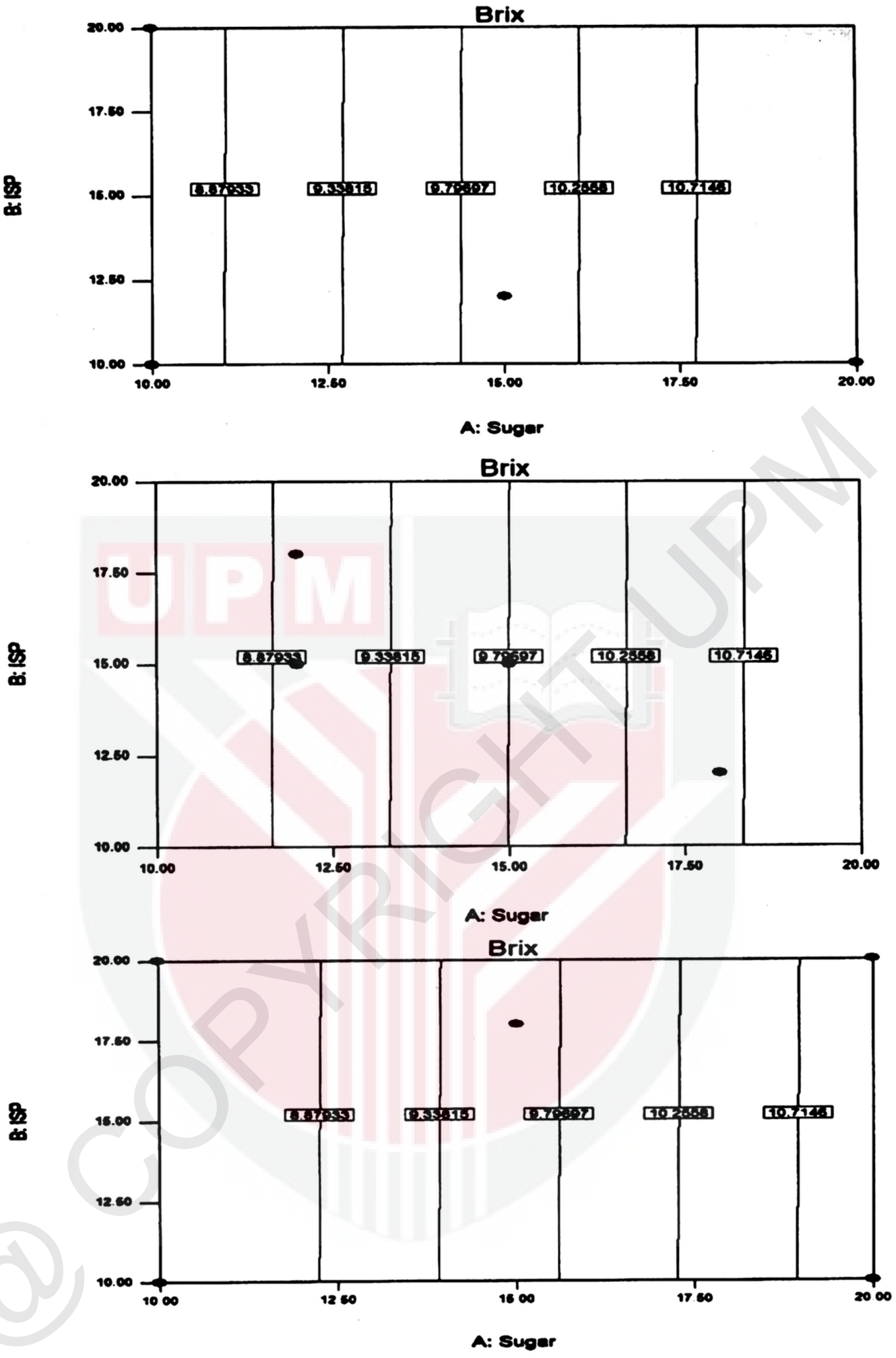

Figure 4.6 Model graphs for the Brix analysis of the samples with different range of values for polydextrose; Range 3% of polydextrose (top), 6% polydextrose (middle) and 9% polydextrose (bottom). Retrieved from Design Expert software.

4.4 Optimization of Samples

In order to obtain the most optimize formulation that are based from the samples, each of the independent variables have to be optimized by using response surface methodology (RSM). The predicted data that was produced based on all the data that were analyses are to have pH values of 6.39, 91.63% solubility, and 9.79% of °Brix, thus the solutions that are the most closest to the predicted responses value were to be selected. This solution will be considered to be the best formulation to produce High Protein High Calorie (HPHC) food product for the cancer patients. The Design Expert software predicted that in order to get the predicted responses value, the model should follow the following conditions:

Table 4.1 The solution suggested by Design Expert software

Solutions	Sucrose (%)	SPI (%)	PD (%)	pH Values	Solubility (%)	°Brix (%)	Desirability
1	15.00	15.00	6.00	6.39	91.63	9.70	1.00

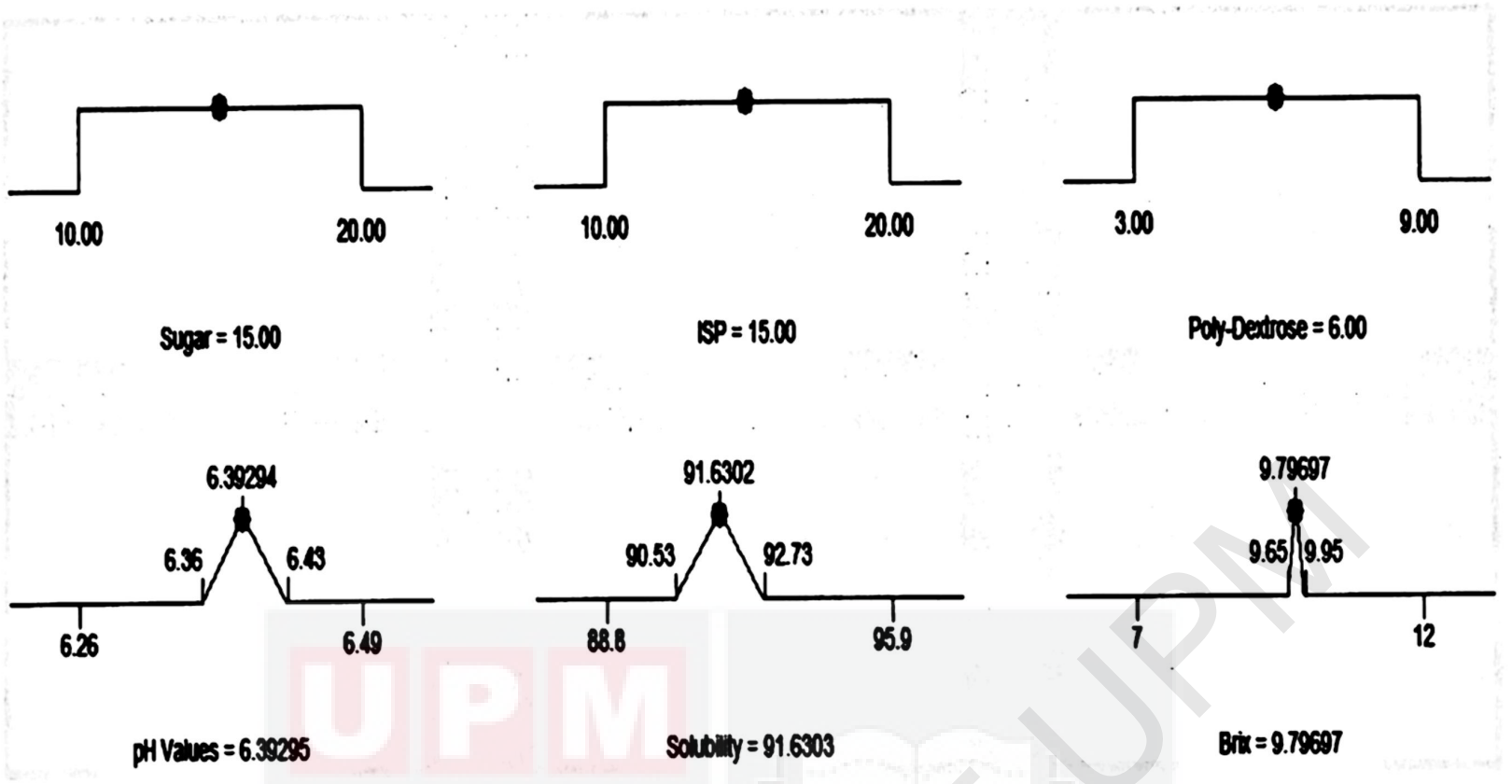

Figure 4.7 Ramps interpretation for the response prediction of the solution. The image was retrieved from Design Expert software.

As shown in table 4.1 and figure 4.1, to obtain the prediction responses value, the optimal value of the independent variables which are sucrose percentage is 15%, SPI percentage to be also 15%, and the ideal polydextrose percentage which are 6.0% needed to be used. With these optimal conditions, a high protein high calorie (HPHC) food product are able to be produce for the cancer patients.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The optimize formulation are proven can achieved for a high protein high calorie (HPHC) food product. It contains high amount of sugar (calorie); sucrose content (15.0 g), and also high amount of protein; SPI content (15.0 g) and with an additional sugar replacement or a dietary fiber; polydextrose content (6.0 g) that aid the mixture in order for an extra boost of energy when consume the food product. This optimized formulation also proven to have consumability properties due to its high solubility index (91.6 %), approximately near to pH 7 (neutral) which can be accepted by most consumers (pH 6.39) and also mid-range value for °Brix value (9.79 °Brix), but more research needed to be done in order for the food product ready to be release for consumers such as stability test, flowability test, sensory test and many more. The value of pH that it exhibit can be accepted for consumer consumption, while the solubility shows that there will be less excess of solutes that became insoluble matter which are very important especially for cancer patients that have sore throat from the side effects of diagnose with cancers. After three analyses (determination of pH, solubility index, and °Brix) were conducted on the formulation that was proposed by response surface methodology (RSM), the content of desired materials (sucrose, soy-protein isolate and polydextrose) have been identified. After analyses, the pH of the mixture solutions are identified to correlates with the amount of sucrose content and polydextrose while not so much bias towards SPI. Besides that, the solubility shows most of the solutions achieved high solubility percentage which can due to the concentration of the solvent or the nature of the solutes before mixing in the solvent. Plus, the brix analysis indicates sucrose to play the major roles in controlling the value of °Brix. Thus, from the analysis that was done, the properties of the formulation that was developed through RSM are able to be understood further which can be used in identifying the optimize formulation of the HPHC food product that especially gave benefits for cancer patients. After that, the analysis are diagnosed and modeled before optimized using the Design Expert software. In a nutshell, the optimized formulation is proposed as the best formulation to produce a high protein high calorie (HPHC) food product for cancer patients.

5.2 Recommendations for Future Study

Future study can be conducted for improvement on the optimize formulation of high protein high calorie (HPHC) food product for cancer patients despite the main objectives of the study were achieved. A detailed list of future work recommendations is given below:

1) The optimized formulation of the HPHC food product can be enhanced by studying and identifying its shelf life. The samples can be place in the desire place and the shelf life can be study such as the samples are place in an open area, in a chiller or freezer and etc. As the samples are made from the mixing of materials in powder form, the samples can be place in an open area while being seal in a zip-lock type bag. The study on the shelf life can also help in identifying the period before caking occurs for the samples. Caking on a food product occurs when agglomeration occurs towards a free flowing materials, such as food powders when the food are stored at rest for a long period of times. Thus, a suitable anticaking agents can be identified in order to prevent the caking process by absorbing any excess moisture and making them become more water-repellent. For an example, calcium silicate ($CaSiO_3$) are a common anti-caking agent that can be added to a food product.

Furthermore, future investigation on the other application of the high protein high calorie (HPHC) food product can be consume by not only critically-ill patients such as cancer patients as a food replacement but can also be consume by athletes as an energy booster in their working regime where by the nutrients in the samples not only have protein for building muscles but also contained high sugar content that aid to supply energy for the working cells in the body.

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APPENDICES

Sample	pH Value			
	Run 1	Run 2	Run 3	Average
A	6.50	6.49	6.46	6.48
B	6.37	6.40	6.35	6.37
C	6.40	6.38	6.41	6.39
D	6.26	6.28	6.26	6.27
E	6.37	6.35	6.35	6.36
F	6.45	6.46	6.46	6.46
G	6.42	6.45	6.43	6.43
H	6.44	6.46	6.45	6.45
I	6.47	6.45	6.46	6.46

J	6.50	6.49	6.48	6.49
K	6.49	6.47	6.48	6.48
L	6.37	6.38	6.37	6.37
M	6.37	6.37	6.38	6.37
N	6.41	6.39	6.38	6.39
O	6.45	6.45	6.46	6.45
P	6.37	6.38	6.37	6.37
Q	6.25	6.27	6.26	6.26
R	6.27	6.30	6.28	6.28

Appendix 6.1 pH measurement at room temperature (25□) in three different runs.

Sample	Percentage Insoluble Matter (%)	Solubility (%)
A	23.5	90.3
B	24.1	89.7
C	22.4	91.4
D	22.0	91.8
E	23.6	90.2

F	20.7	93.1
G	19.5	94.3
H	21.3	92.5
I	17.9	95.9
J	24.8	89
K	20.3	93.4
L	22.4	91.3
M	18.3	95.4
N	18.9	94.8
O	23.1	90.7
P	21.7	92
Q	24.5	89.2
R	24.9	88.8

Appendix 6.2 Solubility index measurement at room temperature (25□) for each sample